



IAVI Report

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Beast in the belly

A new focus on early HIV infection in the gut and other mucosal tissues may generate novel strategies to study, treat, and prevent infection

By Philip Cohen

An untreated HIV infection is often pictured as a long, bloody battle. The virus infects a new host, the immune system strikes back and the fight enters into a prolonged struggle. Then, after many years, the immune system finally collapses and the virus is declared the winner.

At least that was the story told as judged from the perspective of peripheral blood samples, which is how researchers and clinicians have usually followed the infection. But evidence is accumulating that immunity's struggle with HIV may actually be fought—and lost—within the first few weeks of acute infection on battlegrounds in the gut and mucosal tissues.


“Early on, the immune system’s boat is sunk and it is left in the middle of the ocean swimming for its life,” is how Louis Picker of the Oregon Health and Science University describes the new model of HIV infection. “The only question after that is: how long will it last?”

If early HIV infection does deliver a mortal belly wound to the immune system, it suggests that tipping the balance in that first battle will be important for understanding AIDS, directing future research into the disease, as well as holding implications for drug treatment and vaccine design.

Researchers have long known that the primary targets of HIV (and its nonhuman primate-infecting relative SIV) are CD4⁺ T cells. During the chronic phase of infection, peripheral blood levels of these cells can be fairly constant, and it is only when their numbers drop that the infection develops into the symptoms and severe consequences of AIDS. Clinicians and researchers generally followed the battle in peripheral blood because it is a tissue that is easy to collect. But it hasn’t been clear that the picture there was representative of the infection or the status of the immune system, given that the majority of CD4⁺ T cells reside in the gut and other mucosal tissues.

Gut feeling

An early hint that peripheral blood wasn’t telling the whole story came in a study from Ronald Veazey of Tulane National Primate Research Center and his colleagues. They were

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Keystone 2006

Steady progress presented and discussed at the annual Keystone Symposia on HIV


By Kristen Jill Kresge and Simon Noble

This is a milestone year in HIV/AIDS. Although HIV has been within the human population since the 1930s, 2006 marks the 25th year since AIDS was first medically recognized. Tristram Parslow of Emory University, one of the co-organizers of the Keystone Joint Symposia on HIV Pathogenesis and HIV Vaccines, opened the

symposia by referring to the signature year, saying that it was 25 years since HIV “emerged” and ushered in the “dawn of the HIV epidemic,” and reminding the audience that 40 million people are now infected worldwide and that 35 million of those don’t know it. This year also marks the 10th year since the advent of HAART, and he pointed

out that in those 10 years HAART has added, on average, 13 years to each patient’s life, which equates to 3 million life-years saved. Despite this success, that’s no comfort to the large majority of the world’s infected who still don’t have access to ARVs.

The Keystone HIV Symposia are one of the primary showcases for researchers from

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studying the effects of SIV infection in rhesus macaques in the gut-associated lymphoid tissue (GALT), which is host to different developmental stages of CD4⁺ T cells. In inductive sites such as intestinal Peyer's patches, these cells are naïve and resting. But on exposure to antigen, they traffic through the blood and home to effector tissues, the largest of these being the lamina propria (LP) in the intestinal villi.

The researchers infected animals with SIVmac239, a pathogenic strain, and then at one week intervals drew blood and performed biopsies on the animals from peripheral lymph nodes, spleen and the LP. After only one week into the infection, CD4⁺ T cells in the LP dropped to about half the level seen in uninfected controls and continued to slide down after that, nearly disappearing in some animals. However, at the same early time points in the same animals, CD4⁺ T cells in the blood and other tissues remained relatively constant (*Science* **280**, 427, 1998). Later work by Veazey and collaborators showed the killing was focused on memory CD4⁺ T cells that also expressed SIV's (and HIV's) coreceptor CCR5 on their surface. Since these cells account for a high proportion of the cells in the LP, but a smaller percentage of CD4⁺ T cells in the periphery, this helped explain why killing may have been focused in this tissue (*J. Virol.* **74**, 11001, 2000).

While the results suggested a similar rapid, early depletion of CD4⁺ T cells in the gut could play a role in HIV infection, some experts were skeptical. "What I heard from other investigators was: SIV in monkeys wasn't going to be like HIV in humans," says Veazey. "The monkey disease had a faster course, so it could involve a distinct mechanism of pathology."

Eventually though, reports from human clinical studies supported the idea that the *modus operandi* of HIV was very similar to SIV. Satya Dandekar at the University of California, Davis and her colleagues found that levels of CD4⁺ T cells all but disappeared from the gut following HIV infection, even in two patients who were infected only four and six weeks before the biopsies were taken (*J. Virol.* **77**, 11708, 2003). Martin Markowitz and his team at the Aaron Diamond Research Center found the same remarkable depletion of the gut CD4⁺ CCR5⁺ effector memory T cells (*J. Exp. Med.* **200**, 761, 2004). Ashley Haase of the

University of Minnesota Medical School and Daniel Douek of the Vaccine Research Center (VRC) of the National Institute of Allergy and Infectious Diseases and their colleagues looked at the levels of CD4⁺ CCR5⁺ T cells in the gastrointestinal tract, lymph nodes and peripheral blood in patients who had been infected from less than one month to greater than five years. At all points of infection there was massive CD4⁺ CCR5⁺ T-cell depletion from the GALT. In contrast, gut CD8⁺ CCR5⁺ T cells were not depleted and CD4⁺ CCR5⁺ T cells in the blood and lymph nodes were nearly the same as in uninfected people (*J. Exp. Med.* **200**, 749, 2004).

Mechanism of destruction

Then, last year, two high profile papers tackled the question of mechanism by examining in fine detail the early interplay between virus and CD4⁺ T cells in the SIV/rhesus macaque model. The two papers used different techniques and stressed different conclusions. Douek, Mario Roederer (also of the VRC) and their colleagues used PCR capable of detecting single molecules of HIV DNA to examine the extent of viral colonization in the first few weeks of an infection. They concluded that 30-60% of CD4⁺ memory T cells contained SIV DNA at the peak of infection and most of these cells could be detected during a 4-day period. And by using flow cytometry to narrowly focus on CD4⁺ memory T cells (CD45RA⁻ or CD45RA⁺ CD95⁺) throughout the body, they found that the depletion of these cells was apparent at early stages even in peripheral blood, although the largest area of the depletion was the gut mucosa. They concluded that direct infection accounted for CD4⁺ memory T-cell killing, either due to virally-induced cell lysis or cytotoxic T-lymphocyte killing of infected cells (*Nature* **434**, 1093, 2005).

In the second report, Haase's team looked for SIV RNA in GALT sections. They also found that most of the viral gene expression was restricted to CD4⁺ memory T cells. But by their calculations, at the peak of infection only 7% of CD4⁺ T cells had detectable viral RNA, and infected cells could account for only 20% of the measured depletion. Haase's team favors a model where direct exposure to viral particles, but not infection, drives cells to undergo apoptosis (programmed cell death). In support of this

model the researchers found cellular markers of apoptosis in LP but not in inductive sites (*Nature* **434**, 1148, 2005).

Both groups also discovered a surprising population of cells involved in the infection. Douek and Roederer found that more cells were infected with the virus than express FACS-detectable levels of CCR5, suggesting that levels of CCR5 expression below detectable levels can still facilitate viral entry. Haase found that more than 90% of productively infected cells were not activated or proliferating, cells which are normally assumed to be the preferred replication ground for SIV and HIV. Even though these "resting" cells produce 5-fold less virus per cell they are about 10-fold more abundant in the LP, creating a large substrate for transmitted virus to target. All these results suggest that the gut may contain a close packing of cells that are not traditionally thought of as supportive of HIV replication. "What I think is most impressive about the GALT is that it is chock full of viral targets," says Douek. "The virus gets in there and it's like a swarm of locusts going through a cornfield."

While in agreement on the extent of GALT CD4⁺ T-cell depletion, the two different conclusions on mechanism are driven by the limits of each group's technology. PCR can detect a single HIV DNA genome, but can't determine whether that genome is involved in an active infection or an aborted one. HIV RNA production is a direct measure of viral gene activity, but not as sensitive as PCR and therefore might miss low levels of HIV gene expression that could still prove lethal to cells. "It's an interesting and potentially important question whether many cells are graveyards for HIV/SIV genomes or whether many cells are covertly infected," says Haase. He says that he has set up a consortium including the VRC group to combine techniques to follow the fate of the virus in the GALT even more closely. Defining the exact mechanism of killing may have implications for early therapy to treat infections.

Too little, too late

As well as being hard hit in the first wave of viral infection, GALT cells may be ill prepared to defend themselves. In separate work, Haase's team has used the SIV/rhesus macaque model of intravaginal transmission to compare CD8⁺ T-cell responses in GALT,

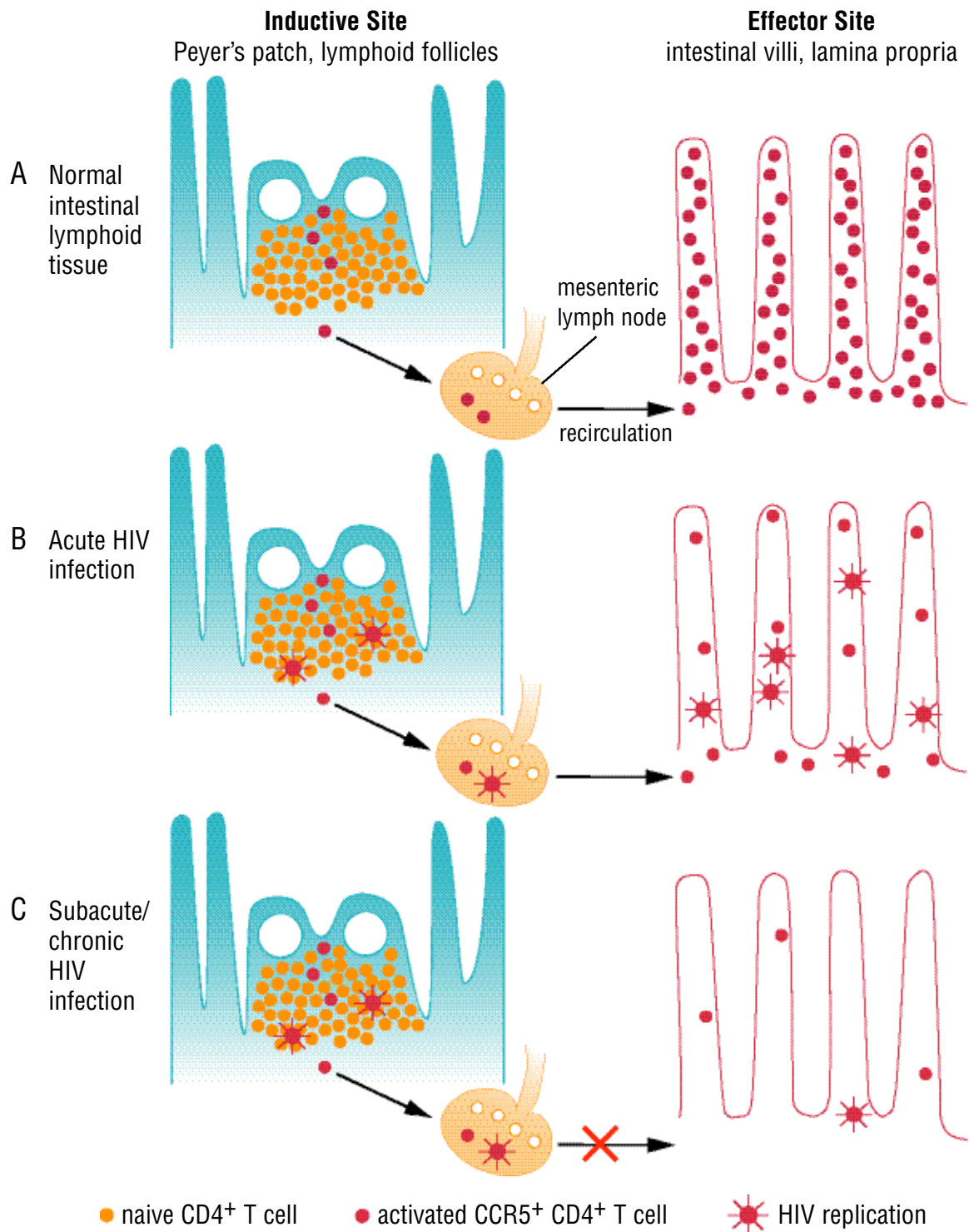


Figure 1. Target cell distribution and HIV replication in the intestinal tract. (A) In normal intestinal lymphoid tissue, naive CCR5⁻CD4⁺ T cells mostly reside in inductive sites. As they are exposed to the variety of antigens normally found in the intestinal tract (dietary antigens or intestinal microbes) these cells are activated, causing them to coexpress CCR5 and then rapidly recirculate and home to effector sites. Large numbers of these activated CD4⁺CCR5⁺ T cells then reside there. (B) In acute HIV infection the activated CD4⁺CCR5⁺ T cells are primary targets for HIV replication and are therefore soon destroyed. This coincides with the peak plasma viremia in early infection, and the subsequent decline in viremia may be due to the loss of this subset of cells. (C) During subacute/chronic HIV infection the extensive loss of CD4⁺CCR5⁺ T cells in the lamina propria means fewer targets and so lower levels of viral replication at effector sites. Steady-state T-cell activation due to antigen encounter in inductive sites maintains viral replication there, providing an inherent mechanism for viral persistence and ongoing replication. The majority of infected cells may be eliminated before homing to effector sites so CD4⁺CCR5⁺ T cells are never restored in chronically-infected patients, even in those given HAART. (Adapted from Veazey and Lackner, *J. Exp. Med.* **200**, 697, 2004)

vaginal and other tissues. They documented strong immune responses in vaginal tissues: the frequency of CD8⁺ T-cell responses against immunodominant SIV Gag or Tat epitopes was above 5% at 21 days post-infection. But this response occurred after the peak of viral infection, doing little to contain the infection until after a persistent infection was well established. In contrast, the CD8⁺ T-cell response in the GALT was consistently the lowest response of any lymphatic tissue and never reached 5%, an immune response Haase and his colleagues characterize as “too late and too little” (*J. Virol.* **79**, 9228, 2005).

Haase has since shown that even in animals infected rectally—a mode of delivery that normally favors the induction of intestinal immunity—the CD8⁺ T-cell response to SIV is weak and still lags behind that in the vaginal tract. “It’s stunning how really crappy the GALT immune response is,” says Haase. The results raise hope for protection of the vaginal mucosa if a vaccine could hasten the development of the immune response there—good news since it is a major entry point for the virus. But the work also suggests new strategies may be necessary to adequately protect the gut mucosa. One possible reason why the GALT may mount a poor defense is suggested by another recent paper from Haase, Picker, and Jeffrey Lifson of SAIC Frederick, Inc. at the National Cancer Institute and other researchers which shows that SIV infection appears to induce a premature regulatory T cell (Treg) response. Normally, a Treg response develops to limit immune responses to prevent damage due to excessive immune activity. But in the case of SIV-infected GALT, Tregs might arise too soon and blunt an immune response before an adequate one even develops (*J. Infect. Dis.* **193**, 703, 2006).


Early insult

There is also evidence that once damaged the GALT may be slow to recover. In their published work Dandekar and Markowitz both studied HIV-infected individuals on HAART. They found that while years of drug therapy was effective at restoring CD4⁺ T-cell levels in blood and lymph nodes, reconstitution of the CD4⁺ T-cell population in the GALT lagged far behind for patients who had entered the chronic phase of infection. Interestingly, for patients treated within the first few weeks of infection, GALT CD4⁺ T-cell restoration was far better but still incom-

plete. Similar results were recently reported at the 13th Conference on Retroviruses and Opportunistic Infections (CROI) based on longitudinal studies of patients. Dandekar reported that the suppression of viral replication and inflammation in gut tissue during therapy best correlated with the degree of mucosal CD4⁺ T-cell restoration during therapy. She has also recently reported that long term non-progressors (LTNPs) actually have slightly higher levels of GALT CD4⁺ T cells than healthy controls, suggesting this may contribute to the lack of disease progression in these individuals (*Proc. Natl Acad. Sci. USA* **102**, 9860, 2005).

Such results have thrown new focus on the issue of when to commence drug treatment for HIV infection. The benefits of early treatment versus delaying exposure to drugs and their possible side effects has long been discussed in the context of chronically infected patients. Now the question is whether initiating treatment in the first few weeks of infection would confer the added benefit of preserving GALT CD4⁺ T cells. Experts agree, though, that it is unrealistic to expect to identify most HIV-infected individuals at such an early stage. One exception is in the case of perinatal HIV infection. A recent study of 205 children found that very early treatment—by age 2 months, as opposed to 3 to 4 months—was associated with delayed and decreased progression of disease. The study did not, however, directly assess the effect on the GALT immunodepletion (*JAMA* **293**, 2221, 2005).

A situation where this goal of exceptionally early treatment might be achieved is within studies designed to identify acute infections. This includes pre-exposure prophylaxis (PrEP) clinical trials, which are testing the ability of tenofovir therapy to prevent HIV infection in volunteers at high risk. Trial participants with breakthrough infections would thus already be on drug therapy before their infection is detected. In an SIV-macaque model, Lifson, Veazey and their colleagues have shown that even early *post*-infection tenofovir treatment can result in preservation of GALT CD4⁺ T cells and low to undetectable blood levels of virus after 30 days of post-infection therapy (*J. Med. Primatol.* **32**, 201, 2003). It isn’t yet clear if there would be any benefit of trying to restore GALT at later phases of disease or even how to achieve restoration. “The gut work tells us that what happens early is actually quite striking,” says Michael Lederman of Case Western Reserve



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Michael Lederman

University. "The question now is if the deterioration of immunity and AIDS that we see later on is related to this early depletion and how." It will be important, for example, to see if the degree of GALT CD4⁺ T-cell depletion is predictive of disease progression.

T-cell depletion

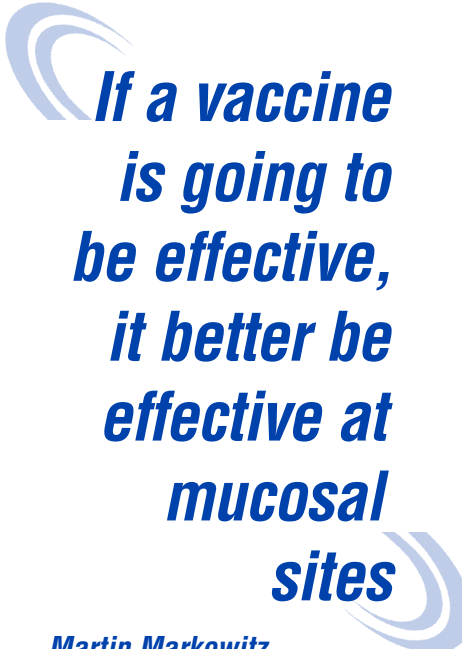
While researchers are trying to make that link by conducting prospective studies of HIV infection, some general theories are emerging of how devastation of CD4⁺ T cells in the GALT may determine the course of disease. As Veazey and his Tulane colleague Andrew Lackner write in a recent commentary (*Nat. Med.* **11**, 469, 2005) the revelation that HIV infection eliminates much of the memory CD4⁺ T-cell population in the first days of infection suggests the subsequent decline of the immune system is the result of a "valiant but futile effort to replace these cells." Indeed Picker, Lifson, and their colleagues have reported that the success of this effort may be a good indicator of disease progression. They assessed the aftermath of CD4⁺ T-cell depletion in SIVmac239 infected macaques using bronchoalveolar lymphocytes obtained by lung lavages as a window into the mucosal immune system. In some animals the depletion was followed by a significant boost in the production of short-lived CD4⁺ memory T cells which migrated to the lungs. In these animals the infection was stable and did not rapidly progress to disease. But in monkeys where this capacity to partially repopulate the mucosal CD4⁺ T-cell compartment quickly failed, disease progression was rapid (*J. Exp. Med.* **200**, 1299, 2004).

This work suggests that the very immune activation that holds off immediate collapse of the immune system in the aftermath of GALT immunodepletion may also have two negative effects: creating activated cells to feed the next round of viral replication and setting the rate at which crucial immune cells are used up and at which disease progresses. "Once the initial depletion occurs, the regenerative capacity of the immune system is likely engaged in a war of attrition it cannot win in the long term," says Lifson. "While it may take a year or two in a macaque and a decade or more in a person to progress to AIDS, much of the eventual outcome is probably determined in the earliest stages of the infection." In another recent perspective article, Douek and other VRC team members also focus on subsequent immune activation as a possible link to disease pro-

gression and suggest how mucosal depletion itself may drive that activation (*Nat. Immunol.* **7**, 235, 2006). These authors cite some of their own unpublished findings that immune activation persists even in HIV-infected people whose viral replication is highly suppressed by drug therapy. They propose that the massive depletion of CD4⁺ T cells during acute infection and possibly other changes in the GALT destroy the integrity of the normally tight immunological barrier in the intestine. As a result the systemic immune system becomes chronically subject to antigens from intestinal flora leading to increased immune system activation and other pathological changes such as fibrosis of lymph nodes.

Clearly, connecting the dots between early events and pathological changes that result in AIDS won't be easy. Many of the crucial events may occur in the GALT and other mucosal tissues that can only be accurately followed with invasive tissue biopsies. "You can't biopsy a thousand patients in a vaccine trial," says Veazey. One alternative strategy now being explored is to catch the cells en route to these destinations by using flow cytometry of T cells in the blood to examine cell surface proteins that determine where effector memory T cells migrate, so-called "homing markers." A number of promising markers are already being explored, among them CCR9, CCR10, and CD103, but a great deal of careful work is still needed to see how expression of these proteins relates to the immunological state of the GALT, especially in the context of HIV infection.

For vaccine designers, an important goal will be vaccines that induce secretion of antibodies and elicitation of cytotoxic T lymphocytes in this tissue. "I think the handwriting is on the wall," says Markowitz. "If a vaccine is going to be effective, it better be effective at mucosal sites." Roederer says that if appropriate markers could be found to monitor the immunological state of the GALT using samples of peripheral blood, it would be a valuable indicator of vaccine effectiveness. "Of course, the ultimate goal is for a completely protective vaccine," says Roederer. "But if people who are vaccinated become infected it's important to know if they're protected in any meaningful way. If we can prove a good correlation between the degree of early GALT depletion and later disease progression, for instance, then we'd have a rapid marker we can use in vaccine trials and know the benefit in weeks, rather than years." ☺



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Martin Markowitz

all corners of the HIV research field to come together and share data, ideas, plans, experiences, and, importantly, develop research collaborations. Presentations range from the intricate molecular biology that HIV employs to go through its lifecycle, to the complex immune effects it elicits as it interacts with its host species, to new findings uncovered as researchers look for ways to prevent infection with vaccine candidates.

Immunology in real time

One of the first presenters began by joking that, since he has never worked on HIV, he originally thought he had been invited by mistake to give one of the keynote opening seminars. Ulrich von Andrian of Harvard Medical School uses intravital microscopy to view immunological processes in living animals in real time. His research group has used 2-photon microscopy to look first-hand at homing and cellular trafficking in the lymphoid tissues, particularly lymph nodes and Peyer's patches in the intestine. After microsurgical exposure of the lymph nodes behind the knee of a mouse that leaves intact the microcirculation of lymph in afferent and efferent lymphatics and blood in high endothelial venules, different immune cell types that have been stained with differently colored fluorophores are introduced.

Von Andrian presented captivating movies showing how these immune cells interact in their natural microenvironment, and revealed just how dynamic and busy a place these tissues are, providing immunologists with the opportunity to see what they usually only get to imagine or represent in cartoon form after inference from indirect experiments. As well as being a realization of what has been previously left to the imagination, his studies provide biological insight and clues to pursue further. He showed cytotoxic T lymphocytes (CTLs) recognizing and interacting with B cells that presented a cognate antigen, the B cells then undergoing lysis, indicated by a color intensity change due to the fluorophore leaking out after cell membrane perforation. He referred to this CTL-B cell interaction as "the kiss of death" and showed that CTL contact suppressed the target B cell's motility prior to lysis. It also seems that CTL can distinguish between viable and dead target cells. The molecular mechanisms behind these two processes will be areas for more traditional analyses.

Von Andrian has also used similar imaging techniques to look at regulatory T (Treg) cells and their interaction with CTLs. His group has found that CTLs under the influence of Tregs fail to degranulate and deliver their lethal payload of granzymes and perforin, and that this defect requires CTL responsiveness to the cytokine TGF- β . They've also found that removal of the Treg cells soon reverses the defect and CTLs regain their lytic potential, raising the possibility that if Treg cells are important sup-

pressors of CTL action in HIV infection (research is ongoing and the jury is still out) then this could form the basis of a corrective therapy.

Antiviral host factors



Host proteins that provide protection from viruses, especially retroviruses, are currently a hot research topic. It's hoped that by learning precisely how these proteins work to prevent infection that similar strategies can be developed for use in prevention or treatment. Joe Sodroski of the Dana Farber Cancer Institute at Harvard Medical School presented a keynote seminar updating current understanding of TRIM5 α , an antiretroviral host factor that acts intracellularly to snuff out HIV or SIV replication (see *Making a monkey out of HIV*, *LAVI Report* 9, 3, 2005). The protein has been identified in a number of mammalian species, most notably

humans and other primates, old and new world monkeys, and cows. As Sodroski noted, "human TRIM5 α does not restrict HIV, and that's why we now have an HIV epidemic." New comparisons of TRIM5 α in different species show that primates and cows have independently evolved their retroviral restriction factors in an example of convergent evolution, which suggests the function of TRIM5 α is a vital one.

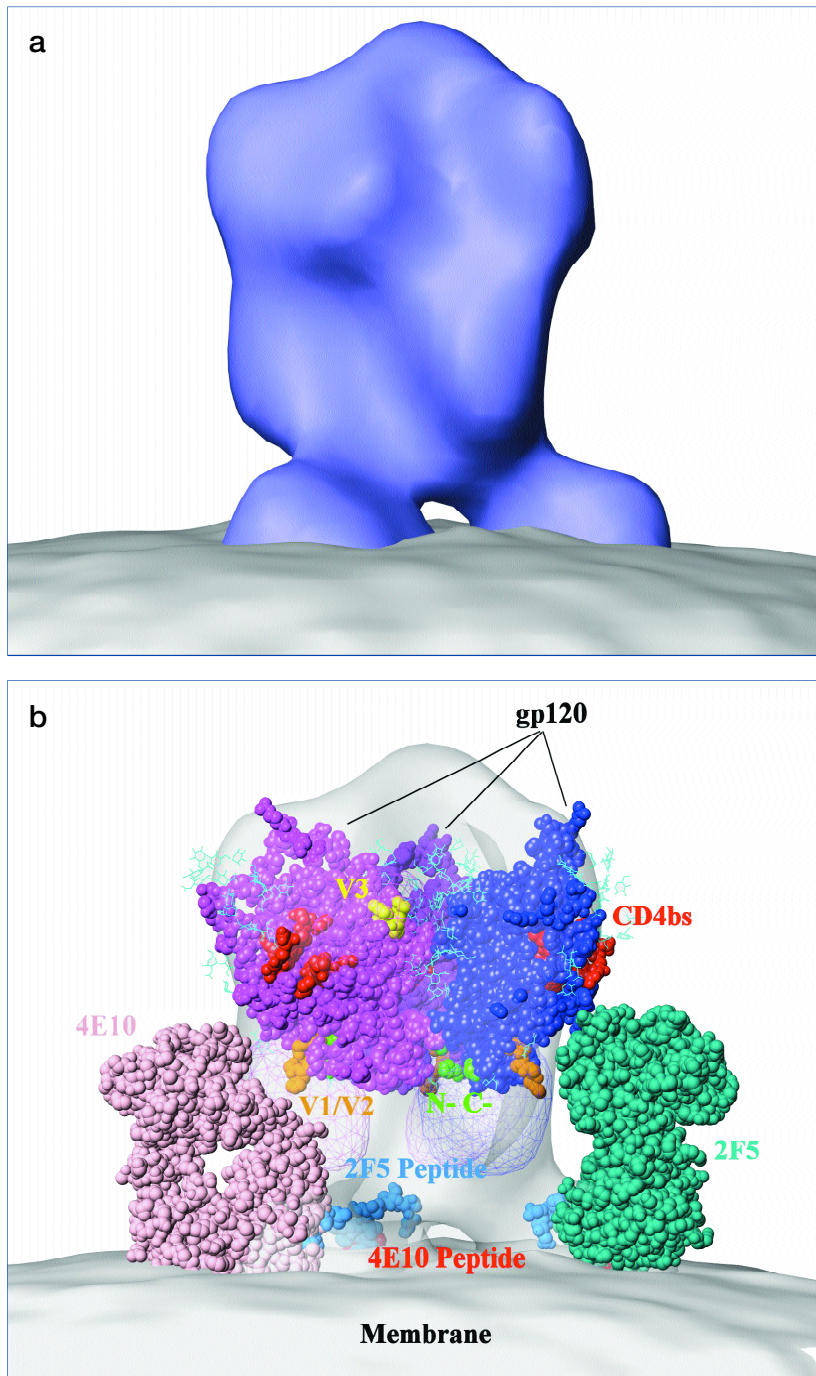
The precise mechanism of action of TRIM5 α is still being worked out. Sodroski's group has been conducting a mutational analysis to discern which domains within the protein are most important for its function. Comparisons of TRIM5 α from different monkey species show that there are four variable regions (V1 to V4) within the SPRY domain, and mutational analyses indicate that the amino acid residue at position 332 within V1 is particularly important; substitution

of the positively charged arginine residue there results in a TRIM5 α that potentially restricts HIV infectivity. Sodroski said that "the problem with human TRIM5 α is that it has an arginine at position 332; if it had just about any other residue there it would be a pretty good inhibitor of HIV." By extension from his earlier statement, that arginine residue at position 332 in TRIM5 α goes a long way to explaining why humans are susceptible to HIV and why there is now a pandemic.

The viral capsid protein is the major determinant of sensitivity to restriction by monkey TRIM5 α proteins, and recognition and binding correlates with the degree of restriction. Structural studies suggest to Sodroski that the pseudotrimeric symmetry of the capsid protein may form a target for the trilobular coiled-coil domain within TRIM5 α . In questions after his talk, Sodroski was asked to comment on the fate of the capsid in TRIM5 α -expressing cells, and he thinks that "the evidence is pointing to acceleration of uncoating." Why this should derail HIV's replication is not clear, but he emphasized the need for further research to fully understand the molecular steps in retroviral uncoating.

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Ping Zhu of Florida University presented an update on his team's latest findings using cryoelectron microscopy to image gp120 on the surface of HIV and SIV particles. Panel A shows a surface rendered model of averaged SIV Env spike (blue) and the associated viral membrane (gray) from CryoEM tomograms. Panel B shows a surface rendered model of SIV Env spike manually docked with SIV unliganded gp120 core atomic model (blue, pink, purple) in the head and gp41 peptides (2F5 epitope peptide, cyan; 4E10 epitope peptide, red) in the leg and foot densities. The proposed positions of the corresponding Fab, 2F5 (teal) and Fab 4E10 (pink), each to one leg, are also shown. The CD4 binding sites (red) and N- and C-terminal peptide stems (green) are highlighted in the gp120 core structure. The V1/V2 stems are highlighted in orange and the densities proposed to be occupied by the missing V1/V2 loops are enveloped in colored mesh. The V3 stems (yellow) and associated densities proposed to be occupied by the V3 loops (mesh) are similarly highlighted. **Sources:** gp120 core structure: PDB ID: 2BF1, Chen et al., *Nature* **433**, 834-41, 2005; 2F5 FAb and epitope peptide: PDB ID: 1TJI, Ofek et al., *J. Virol.* **78**,10724-37, 2004; 4E10 FAb and epitope peptide: PDB ID: 1TZG, Cardoso et al., *Immunity* **22**, 163-73, 2005

That potentiation of restriction correlates with an increase in capsid binding leads him to believe that pharmacological intervention may be attainable. Jonathon Stoye of the National Institute for Medical Research, London, pointed out later in his own presentation, that the restriction factors themselves are unlikely to represent useful drugs—"for a start, they're very large proteins, which disqualifies them." He suggested some conceivable alternatives: the use of gene therapy to deliver the restriction factors, developing small molecule drugs that mimic restriction factors, or developing drugs that can either re-target or stimulate expression of endogenous restriction factors.

Nucleic acid scrambler

Another important, recently-discovered host restriction factor is APOBEC3G (A3G), a ribonucleoprotein complex with cytidine deaminase activity that can effectively scramble RNA or DNA and render it nonsensical, thereby stopping HIV replication in its tracks (see *Guardian of the genome, LAVI Report 9, 2, 2005*). This cellular protein is the long sought-after target of the HIV protein Vif (viral infectivity factor) that is essential for virus infectivity and acts by binding to A3G and tagging it for degradation by the proteasomal pathway. Although A3G is a cellular protein, it is packaged within virions and acts soon after uncoating when the virion enters a new cell.

Warner Greene of the Gladstone Institute at the University of California, San Francisco, gave an update on new insights into A3G. The biology of A3G is intrinsically linked to its ability to exist in a high- (HMM) or low-molecular mass (LMM) complex. In resting CD4⁺ T cells and monocytes that are not permissive for HIV replication, the enzymatically-active LMM form of A3G is expressed and it acts as a potent post-entry restriction factor for HIV, whereas in activated CD4⁺ T cells and macrophages the enzymatically-inactive HMM form of A3G is expressed and the HIV replication cycle can proceed.

Greene and his research group have looked at virion-packaged A3G. Surprisingly, this virion A3G is in an enzymatically-inactive HMM form, although distinct from the cellular A3G HMM complex. They've also determined that it is bound to HIV RNA and requires HIV's RNaseH enzyme to trigger its cytidine deaminase activity. So HIV's RNaseH activity is required for both the generation of the ssDNA template that is the target of A3G activity, and for the activation of HMM virion-packaged A3G activity. Greene noted that a host restriction factor (A3G) that requires activation by a virus enzymatic activity (HIV RNaseH) is an example of "an unusual host-pathogen relationship."

As to the precise nature of the HMM complex itself, co-precipitation studies indicate that A3G associates with a diverse mix of over

75 different proteins and that it contains cellular RNA components, a class of retrotransposon RNAs, Alu, and hY. Alu is one of the most prolific mobile genetic elements in mammalian genomes, and the finding of such elements raises the possibility that the HMM A3G is defending against the "threat within" posed by retrotransposons after T-cell activation. Unfortunately this switch to the HMM A3G complex could open the door to external threats like exogenous retroviruses.

Identification of the proteins in the HMM A3G by Greene's group prompted them to search databases for comparisons and they find similarities to the make-up of Staufen RNA granules (important in RNA localization), Ro-La ribonucleoproteins (which have roles in RNA processing and possibly as chaperones), and pre-spliceosomes (an intermediate in mRNA processing). Indeed Greene went on to speculate that HMM A3G could be a collection of related complexes.

Greene rounded off his talk considering the therapeutic implications of recent findings about A3G, and said it would be nice to identify small-molecule inhibitors of the HMM complex, or ones that promote its dissociation, to preserve the restrictive LMM A3G in activated CD4⁺ T cells.

Alternative models

Because of the limits to what researchers can learn from human subjects infected with HIV-1 or the best monkey models of acquired immunodeficiencies, they are always looking for other models of disease that perhaps hold important lessons for HIV-1 infection in humans. Sarah Rowland-Jones of the UK Medical Research Council Laboratories in the Gambia and in Oxford, UK, has been studying HIV-2 infection, which she considers a "neglected model of naturally-attenuated HIV infection in humans."

HIV-2 evolved from SIV found in sooty mangabeys (SIVsm; whereas HIV-1 evolved from SIV found in chimpanzees) and is endemic in West Africa with a seroprevalence around 1%, meaning about 1 million people are infected. HIV-2 infection

provides no protection from infection with HIV-1 and is probably a risk factor. A minority of those infected with HIV-2 will develop AIDS and could benefit from ARVs, but the majority, 80-85%, will not progress to AIDS and it is these natural long-term non-progressors (LTNPs) that may provide clues to what is protective in immunodeficiency virus infection.

The CD4⁺ T-cell depletion in HIV-2-infected individuals is less rapid than in HIV-1 infection, and while most HIV-2-infected people have an undetectable to low plasma viral load, their proviral burden is similar to that seen in HIV-1 infection. Strong HIV-2-specific CTL responses are evident in infected individuals and these frequently show cross-reactivity with HIV-1 epitopes, but the overall magnitude and cytotoxicity of HIV-2-specific CTLs and the magni-

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tude of interferon (IFN)- γ responses does not differ markedly from HIV-1 infection.

However Rowland-Jones and her colleagues have found that there are significant differences in natural killer (NK) cell activity (in terms of circulating numbers, cytotoxicity, and cytokine and chemokine secretion) between HIV-1- and HIV-2-infected individuals who have normal CD4⁺ T-cell counts, with the most striking difference being much higher production of the chemokine MIP-1 β by NK cells in HIV-2-infected individuals. These differences are not seen in individuals with lower CD4⁺ T-cell counts.

Rowland-Jones' team have also found that the magnitude and breadth of CD4⁺ T-cell responses are greater in healthy HIV-2-infected individuals and that they also have a functionally distinct population of IFN- γ ⁺/interleukin (IL)-2⁺ CD4⁺ T cells that are not seen in HIV-1 infection, supporting again the notion that T helper cell responses are important in HIV infection. Rowland-Jones postulated that HIV-2-infected individuals with a normal CD4⁺ T-cell count may represent a distinct LTNP population with broad and sustained CD4⁺ T-cell and preserved NK-cell responses, and concluded that "HIV-2 is a potentially informative model" with regard to HIV-1 infection.

Sooty mangabeys

In a similar vein, Mark Feinberg of Emory University is studying host-virus relationships in non-pathogenic SIV infection of reservoir hosts to learn more about the nature of successful immune responses to immunodeficiency virus infections. His research team is grappling with some fundamental questions about AIDS pathogenesis, specifically asking whether chronic immune system activation is the primary mechanism driving CD4⁺ T-cell depletion, and therefore AIDS progression, in HIV-1 infection. He noted that chronic HIV infection is associated with chronic immune activation, while other chronic viral infections of humans (such as hepatitis B and C) are not.

Feinberg's team is intrigued by the absence of disease in SIV-infected sooty mangabeys (SMs), the natural reservoir species for SIVsm that is commonly naturally-infected at sexual maturity both in the wild and in captivity. SMs show no signs of immunodeficiency, neuropathology, or wasting syndromes despite high levels of chronic viremia, and they have normal levels of naive CD4⁺ and CD8⁺ T cells, no increase in CD8⁺ T-cell proliferation or evidence of pathologic CD8⁺ T-cell activation, suggesting that perhaps this attenuated immune activation may protect SMs from developing AIDS.


Feinberg showed that SMs have lower virus-specific CD8⁺ T-cell responses than humans with HIV infection, and there is no correla-

tion between the magnitude of SIV-specific CD8⁺ T-cell responses and the level of viremia in infected SMs, suggesting that these responses are not all that important.


His group has developed a comparative infection model where they can compare SIVsm infection in SMs and in rhesus macaques, in which SIVsm is pathogenic, to try to find what underpins the different infection outcomes. Feinberg noted that a large difference early in infection between SMs and macaques in their levels of CD8⁺ T-cell proliferation implicates perhaps an underlying altered innate response, and went on to show that macaque NK cells proliferate more than SM NK cells during primary and chronic SIVsm infection.

They have also found that plasmacytoid dendritic cells (pDCs) in infected SMs are not activated and don't migrate to the lymph nodes that are the key generative sites for antiviral immune responses, "so it's not surprising that an active immune response is not initiated" said Feinberg. That lack of pDC activation appears to be due to a specific defect in TLR signaling; SM pDCs do not produce IFN- α in response to *ex vivo* TLR7 or TLR9 stimulation, nor do they produce IFN- α in response to *ex vivo* stimulation with inactivated SIV. This defect is not general though, since SM pDCs do produce pro-inflammatory cytokines like IL-12 and TNF- α after various *ex vivo* stimuli, and do produce IFN- α following exposure to other stimuli such as influenza virus. Feinberg thinks that this failure of pDC activation in SMs may have a large impact on activation of downstream innate and adaptive immune responses, and said that SMs "see the virus but respond in a different way." He thinks the defect in type 1 IFN production in response to SIV might be particularly important.

To further illustrate the lack of global immune activation in SMs, Feinberg's team has compared gene expression profiles in CD4⁺ and CD8⁺ T cells and PBMCs taken from infected and non-infected humans with HIV and SMs with SIVsm. This analysis reveals that SMs have a far more quiescent immune system in terms of upregulation of genes, and that type 1 IFN response genes are among the most strongly upregulated genes in T cells of HIV-infected humans but not in SIV-infected SMs. Feinberg concluded by suggesting that this failure to activate pDCs in response to viral infection, which then avoids aberrant immune activation, is likely the primary mechanism protecting SMs from AIDS. By extension, he thinks that the chronic CD8⁺ T-cell activation and bystander immunopathology characteristic of human AIDS might be the result



The chronic CD8⁺ T-cell activation and bystander immunopathology characteristic of human AIDS might be the result of chronic activation of innate host responses



On trials

Researchers at Keystone provided a comprehensive roundup of ongoing AIDS vaccine trials and related activities. Bamey Graham of the Vaccine Research Center (VRC) at the US National Institutes of Health (NIH) kicked off this series of updates with a look at the many ongoing trials with the VRC's lead vaccine candidate—a DNA plasmid vaccine containing clade B *gag*, *pol*, and *nef* and *env* genes from clades A, B, and C, followed by an adenovirus serotype 5 (Ad5) vector vaccine boost carrying the same genes excluding *nef*. This DNA/Ad5 candidate entered Phase II testing late last year and is now in clinical trials in several countries in partnership with the HIV Vaccine Trials Network (HVTN), IAVI, and the Walter Reed Army Institute of Research (WRAIR).

IAVI and WRAIR are evaluating this prime/boost regimen in four east African countries. Two sites working with IAVI in Kigali, Rwanda and Nairobi, Kenya are now preparing to increase the number of volunteers participating in this trial, after target enrollment was quickly met (see *Vaccine Briefs*, this issue). WRAIR and partners recently began recruitment at its sites in Uganda and Kenya, and enrollment will begin in May at another in Tanzania, according to an update provided by Nelson Michael of WRAIR. He also

reported on a trial they are conducting in Uganda involving 31 volunteers that received the VRC's DNA candidate in a previous trial and will now be boosted with Ad5 in this subsequent trial.

In Phase I trials, Graham reported that the response after the DNA prime vaccination strongly correlates with peak CD4⁺ and CD8⁺ T cells responses after the Ad5 boost. The antibody responses, measured by ELISA, were dramatically increased following the Ad5 vaccination.

But Graham faced some tough questioning from Bruce Walker on the decision to include *env* genes in this vaccine, which primarily induces cellular immune responses. In his work with elite controllers, individuals that are HIV infected but control the virus below detectable levels, Walker found that responses to Gag and not Env correlated with slow disease progression. And since Env is so variable, he predicted that its presence in the vaccine may distract the immune system by drawing responses to a moving target. This point was quickly countered by Richard Koup, also of the VRC, who was in the audience. Koup emphasized that debate over gene inserts is merely an indicator of advances in the AIDS vaccine field. "We should be happy we have responses at all," he said.

These immune responses may be tested soon in a preliminary efficacy trial. Preparations for a Phase IIb "test of concept" trial are underway for the VRC's DNA/Ad5 candidate. WRAIR sites in Uganda, Kenya, and Tanzania are now being readied for this larger trial, according to Michael.

Progress was also reported on other trials at the WRAIR. Enrollment is now complete in the only ongoing Phase III trial, taking place in Thailand, evaluating the efficacy of a prime-boost administration of the ALVAC canarypox vaccine and the VaxGen gp120 vaccine, the latter having already been tested in a previous efficacy trial. The final round of immunizations with this combination will occur in July, but volunteers will be followed for an additional three years.

Michael also reported on a series of clinical trials planned by WRAIR to test prime/boost regimens of various DNA constructs and the modified vaccinia Ankara (MVA) vaccine developed by WRAIR and the NIH. These trials will take place at WRAIR sites in the US, Thailand, and Africa. Data from the Phase I trial, RV 158, testing the safety and immunogenicity of WRAIR's MVA-CMDR candidate will be presented at the 2006 AIDS vaccine meeting in

of chronic activation of innate host responses, rather than the primary defect in itself.

Elite controllers

Bruce Walker of Massachusetts General Hospital and Harvard Medical School is looking at HIV-1 infection in humans, but he is refining the cohorts of infected individuals he is studying to try to tease out new observations. His research group is interested in determining what accounts for the differences in time from initial HIV infection to AIDS, which on average is about 10 years but can actually be anywhere from 6 months to more than 28 years and counting. They are studying asymptomatic HIV-1-infected individuals who control their virus without the need for ARV intervention, and have further classified individuals to distinguish viremic controllers (VCs), who have plasma HIV RNA levels below 2000 copies/ml blood, from elite controllers (ECs), who have undetectable plasma HIV RNA (<75 copies/ml blood by bDNA or <50 copies/ml blood by ultrasensitive PCR). These criteria must be met for at least one year.

So far Walker's group have not found viral or host genetic factors that are strongly associated with an individual's controller grouping.

But they have found host immunologic factors, and Walker showed in his presentation that ECs have less robust CD8⁺ T-cell responses (lower magnitude and to fewer epitopes) than VCs or chronically-infected individuals. ECs do, however, have more focused Gag-specific CD8⁺ T-cell responses. ECs also have a significantly higher percentage of HIV-specific, and especially Gag-specific, CD4⁺ T-cells that secrete both IL-2 and IFN- γ .

Walker is now looking to expand his EC cohort to 1000 individuals (he has already identified 200) and issued a recruitment call for his Elite Controller Collaborative Project that currently includes more than 45 collaborators from all over the US and some in Europe. He hopes to define haplotype maps of ECs in collaboration with investigators at the Broad Institute, Boston, and harness the power of the Human Genome Project.

Mucosal focus

There have been a flurry of recent publications and presentations on how HIV wreaks havoc on the cells of the gut during the very earliest stages of HIV infection (see *Beast in the belly*, page 1 and *CROI covers advancements from start to finish*, *IAVI Report* 9, 6, 2006). The importance of understanding the effects of the virus on the

Amsterdam, which will take place in August/September.

This same MVA-CMDR construct is also being evaluated in clinical trials at the Karolinska Institute (KI) in Stockholm. These Phase I trials are designed to compare the safety and immunogenicity of either intramuscular or intradermal injections of a prime vaccination with a multiclade, multigene, DNA plasmid vaccine developed at the Swedish Institute for Infectious Disease Control (SMI) followed by a boost with the MVA-CMDR from WRAIR. Forty participants receive either three DNA immunizations followed by a single MVA boost or placebo.

The final volunteer will complete the vaccination schedule this May and Eric Sandström of KI says results from this trial will also be presented in Amsterdam. “We look forward to a very pleasant presentation,” he said, modestly hinting at the results. “Both immunizations have been safe and well tolerated and, compared to published results on this approach, we are encouraged by the data.”

Sandström and his colleagues in Tanzania, Sweden, Germany, South Africa, and the US are also starting a trial with the same vaccines in 60 volunteers in Dar es Salaam, Tanzania, once they receive final approval from the local authorities. As part of a program sponsored by the European Union, the Karolinska Institute and SMI


have been assisting with the development of clinical and laboratory capacity in Dar es Salaam.

Detailed plans for another new trial were also presented by John Hural of the HVTN. Merck and the HVTN are now completing site preparations for a Phase II “test of concept” trial with Merck’s Ad5 vaccine candidate in South Africa. This candidate is already being tested in a Phase IIb trial, known as the STEP study, in North America, South America, the Caribbean, and Australia (see *Renewed Promise, LAVI Report* 9, 4, 2005). But the South African trial marks the first time this candidate will be evaluated in a population where the predominantly circulating clade of HIV does not match that in the vaccine. The HIV antigens in the vaccine are from clade B, while the epidemic in South Africa is mainly clade C. Immune responses induced by the vaccine will be measured by ELISPOT against both clade B and C peptides for the first 600 volunteers. The panel of clade C peptides was designed by Steve Self of the HVTN to span all possible T-cell epitopes found in >15% of HIV isolates.

The trial will enroll 3000 volunteers at 5 HVTN sites in South Africa, 40% of whom are required to have low levels of pre-existing Ad5 immunity (antibody titers <1:200). Due to the high prevalence of pre-existing immunity to Ad5, initial expectations are that 6000 people will need to be

screened across the sites in order to enroll just 1200 volunteers that meet this criterion. Hural acknowledges that this will be a huge task but emphasizes that all of the South African HVTN sites are now undergoing expansion in order to handle this number of volunteers, and he said that by the time it begins, each site will be capable of processing volunteer specimens and preparing them for shipment.

Recruitment efforts for the South African trial, HVTN 503, will also focus more on women. In the STEP study only 8-900 of the 3000 total participants are women, but in this second Phase II trial, HVTN plans to enroll equal numbers of male and female volunteers. This is reflective of the high number of infections among women in South Africa. A study of almost 12,000 15-24 year olds living in South Africa in 2003 reported that HIV prevalence in women was 15.5% and only 4.8% in men of the same age group (*AIDS* 19, 1525, 2005). And this is not an isolated example. “I don’t think there’s a case from Africa where you don’t see this gut-wrenching stratification by gender,” said Michael.

This study is expected to start once Merck’s Ad5 neutralization assay, which will be used to determine baseline eligibility for the trial, is successfully transferred to the South African sites. Merck expects that enrollment will begin before the end of the year. 

intestinal tissues and the mucosal immune responses at this site were a recurring theme at Keystone. “It’s an important lymphoid compartment and it needs to be looked at, regardless of route of HIV transmission,” said Barbara Shacklett from the University of California, Davis.


Information is now building on the loss of CD4⁺ T cells in the gut of humans during acute infection that was first reported with SIV infection in rhesus macaques. Researchers, including Shacklett, are also studying HIV-specific responses in mucosal tissues of chronically HIV-infected humans. Her group compared the immune responses in peripheral blood samples from 13 HIV-infected individuals with those in the gut, as obtained by rectal biopsy, in several epitope mapping studies. They found the magnitude and antigenic specificity of these responses are quite similar when measured by IFN- γ ELISPOT. Although not statistically different, the trend in these individuals was towards a higher breadth of immune response in the gut, which Shacklett said is expected with such a large amount of virus in these tissues.

Her laboratory then looked at the differences in the functionality of the CD8⁺ T-cell responses in the two compartments in 22 HIV-infected individuals, 6 of whom were taking ARVs, to see if the


functional diversity of the mucosal immune responses correlated with clinical status. Analysis was done for different cytokines, including expression of MIP-1 β , TNF- α , IFN- γ , IL-2, and expression of CD107a, a protein present in the membrane of cytotoxic granules that is transiently expressed as a result of degranulation and therefore reflects their cytotoxic capabilities (*J. Immunol. Methods*, 281, 65, 2003). Here Shacklett found a significant difference. Although overall the functional profiles of the immune responses in the peripheral blood and gut were similar, the Gag-specific CD8⁺ T-cell mucosal responses were greater and were characterized by CD107a expression.

There is some debate in the field about the correlation between CD107a and cytotoxicity, but this finding suggests that there is an active CTL response in the gut mucosal tissues during chronic infection, and alludes to an important difference between the gut mucosa and blood.

Shacklett also reported that the functionality of CD8⁺ T cells within the mucosal tissues of the gut during chronic HIV infection varied with viral load. Individuals with higher viral loads (>30,000 viral copies/ml of blood) were more likely to have CD8⁺ T cells expressing a single cytokine than those with viral loads below 5,000 copies/ml, who were



**Researchers
at Wyeth
have found
that intra-
muscular
delivery
of their
recombinant
vesicular
stomatitis
virus vaccine
may be a
promising
inducer of
immune
responses at
mucosal
tissues**



more likely to have CD8⁺ T-cells in mucosa that expressed multiple cytokines.

Shacklett's group also looked at secretion of perforin, a protein associated with cytotoxicity, by CD8⁺ T cells in the gut of rhesus macaques during acute SIV infection and found that the quantity peaked in the gut around 21 days after intravaginal infection. But this is "too little, too late" since the bulk of the damage to this compartment occurs within 14 days. After around 180 days the levels of perforin dropped to the same levels as those in uninfected control animals. "There are still CD8⁺ T cells in the gut, but they aren't able to secrete perforin," said Shacklett. There is also evidence that CD8⁺ T cells in lymph nodes are low in perforin during chronic infection.

The low levels of this protein in the gut may be the result of a mechanism that evolved to protect the gut from what Shacklett refers to as "friendly fire." Perforin's ability to poke holes in cell membranes could be potentially damaging to the thin intestinal lining and reducing quantities of the protein may be one way the immune system keeps it in check. However it could inadvertently offer an advantage to HIV. "This is one of the things that could help HIV survive as a chronic infection," Shacklett suggested.

Considering the importance of mucosal immunity, the development of AIDS vaccine candidates that stimulate strong immune responses at these tissues remains a priority. To this end several researchers are working on both adjuvants and vaccine vectors that may augment mucosal immune responses.

To date there are no mucosal adjuvants that are approved for human use. Of the known mucosal adjuvants, cholera toxin is by far the most potent but its safety profile makes it unsuitable for evaluation in human volunteers. Therefore many groups have been hard at work modifying bacteria to make them safe while preserving their capabilities to stoke a mucosal immune response. Susan Barnett from Chiron presented work on the company's mucosal adjuvant, known as LTK63, which is a non-toxic mutant of heat-labile enterotoxin (LT) from *Escherichia coli*. This LT mutant carries a single point mutation and in extensive preclinical studies in mice and rabbits it was found to be safe and immunogenic, producing strong serum and mucosal antibodies (serum IgG and vaginal IgA).

This adjuvant is now being evaluated in a clinical trial for an influenza vaccination,


where it is being administered intranasally. Chiron is also studying the immunogenicity of the LTK63 adjuvant with both mucosal and parenteral immunizations in female rhesus macaques in collaboration with Chris Miller at the University of California, Davis.

Other methods investigators are exploring to induce robust immune responses at mucosal tissues is the type of vaccine vector or route of delivery. Intramuscular immunization is generally considered an ineffective way to provoke mucosal immunity, according to Stephen Udem of Wyeth, but results presented by the company at Keystone indicate otherwise. Researchers at Wyeth have found that intramuscular delivery of their recombinant vesicular stomatitis virus (rVSV) vaccine encoding HIV Gag may be a promising inducer of immune responses at mucosal tissues (see *Renewed Promise, IAVI Report 9*, 4, 2005).

Although this viral vector has been used in research for years, "nobody has really looked at VSV as a mucosal immunogen," said Udem. "There's extraordinarily little already known."

In a poster at Keystone, researchers detailed the HIV-specific T cell immunity induced at three different mucosal tissues. Mice immunized intramuscularly with a prime and boost of rVSV vector expressing HIV Gag developed strong CD8⁺ T-cell responses to both HIV Gag and the N protein of VSV in splenocytes and lamina propria lymphocytes. These CD8⁺ T cells secreted IFN- γ when exposed to antigenic peptides and persisted for at least a month after the booster immunization. The vaccine fared less well in intraepithelial lymphocytes where only weak immune response were observed.

Wyeth is planning to evaluate the mucosal responses induced by rVSV more closely in rhesus macaques given the vaccine intramuscularly. Even more interesting data might come from intranasal administration, a route thought to induce more potent responses at mucosal tissues, but according to Udem this approach is currently not being studied. Wyeth's priority is establishing the safety of live, but attenuated VSV-based vaccines. "So much of what we're doing right now is trying to get a sufficient level of comfort with regulatory agencies," he says.

The US Food and Drug Administration has agreed to a Phase I clinical trial with an intramuscular administration of Wyeth's VSV-based AIDS vaccine and Udem expects this will start in early 2007. This trial will be conducted with the HIV Vaccine Trials Network (HVTN). 

The polio eradication endgame

As polio eradication nears realization, such real-world vaccination strategies could hold lessons for the future in AIDS vaccine development

By David L. Heymann and R. Bruce Aylward

Recently, an 18 month old girl, one of the few children paralyzed by poliomyelitis last year in India, was filmed by an international camera crew documenting the final human-to-human chains of polio transmission. She had been hidden by her parents from the polio vaccination team each time it passed because of a misunderstanding about the safety of the vaccine, and her left leg had been paralyzed two months earlier by polio. Already by the time of filming, in the arms of her mother, she had learned to move her paralyzed leg by slipping her non-affected leg behind and lifting upward. This is surely the first of many other self-taught mechanisms she will use as she learns to move around the household and then in the community, as she copes for the rest of her life with a disability that could have been prevented. The irony and tragedy of her fate is to have lived at a time when polio vaccines were made accessible to every child in all countries, but missed out because her parents misunderstood.

In 1988 polio paralyzed approximately 1000 children each day in 125 endemic countries (Figure 1). The polio eradication strategy that was developed over the following years includes three major activities aimed at establishing the levels of herd immunity required to interrupt transmission of wild poliovirus. The first of these activities is routine immunization of infants under one year of age using trivalent oral poliovirus vaccine (tOPV). The second is mass vaccination campaigns—national or sub-national immunization days in areas where wild poliovirus is identified and/or where the risk of infection is considered high—using tOPV targeted at all children under five years of age. Prior to conducting mass vaccination campaigns, district level micro-planning and mapping identify where children under the age of five years live and provide a framework for social mobilizers and vaccinators as they pass from community to community and house to house. The third activity underlies the first two, and is national and global surveillance of acute flaccid paralysis (AFP) among children under 15 years of age to identify all children paralyzed by polio virus,

thus indicating the geographic areas where activities to interrupt polio transmission must be targeted or intensified.

Polio eradication activities have made tremendous strides since 1998. By 2000, the number of polio-endemic countries had decreased from over 125 to 20, and by mid-2003 the number had decreased to 6, with 784 children reported paralyzed that year by polio. Those six countries that still had endemic transmission of the wild poliovirus in 2003 were India, Pakistan, Afghanistan, Egypt, Niger, and Nigeria.

As long as one country is infected with wild poliovirus, all countries in the world are at risk

International spread of poliomyelitis

In August 2003, polio vaccinations were suspended throughout northern Nigeria because of false rumors that polio vaccine was contaminated—either with HIV at the time of manufacture, or from the deliberate addition of hormones to permanently sterilize young girls¹. Vaccinations remained suspended for approximately 12 months. During that period and since, genomic sequencing of the type 1 poliovirus that has caused outbreaks in 18 polio-free countries in Africa, the Middle East, and Asia has genetically linked these outbreaks to parent viruses in northern Nigeria. During this same period, three additional polio-free countries—one in Africa, two in southeastern Asia—were re-infected by type 1 polio virus genetically linked to India, reinforcing the understanding that as long as one country is infected with wild poliovirus, all

countries in the world are at risk.

The response to imported wild poliovirus has been rapid and effective. Five synchronized vaccination campaigns using oral poliovirus vaccine were conducted in West and Central Africa, and serial campaigns were conducted by all the countries with imported virus. At the same time, serial campaigns continued in the six polio-endemic countries, and Saudi Arabia participated in control efforts by establishing polio vaccination requirements for those less than 15 years of age traveling to Saudi Arabia for work, tourism, or religious pilgrimage. By April 1, 2006 Egypt and Niger had become polio free, leaving four polio-endemic countries—India, Pakistan, Afghanistan, and Nigeria—and nine

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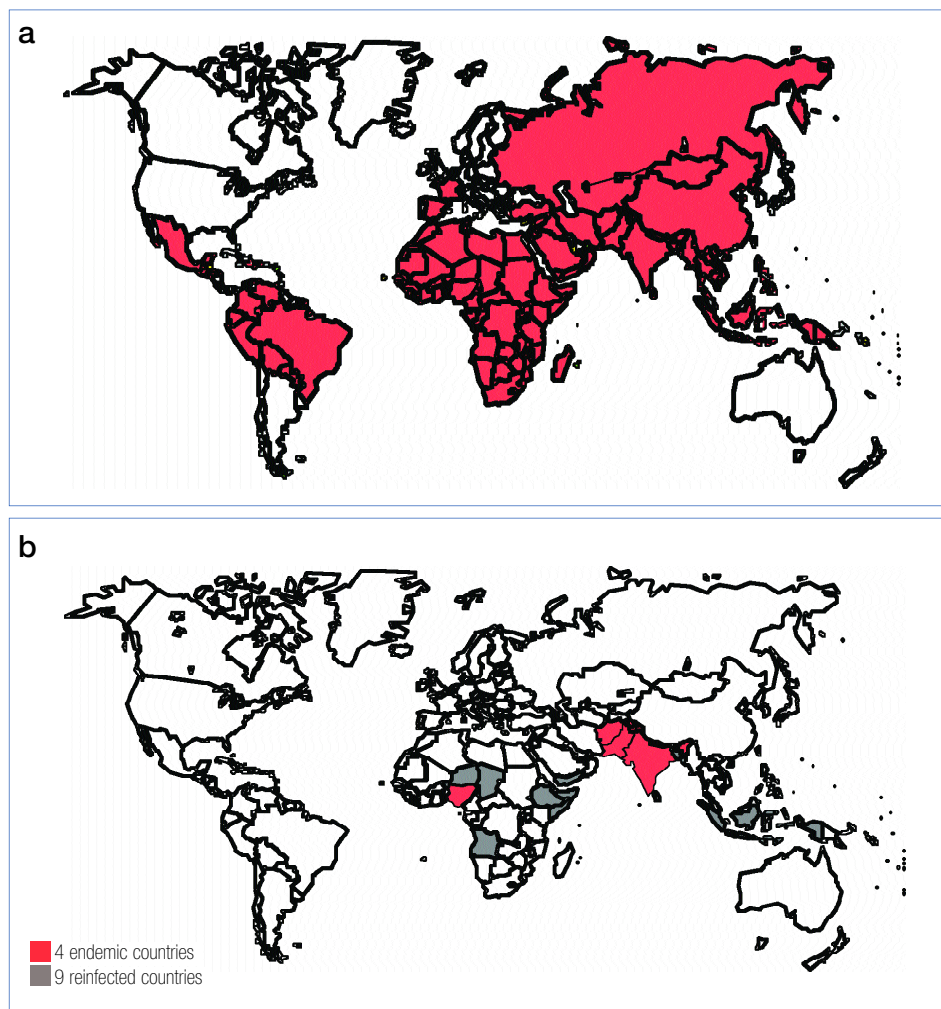


Figure 1. Countries with transmission of wild poliovirus, (a) June 1988 and (b) April 2006.

countries to which poliovirus has been imported—Yemen, Indonesia, Angola, Somalia, Niger (repeatedly re-infected across its common border with Nigeria), Chad, Somalia, Ethiopia, and Bangladesh (Figure 1).

Vaccine formulation

At the same time that poliovirus spread internationally from Nigeria and India, observations within India and Egypt—two high population-density countries with continued indigenous transmission of poliovirus—suggested that the vast majority of children with paralytic polio had been vaccinated with at least three doses of trivalent oral poliovirus vaccine, most with many more. It has been known since the mid-1970s that seroconversion to types 1 and 3 polio virus after three doses of tOPV

was significantly less than that for type 2 (ref. 2). The higher type 2 seroconversion rates provide an explanation for the successful interruption of human-to-human transmission of type 2 poliovirus worldwide that occurred in 1999, while types 1 and 3 poliovirus continue to circulate. A call was therefore made to oral poliovirus vaccine manufacturers in October 2004 to develop and license monovalent type 1, and then type 3 monovalent oral poliovirus vaccines. By May 2005—using historical data from previously licensed monovalent vaccines—two companies licensed monovalent oral poliovirus vaccine type 1 (mOPV1), followed shortly afterwards by additional licensed mOPV1 vaccines, and by September 2005 a licensed monovalent type 3 oral poliovirus vaccine as well (mOPV3).

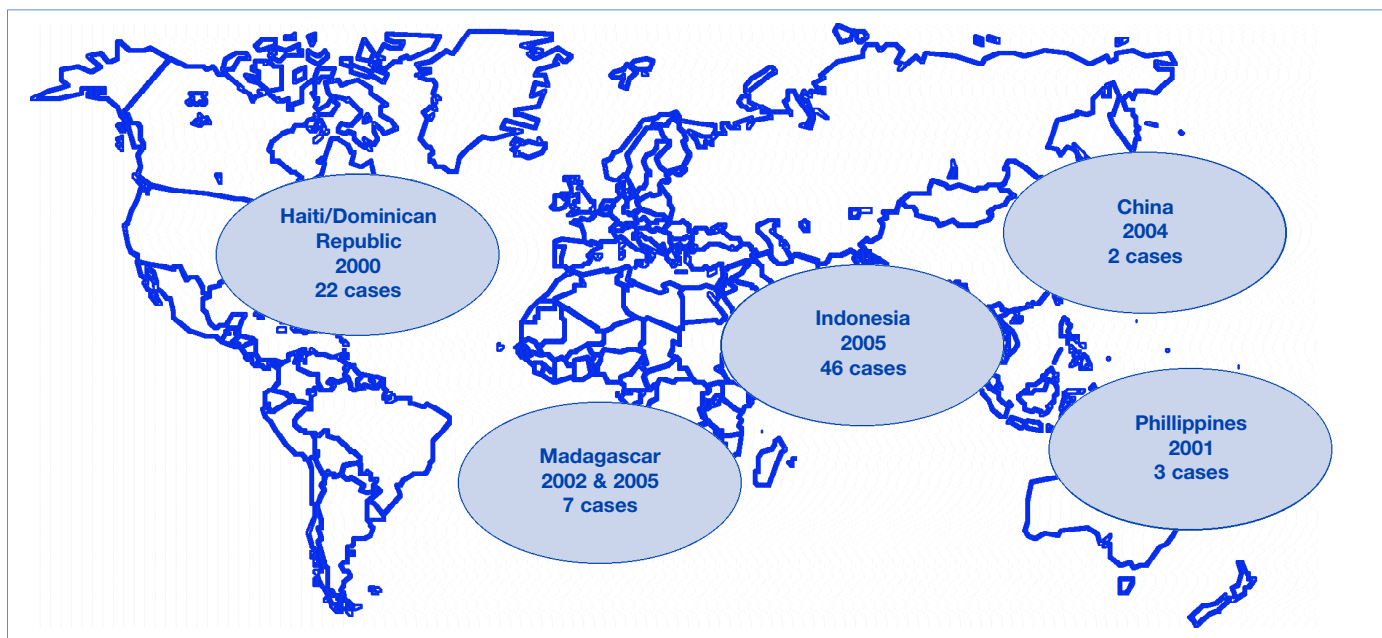


Figure 2. *Outbreaks of circulating vaccine-derived poliovirus, 2000 - 2005.*

These monovalent vaccines are currently being used in campaigns in the four countries that have never interrupted transmission of the wild poliovirus, and in eight of the nine countries with outbreaks from imported wild poliovirus. In countries that have only type 1 virus circulating, mOPV1 is used exclusively; while in countries with both type 1 and 3 virus, mOPV1 and mOPV3 are used sequentially, guided by the local epidemiology. Seroconversion studies to better understand the full potential of monovalent vaccines are being conducted in India and Egypt, but epidemiologically these vaccines have proven their worth, shortening the time for full containment of type 1 poliovirus outbreaks in polio-free countries and interrupting transmission in densely populated areas such as Mumbai. With use of monovalent polio vaccines, continued government commitment and availability of funds, the goal of polio eradication remains fully in sight.

Vaccine instability

During early 2004 an outbreak of polio caused by type 2 poliovirus occurred in Guizhou Province, China, which was free of indigenous polio transmission for the previous 10 years, and genetic sequencing of this virus indicated that it was a circulating vaccine derived poliovirus (cVDPV), a virus with >1% difference from a parent oral poliovirus vaccine (OPV) virus strain by full VP1 sequence homology. With rapid and appropriately targeted vaccination campaigns using tOPV, the outbreak was rapidly contained.

It has been recognized since the late 1990s that polio outbreaks can be caused by cVDPVs. As of April 1, 2006, outbreaks arising from Sabin-derived poliovirus strains have been documented in Haiti and the Dominican Republic (2000-2001), the Philippines (2001), Madagascar (2002 and 2005), China (2004),

and Indonesia (2005), with others such as in Egypt during the 1980s described retrospectively (Figure 2)³. Although the conditions that give rise to cVDPVs are still being studied, it is clear that attenuated vaccine viruses are able to regain neurovirulence and the capacity to circulate and cause outbreaks, but it appears that this is a rare event.

In addition, the prolonged excretion of vaccine-derived polioviruses by some persons with primary immunodeficiency syndromes (iVDPVs) has now been well documented, with 6 of the 30 known iVDPVs having had excretion longer than 60 months⁴. The true incidence of such chronic iVDPVs remains uncertain, primarily because it has been documented mainly in persons with common variable immune deficiency, a syndrome that is often asymptomatic into early adulthood and therefore not fully detected because global and national surveillance for AFP targets children under 15 years of age. To date, all those who have excreted virus for longer than 60 months have lived in high income countries and iVDPV, like cVDPV, appears to be rare.

Of significance is the observation that acquired immunodeficiency syndromes, such as that caused by HIV infection, have not been associated with prolonged poliovirus excretion, and no iVDPV is known to have generated secondary infections with paralysis⁵.

In September 2003, a WHO consultation group on oral poliovirus vaccines and vaccine-derived polioviruses concluded that the continued occurrence of 250-500 vaccine-associated paralytic poliomyelitis (VAPP) in OPV recipients or their contacts, along with regular outbreaks of cVDPV, would be an unacceptable risk for most, if not all, countries. Based on an evidence-based risk-benefit analysis, the group recommended eventual simultaneous OPV cessation in all OPV-using countries

once polio transmission has been confirmed as interrupted worldwide⁶.

In addition to the risk of cVDPV emergence and the long-term risk of iVDPV secretion after OPV-cessation, each of which would require an immediate outbreak response, there is the longer term risk of re-introduction of a wild, vaccine-derived or Sabin poliovirus strain from a laboratory where they are stored, or from an inactivated polio vaccine (IPV) production site where wild virus is grown for vaccine production. These risks must be reduced to an absolute minimum by decreasing the number of facilities storing, handling, and/or amplifying these viruses by destruction of living viruses, or in a very few countries ensuring that living polioviruses are placed and maintained under polio-biocontainment conditions that have been developed by an international consensus process.

To reach this goal, 158 countries have initiated and/or completed both a laboratory survey for wild poliovirus and infectious or potentially infectious materials (to be followed by similar surveys for Sabin polioviruses), and an industry survey to determine which IPV production sites will continue manufacture after eradication⁷. Five countries have been identified that plan to maintain IPV manufacturing facilities after OPV-cessation: France, Canada, Belgium, Denmark, and the Netherlands, and facilities in these countries have begun to adapt their manufacturing processes to the required level of polio-biocontainment.

Based on perceived and real risks as outlined above, countries must develop national post-OPV polio vaccination strategies. It is believed that many countries with minimum or no risk will decide not to introduce IPV and completely stop polio vaccination. These countries will depend on a global surveillance capacity for polio, a stockpile of monovalent polio vaccines against types 1, 2 and 3, and an outbreak response mechanism that is being put in place under the International Health Regulations (2005), where polio is among four specific infectious diseases for which reporting is required⁸. Countries that contribute a real international biohazard risk—because of continued handling, storage, and/or amplification of poliovirus—will be required to develop, fully implement, and maintain an IPV vaccination policy that will


ensure high levels of polio immunity among laboratory workers, IPV production operators, and the general population.

The polio eradication endgame, including the complexities of OPV cessation, was not entirely foreseen in 1988 when the world embarked on the eradication of polio. tOPV, the workhorse of polio eradication, has nearly reached the end of its life-cycle, 50 years after its initial development, and IPV will be the vaccine of choice for those few countries that continue polio vaccination after OPV-cessation. As research and development continue for AIDS vaccines, the lessons from oral polio vaccine are clear: Vaccinology is often an evolutionary process, requiring successive generations of vaccines that are adapted to the changing epidemiology of the disease, and that compensate for any inherent risks of the vaccine itself.

Postscript

Vaccine cessation also occurred after the certification of eradication of smallpox (variola), the only infectious disease to have been eradicated to date. Smallpox vaccine is made from vaccinia, and primary vaccination is associated with complications that range from vaccinia eruption at sites that are, or have previously been, eczematous to generalized vaccinia infection and post-vaccinal encephalitis leading to permanent neurological disability or death. With a case fatality ratio for post-vaccinal encephalitis of approximately 30%, the risk of fatal complications from smallpox vaccine is approximately one per million doses of vaccine administered, depending on the strain of vaccinia used in vaccine preparation⁹. Because the risk of complication from vaccinia was considered greater than the risk of smallpox after eradication had been certified, cessation of smallpox vaccination was universal.

Stocks of variola virus held in laboratories around the world were then considered to be the greatest risk to smallpox eradication and this risk was dramatically illustrated by a laboratory accident resulting in a fatal case of smallpox in the UK in 1978¹⁰. That highly-publicized event persuaded national authorities to either destroy virus stocks or transfer them for safe-keeping to designated high-security WHO-collaborating centres that were required to continue vaccinating all those who worked at these facilities.




**Vaccinology
is often an
evolutionary
process,
requiring
successive
generations
of vaccines
that are
adapted to
the changing
epidemiology
of the
disease**



In 1981, within a year following the certification of smallpox eradication, AIDS was identified for the first time in the US, after the international spread that would rapidly lead to endemicity had already begun. In 1984 the US practice of vaccinating military personnel as protection against the possible use of variola virus as a biological weapon led to recognition of a fatal link between smallpox vaccination and HIV infection; a young military recruit with latent HIV infection developed generalized vaccinia and AIDS following smallpox vaccination that led to death six months later¹¹. This demonstration of the fatal potential of smallpox vaccination in HIV-infected persons suggests that, had AIDS emerged earlier, it would have undermined chances for the eradication of a disease that depended on vaccination as the cornerstone for control.

It is fortunate that HIV infection does not create the same obstacle to polio eradication as it would have for smallpox—the window of opportunity to complete polio eradication remains open, and new and better adapted vaccines are now available. The challenge is to complete the job while the window remains open, and through a series of clearly thought out evidence-based OPV-cessation activities, safeguard against the recurrence of a disease that shatters the dreams of parents and the lives of their children.

Lessons from the final challenges in polio eradication are important for the future in AIDS vaccine development. They suggest that optimizing the impact of future AIDS vaccines will require planning to ensure full acceptance and uptake, as well as the capacity to collect and assess the evidence required to adapt vaccines to the emerging AIDS epidemiology, and to compensate for any undesirable vaccine-related effects. 

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Chemokines enhance CD8⁺ T-cell memory

CD8⁺ T cells are often thought of as the frontline weaponry in the battle to fend off pathogens and malignant cells, but crucially they require helper activity from CD4⁺ T cells. This help is especially important in assisting the development of long-term CD8⁺ T-cell memory. The antigenic ligands for the CD8⁺ and CD4⁺ T cells involved in help-dependent responses must be co-presented by the same antigen-presenting dendritic cell (DC). Precisely how this is accomplished has not been fully understood.

Two different models have been proposed to explain how this holy trinity of DC, CD8⁺, and CD4⁺ T cell manage to pass the relevant signals between them. The first suggests that the two T cell types bind simultaneously to a single DC and that local cytokine production or direct interaction promote their cooperation. But since T cells specific for any particular antigen are rare in the context of the naïve T cell pool, it has been argued that there would be a very low probability of this happening. The second model therefore proposes that the DC-CD4⁺ T-cell interaction induces differentiation of the DC (called 'licensing') that then supports subsequent CD8⁺ T-cell priming.

Now a group of researchers led by Ron Germain provide convincing evidence that the chemokines MIP-1 α and MIP-1 β (also known as CCL3 and CCL4) are key players in actively guiding the recruitment of CD8⁺ T cells to join the DC-CD4⁺ T cell interaction (*Nature* **440**, 890, 2006). They transferred transgenic CD8⁺ and CD4⁺ T cells specific for OVA antigen into immunocompetent mice, immunized the mice subcutaneously with either OVA or an irrelevant antigen in opposite flanks, and then compared the distribution of CD8⁺ T cells in the two draining lymph nodes. They found a sig-

nificantly greater accumulation of the CD8⁺ T cells in the lymph node on the OVA-immunized side.

The researchers then used intravital 2-photon microscopy of intact lymph nodes in living animals and fluorescently dyed T cells and DCs to see if this increased accumulation was characterized by enhanced direct interactions between the cell types. They found that when CD4⁺ T cells were present, CD8⁺ T cells were much more likely to interact with OVA-pulsed DCs as opposed to unpulsed DCs, suggesting that the CD4⁺ T cells were augmenting the interaction specifically with DCs presenting cognate antigen. The researchers suspected that this process might be chemokine dependent and so injected the mice with various chemokine-blocking antibodies; antibodies against MIP-1 α and MIP-1 β reduced the accumulation of CD8⁺ T cells in the lymph node whereas antibodies against other chemokines had no effect.

The researchers then looked at the functional significance of these findings. MIP-1 α - and MIP-1 β -blocking antibodies eliminated the enhancing effect of CD4⁺ T cells on both the number and the effector activity of CD8⁺ memory T cells measured several weeks later. However, blockade of these chemokines did not affect the acute expansion or the acquisition of effector functions of CD8⁺ T cells, suggesting that they are not simply augmenting co-stimulatory signals involved in CD8⁺ T-cell activation or other trafficking events.

The findings increase fundamental understanding of the immune system, indicating that inflammation leads to CCR5 expression by naïve CD8⁺ T cells that then enables them to be actively attracted to sites of productive DC-CD4⁺ T cell interaction. The research could open up new avenues to augmenting CD8⁺ T-cell memory in vaccination strategies, perhaps using chemokines as adjuvants.

HIV Nef strikes again

A huge assortment of effector functions have been attributed to HIV's Nef protein, and now it seems another can be added. It has been reported that Nef is required for high levels of viral replication, for progression to AIDS in the infected host, helps in immune escape, affects numerous cellular signaling pathways, increases infectivity of progeny virions, downregulates cell surface expression of important immune molecules and helps immune evasion, helps prevent infected cells from apoptosis, and helps facilitate the infection of T cells. New research from a team led by Andrea Cerutti (*Nat. Immunol.* **7**, 302, 2006) now suggests that Nef can also affect the antibody response against HIV by inhibiting immunoglobulin class switching, thereby preventing the maturation of the antibody response.

Class switch recombination is a process that occurs after B lymphocytes have been activated by CD4⁺ T cells through CD154 and the cytokines interleukin (IL)-4 and IL-10 and they migrate to the germinal centers of secondary lymphoid organs. This class switching substitutes the heavy-chain constant region of IgM and IgD with that of IgG, IgA, or IgE, giving the new classes of antibodies different effector functions

that enhance their ability to counteract pathogens; IgG and IgA can neutralize viruses both systemically and at mucosal entry sites.

It is known that the humoral response to HIV is defective and that it is improved by antiretroviral therapy, and that B cells are intrinsically poorly responsive to CD4⁺ T-cell help in HIV infection. However, HIV does not infect B cells so how are these effects mediated? Nef, like Tat, is secreted extracellularly, and the researchers first showed that Nef can accumulate in B cells both *in vivo* and *in vitro*. This intracellular accumulation interfered with a number of signaling pathways by increasing the regulatory proteins I κ B α , SOCS1, and SOCS3, which in turn blocked signaling through NF- κ B and STAT and, ultimately, CD154 (also known as CD40L), IL-4, and IL-10, resulting in inhibition of class switch recombination.

The researchers only demonstrate these effects *in vitro* and it will be important to see if the findings hold up in HIV-infected individuals and monkey models of infection. And since Nef has been included in some vaccine candidates, what are the implications in light of these new findings? The authors end by speculating that Nef-blocking agents might be useful in improving the antibody response to HIV.

Study of early HIV infection begins enrollment

Active recruitment and enrollment of volunteers has now started at research sites in Lusaka, Zambia; Masaka, Uganda; Kigali, Rwanda; and Kangemi, Kenya for a multi-centre, epidemiological study of newly HIV-infected individuals being conducted by IAVI. Recently infected individuals for this research study are identified through participation in incidence studies where they are counseled on risk-reduction practices and tested for HIV at least four times a year.

This new study will track these HIV-infected individuals for up to five years to follow the natural course of HIV infection in these cohorts. All volunteers will receive counseling and care and will be referred to a program offering antiretroviral (ARV) treatment when needed. Investigators will also analyze samples of the newly-transmitted virus.

Data from this study could provide researchers with information about the virus that is transmit-

ted and the early immunological events that take place during acute stages of HIV infection. This may offer several clues to help inform preventive vaccine design since ultimately this is the virus challenge that a vaccine would need to protect against.

The IAVI research study will also begin recruiting recently HIV-infected volunteers at other sites in Entebbe, Uganda; Cape Town, South Africa; and Kilifi, Kenya. Other groups, including the recently established Center for HIV/AIDS Vaccine Immunology (CHAVI) are studying individuals during the earliest stages of acute HIV infection (see *An Interview with Barton Haynes, IAVI Report 9, 4, 2005*). Links between these efforts and the IAVI-led research studies have already been established. Myron Cohen and others at the University of North Carolina are now also creating collaborations with several research sites in South Africa, Malawi, Uganda, Tanzania, the UK, and the US to identify individuals as soon as possible after the establishment of HIV infection.

African Union launches prevention campaign

The African Union in partnership with the Joint United Nations Programme on HIV/AIDS (UNAIDS) initiated a unified call from many African leaders to increase and improve HIV prevention services on the continent. On April 11 leaders from several countries kicked off this initiative. Among these was Meles Zenawi, the Primer Minister of Ethiopia, who emphasized how the scale up of prevention services can have profound effects in dealing with the epidemic.

Elements of this comprehensive prevention plan include addressing the root causes of HIV transmission, increasing access to HIV testing and counseling services to increase knowledge about the virus and prevent transmission, and developing strategies specifically targeting women and youth with important messages on HIV prevention and behavior. At the launch, First Lady

Jeannette Kagame of Rwanda spoke about the disproportionate number of women in Africa who are HIV infected. Other components of the effort include the need to strengthen and expand existing healthcare systems and programs on mother-to-child transmission of HIV.

Of the 5 million new HIV infections in 2003, 3.2 million occurred in sub-Saharan Africa, according to the latest UNAIDS statistics. And although access to HIV prevention and care has increased in recent years, the huge number of new infections can cause a significant burden on existing programs.

The World Health Organization (WHO) and UNAIDS predict that implementation of broad prevention programs such as these could help avert 63% of new HIV infections that are expected to occur in the next six years. This initiative was launched in advance of a Special Summit on HIV/AIDS, tuberculosis, and malaria that will convene next month in Abuja, Nigeria involving African Union heads of state.

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IAVI is a scientific organization founded in 1996 whose mission is to ensure the development of safe, effective, accessible, preventive HIV vaccines for use throughout the world. IAVI focuses on four key areas: Accelerating scientific progress; education and advocacy; ensuring vaccine access; and creating a more supportive environment for industrial involvement in HIV vaccine development.

The International AIDS Vaccine Initiative (IAVI) is a global not-for-profit organization whose mission is to ensure the development of safe, effective, accessible, preventive HIV vaccines for use throughout the world. Founded in 1996 and operational in 23 countries, IAVI and its network of collaborators research and develop vaccine candidates. IAVI's financial and in-kind supporters include the Bill & Melinda Gates Foundation, the New York Community Trust, the Rockefeller Foundation, the Starr Foundation; the Governments of the Basque Country, Canada, Denmark, the European Union, Ireland, the Netherlands, Norway, Sweden, the United Kingdom, and the United States; multilateral organizations such as the World Bank; corporate donors including BD (Becton, Dickinson & Co.), Continental Airlines, DHL and Pfizer; leading AIDS charities such as Broadway Cares/Equity Fights AIDS, Crusaid, Deutsche AIDS Stiftung, and the Until There's A Cure Foundation; and other private donors such as the Haas Charitable Trusts. For more information, see www.iavi.org.

Trial sites in Kenya and Rwanda expand recruitment

The projected number of individuals participating in a Phase I AIDS vaccine trial in Kenya and Rwanda, conducted by IAVI in partnership with the Vaccine Research Center (VRC), will be increased based on early successes in recruitment. Project San Francisco began enrolling volunteers at the site in Kigali, Rwanda late last year—marking the start of the first AIDS vaccine trial in the country—and the Kenya AIDS Vaccine Initiative (KAVI) at the University of Nairobi began recruitment in January. Total enrollment for both sites was initially set at 64 volunteers but will now be increased to 104.

This trial is one of three closely coordinated trials testing the safety and immunogenicity of a “prime-boost” vaccination regimen with the VRC’s DNA plasmid and adenovirus serotype 5 (Ad5) vaccine candidates (see *On trials*, page 10). Two sites affiliated with the Walter Reed Army Institute of Research and its partners began recruitment recently in Kampala, Uganda and Kericho, Kenya for a trial with the same candidates.

Sabina Wakasiaka, a nurse counselor from KAVI, credits the successful enrollment rates to outreach programs conducted in the last few years, which have helped to increase the vaccine literacy among many community organizations. The staff is also targeting more women for recruitment in this trial.

Other developments in Kenya include the opening of two new community clinics in Kilifi by the Kenya Medical Research Institute (KEMRI), with support from IAVI. One of these clinics, the Comprehensive Care and Research Clinic, will offer HIV testing and counseling services that can help facilitate future AIDS vaccine trials in the country, as well as house a clinical trials laboratory. Part of this building has also been reserved for provision of HIV treatment and care through the District Hospital, including a program for the prevention of mother-to-child transmission that tests over 4000 pregnant women each year.

The other newly-established clinic will focus mainly on couples voluntary counseling and testing, which can help identify individuals in serodiscordant relationships and who are therefore at high risk for HIV infection within their marriage or partnership. Couples counseling is an established practice at sites in Rwanda and Zambia that partner with IAVI, but this clinic is one of only a few to utilize this approach in Kenya. KEMRI also opened a new drop-in center and clinic for high-risk, HIV-uninfected individuals in Mtwapa. More than 300 uninfected volunteers have already been enrolled in a study to help promote an understanding among individuals at high risk of HIV infection of ways to lower this risk. Collaborators from the University of Washington will treat volunteers for sexually-transmitted diseases, including offering treatment to those who are incidentally infected with HIV during the course of the study.

WHO/UNAIDS convene meeting on vaccine clinical trial design

The World Health Organization (WHO) and the Joint United Nations Programme on HIV/AIDS (UNAIDS) recently sponsored a technical consultation with several experts in the AIDS vaccine field to discuss the design and implementation of Phase IIb “test of concept” trials, also referred to as “proof of concept” trials, in evaluating AIDS vaccine candidates and their implications for future approval and licensure.

This meeting, hosted by IAVI, was held in New York City and brought together representatives from a diverse range of organizations to consider how to design test of concept trials and the role they may play in the development of vaccines. Attendees included representatives from the Botswana Harvard AIDS Institute Partnership, Medical Research Council of South Africa, Chinese Center for Disease Control and Prevention, Project San Francisco in Rwanda, HIV Vaccine Trials Network (HVTN), US National Institutes of Health (NIH), US Centers for Disease Control and Prevention, US Food and Drug Administration, IAVI, Johns Hopkins University, as well as other representatives from India, Thailand, and Zambia.

The results of a single Phase IIb trial would not be expected to provide sufficient evidence of safety or efficacy for licensure. However experts at this meeting considered the pressure that could arise, particularly in regions hardest hit by AIDS, if a Phase IIb trial showed impressive efficacy. The group largely agreed that full Phase III trials would still be necessary to ensure the vaccine was safe and effective, but emphasized that governments and organizations conducting trials should consider these issues now.

Merck was the first company to begin a Phase IIb trial, in collaboration with the HVTN, for their lead AIDS vaccine candidate and they recently announced plans to begin another “proof of concept” trial with the same candidate in South Africa (see *On trials*, page 10). Other organizations, including the VRC at the US National Institutes of Health (NIH), are also considering using these trials to evaluate preliminary efficacy of their candidates.

The recommendations of this group will be presented to the WHO/UNAIDS Vaccine Advisory Committee and the resulting position paper will help these organizations evaluate the utility of Phase IIb test of concept trials for AIDS vaccines.