

# HIV Report

## Contents

1 Guardian of the Genome

1 The Role of the SIV Model in AIDS Vaccine Research

9 New Strides in Protecting Infants from HIV

13 Obituary: Maurice Hilleman

14 An Interview with Frances Gotch

18 Research at the Extremes: Keystone 2005 Coverage

26 HIV in the Aftermath

29 Research Briefs

31 Vaccine Briefs

## Guardian of the genome

*Research into a different kind of viral defense system may yield powerful treatments and solve some mysteries about HIV*

**By Philip Cohen**

**F**our years ago, when Ann Sheehy took a trip to her local cinema to see the movie *Gladiator*, she knew she was in for one hell of a story.

Actor Russell Crowe had recently won an Academy award for his portrayal of Maximus, a once-great Roman general reduced to slavery, seemingly destined to die off the pages of history as a nameless gladiator. But after many gory encounters, Maximus rises from obscurity to battle and defeat his nemesis, the vile Emperor of Rome.

"I'm a history buff so the story of ancient Rome appealed to me, even if it was a bit bloody," remembers Sheehy, a postdoctoral researcher in Mike Malim's laboratory in King's College London. "I know for certain I wasn't thinking about HIV. When I see a movie it's one of the few times I try to forget about what I work on." Ironically, the same transformation of invisible serf into celebrated soldier would soon take place in her own laboratory—albeit on a molecular level.

In this version the role of the unsung hero was to be played by a human protein known as a cytosine (or cytidine) deaminase. These enzymes can directly mutate genetic material, morphing the cytosine present in RNA or DNA into uracil by twisting around a few atoms. Back when Sheehy watched Crowe strut into the Coliseum, these enzymes seemed of little direct relevance to HIV's lifecycle. "I had never even heard of these enzymes," she says.

But pioneering work by Sheehy, Malim and their colleagues soon revealed that one of these mutator proteins, dubbed APOBEC3G, is actually on the front line in the battle against HIV, engaged in molecule-to-molecule combat nearly from the moment the virus enters a human cell. After a few years, a flurry of publications from top labs, and many recent twists in the tale, the story of APOBEC3G and similar enzymes has become one of the hottest areas of HIV biology—and researchers speak of these proteins in heroic, one might even say cinematic, terms. "They are intracellular guardians and defenders of genomes," Malim recently told scientists gathered at the Keystone Symposium on HIV Pathogenesis held in Banff, Canada.

*continued on page 2* 

## Perspective

## The role of the SIV model in AIDS vaccine research


**By Thomas C. Friedrich and David I. Watkins**

**D**espite over twenty years of research, there is still no effective vaccine against HIV. Only one vaccine candidate has been through a Phase III (large-scale efficacy) trial: AIDSVAX<sup>1,2</sup>, a gp120-based candidate that was intended to induce antibody responses against the virus that would prevent individuals from becoming infected with HIV. Although this vaccine candidate

elicited antibodies in most volunteers, it failed to prevent infection.

The failure of AIDSVAX was likely due to its inability to induce potent neutralizing antibodies (NAbs). A vaccine that elicits both a broadly-specific NAb response and a robust cellular response is the Holy Grail of AIDS vaccine research since it might impart sterilizing immunity, preventing the virus

from binding to and entering target cells and thereby completely protecting vaccinated individuals. Unfortunately, very few antibodies have been identified that bind the viral envelope and are capable of neutralization, so while the handful of characterized NAbs are the subject of intensive research, a vaccine based on NAbs remains a distant hope for the clinic.

*continued on page 6* 

One reason APOBEC3G is generating such excitement is that since its discovery researchers have been finding more and more human proteins with latent abilities to oppose HIV (see *Getting acquainted with the host factors*, page 23). “We are just starting to scrape the surface of the cellular defenses that are out there,” says Mario Stevenson of the University of Massachusetts Medical School.

The science of these antiviral factors is at such an early stage that researchers haven’t even agreed on what to call them. Some see these anti-HIV proteins as an extension of the traditional innate immune system. Others see them as something altogether different and prefer to call them host restriction factors or factors of intrinsic immunity. But whatever moniker they use, many researchers think that therapies based on this different kind of defense may help yield powerful treatments and studying them may solve some longstanding mysteries about the biology of HIV.

One of these mysteries, the function of the HIV protein Vif, is what originally drew Sheehy to Malim’s laboratory. Vif is encoded by one of only nine genes HIV possesses, but the precise role of this protein had remained elusive for more than a decade. It was known that the *vif* gene is required to cause disease in animal models but viruses with the *vif* gene deleted can replicate normally in certain “permissive” cell lines, while infection of “non-permissive” cells produces only defective virions. Genetic evidence suggested that an unidentified factor in non-permissive cells inhibited propagation of the virus, but this factor was normally inactivated by Vif.

In an effort to unmask that antiviral factor and how it worked, Sheehy took advantage of two human T cell lines known to differ at much less than 1% of their genomes. Importantly, one of these cell lines was permissive to replication of *vif*-deleted virus while the other was not, suggesting that a defect in the unknown anti-HIV factor was one of the few genetic differences between them.

In 1999 she pulled out several promising candidate genes, the fifteenth of which was the one that would later be known as APOBEC3G. Sheehy initially called it CEM15 after the cell line from which it was cloned. After running CEM15’s sequence through a human database, she threw it in the freezer. “I hate to be reminded of that,” jokes Sheehy. “Nothing informative came out in that search, and there were some other genes that seemed promising. So I put it in a separate pile of things to look at later.” Luckily, the impending completion of the human genome sequence was causing a gene cloning frenzy and new human gene sequences were being deposited in databases on a daily basis. She only had to wait for the right group to sequence the right genes to reveal the connection between CEM15 and other cytosine deaminases. Even so, it took three more years.

But it was worth the wait. As Sheehy, Malim and their colleagues wrote in their now classic paper on APOBEC’s discovery (*Nature* **418**, 646, 2002), “The sequence similarity between CEM15 and cytosine deaminases is potentially provocative.” Among the known cytosine deaminases were APOBEC-1, a protein that changes a single cytosine (C) into uracil (U) in the messenger RNA for the intestinal protein apolipoprotein B, and Activation-Induced Deaminase (AID), which is involved in generating antibody gene diversity through the enzyme’s ability to mutate DNA. The immediate implication was that human cells had somehow transformed the power of APOBEC enzymes to alter the cell’s own genetic information into an antiviral weapon.

As the King’s College London team and their colleagues, led by

Michael Neuberger at the Medical Research Council Laboratory of Molecular Biology in Cambridge, rushed to test the model, many other top HIV labs joined the fray. Over the next year papers or reviews on APOBEC enzymes and HIV were being published monthly. In 2004 that pace surged to once a week. From this flood of data a compelling picture quickly emerged of the life and death struggle of APOBEC and HIV (Figure 1).

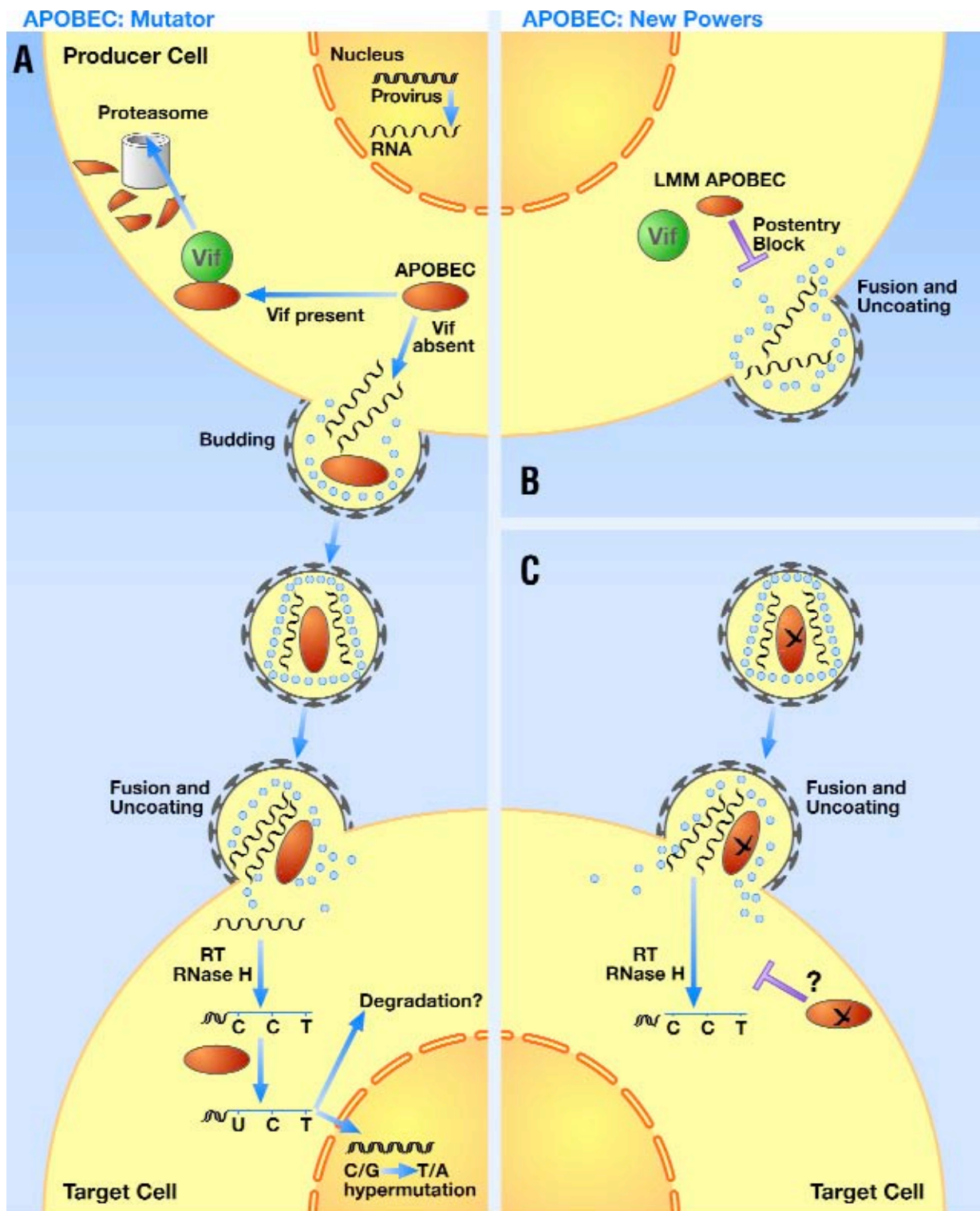
In this model the APOBEC mutator enzyme effectively sterilizes viral progeny. It lies in wait in human cells for an invading retrovirus to reproduce and then sneaks inside nascent virions. When one of those newly minted viruses infects another cell and switches on its reverse transcriptase enzyme to copy its RNA genome into a strand of DNA, the APOBEC stowaway strikes. It riddles the DNA strand with C to U mutations, potentially creating stop codons in the viral protein sequence and fouling splicing sites when the viral genome is later transcribed into RNA. If that isn’t enough, since DNA doesn’t usually contain U, this copy of the HIV genome can be recognized by cellular DNA repair enzymes which may chew it to bits.

Or at least that’s what happens if HIV lacks the Vif protein. Before APOBEC can strike Vif disarms it by branding its would-be attacker with ubiquitin molecules, chemical tags which mark it for destruction by the proteasome machinery that recycles the cell’s own damaged proteins. With APOBEC removed new virions are free to infect at will, create DNA copies that integrate into its host’s chromosomes and spawn the RNA genomes of the next viral generation. So the invading viral army proceeds unhindered.

“It’s a model that makes a lot of sense, that’s straightforward and simple,” says Malim. This model of APOBEC’s mechanism also explained a curiosity about HIV: it is one of the most adenosine (A)-rich retroviral genomes known. This makes sense considering that the C to U DNA changes that are the signature of APOBEC action would result in guanine (G) to A mutations in the RNA genome of new viruses. So a tendency to A-richness would seem a reasonable adaptation for a virus that has done battle with APOBEC-like enzymes for eons. But while the model of APOBEC as mutator turned viral birth control agent is appealing, it is also incomplete. APOBEC, it turns out, has other tricks up its sleeve.

One of the first hints that APOBEC had other talents came last year from the study of the effect of APOBEC on hepatitis B virus (HBV), which depends on the conversion of RNA to DNA in part of its lifecycle just as retroviruses do. It isn’t clear if APOBEC plays a natural role in defending against HBV since the enzyme hasn’t been found in liver cells. But Didier Trono’s laboratory at the University of Geneva, Switzerland showed that, given the chance, APOBEC is fully capable of fighting HBV. His team genetically engineered tissue culture cells to express APOBEC3G and then infected them with HBV. As might have been expected, APOBEC was able to cripple the virus. But what caught the researchers off guard was that in at least in some of the cell lines they engineered, APOBEC3G managed to cripple the virus without causing an appreciable increase in C to U mutation (*Science* **303**, 1829, 2004).

Researchers were still scratching their heads over this result last January when Sheehy, Malim and their colleagues dropped another paradigm-challenging paper in their laps. Their team was trying to understand exactly how APOBEC3G unleashed its mutational weapon. In similar cytosine deaminase proteins, a quartet of amino acids—histidine, glutamic acid and two cysteines—bound to a zinc



**Figure 1. Original and new models of APOBEC3G antiviral action.**

Panel A depicts the original model of APOBEC3G action. In a cell producing virus lacking functional Vif protein, APOBEC3G incorporates into nascent viruses budding from the producer cell (top of panel). When the virus infects a target cell (bottom of panel), APOBEC3G is released and mutates the nascent DNA, leading to its degradation or hypermutation. If Vif is present in the producer cell (top of panel), APOBEC3G is tagged with ubiquitin for transport to the proteasome and destruction.

Panel B depicts a new mechanism by which a low molecular mass form of APOBEC3G (LMM APOBEC) inhibits virus. LMM APOBEC is found in resting CD4<sup>+</sup> T cells and can block events in viral replication immediately following fusion through a mechanism yet to be determined. It can operate on wild-type virus in the presence of functional Vif protein.

Panel C depicts another Vif-sensitive mechanism of APOBEC antiviral action uncovered by engineering a form of APOBEC that lacks DNA-mutating activity (APOBEC oval with X). Even in the absence of its mutational powers, APOBEC3G can demonstrate potent antiviral activity in the target cell. Where this block occurs in the viral replication cycle is still unknown.

atom are responsible for carrying out the alchemy of converting Cs to Us. APOBEC3G has two of these domains. As might be expected then, the researchers found that crippling both domains stripped the protein of its anti-HIV powers, while keeping either intact largely preserved them. All that made good sense, until the team directly assessed the ability of each domain to mutate DNA, and found that only the C-terminal one had this ability (*Current Biology* **15**, 166, 2005). That means that when the C-terminal domain was inactivated by mutation, APOBEC was completely stripped of its mutational power, and yet remained a formidable force against HIV. "This surprised us, to say the least," says Malim. APOBEC appeared to have the power to mutate HIV to death. Yet that might not be its only weapon, or even its most powerful.

And APOBEC had at least one more surprise in store. Another hallmark of APOBEC's particular kind of combat was supposed to be that it strikes not directly at incoming virus, but its progeny. But in April, Warner Greene's group at the University of California, San Francisco reported an exception to this rule. His team was studying another classic HIV puzzle. Why does the virus replicate efficiently in activated T cells but very poorly in resting T cells? Many explanations have been offered over the years but Greene's team decided to investigate whether APOBEC enzymes play a role.

They were prompted to do so when they discovered that in most cells APOBEC3G doesn't stand on sentry duty alone but is part of a large or high molecular mass complex that includes uncharacterized RNA molecules. However they found that in resting T cells APOBEC3G is primarily found in a much more streamlined, low molecular mass form. Greene's team recently reported that if this APOBEC3G-lite is eliminated in resting T cells, a known block to HIV reverse transcription largely disappears. And although slimmed-down APOBEC3G appears to have cytosine deaminase activity, Greene's team found that only about 8% of viral sequences isolated from these cells showed evidence of C to U hypermutation (*Nature* **435**, 108, 2005).


The finding is remarkable for three reasons. It shows that, in some circumstances at least, the APOBEC enzyme can act directly and immediately on the incoming virus, that it can stop the replication of wildtype HIV even if functional Vif is present, and that this activity doesn't appear to require mutation.

But the work also raises an interesting question. If APOBEC has all these powers to stop HIV in its tracks, why didn't our cells evolve to use it? Harmit Malik at the Fred Hutchinson Cancer Research Center in Seattle thinks one possibility is that cells didn't optimize APOBEC to fight HIV because the enzyme evolved for another purpose. His team, together with that of his colleague Michael Emerman, has found evidence that APOBEC proteins have been active guardians of primate genomes for 35 million years, well before lentiviruses evolved about one million years ago. To understand what APOBEC was doing all that time their two labs studied the selective forces on different members of this enzyme family in primates.

The prediction of this type of analysis is that proteins which perform a physiological role for the cell, such as AID, would be driven by the selective forces of evolution to perform that function as efficiently as possible. Once the sequence of the protein has been pushed to near perfection, any change in its sequence caused by random mutation tends to lower its efficiency and it is said to be under negative selection. In contrast, some proteins provide functions that are under constant pressure to change, a feature called positive selection. A good example is a protein like APOBEC3G, which is suspected to interact with proteins from a pathogenic virus. Any change in this type of protein will eventually be countered by a deflecting change in its target. This arms race leads to a genetic conflict where both combatants constantly explore new sequences to cope with each others shifting tactics.

In addition, if it was true that some members of the APOBEC3 gene family (primates have a total of eight similar enzymes designated APOBEC3A, APOBEC3B, etc.) evolved to deal with HIV or similar viruses, then this leads to three predictions. First, some of these proteins should experience positive selection. Secondly, the positive selection should be sporadic, present in lineages whose natural history included a devastating encounter with such viruses while absent in others. Third, the positive selection should be restricted to just the region of each APOBEC gene which interacted with viral proteins like Vif that directly engage it.

Unexpectedly, only one of these predictions turned out to be correct. Six of the APOBEC3 proteins were under positive selection, but that was true in all ten primate line-



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**Mario Stevenson**



ages the researchers examined. This suggested these proteins were not fighting sporadic viral infections but some ever-present danger. And when they examined the APOBEC3G sequence in fine detail they found that areas of positive selection were not limited to those mapped biochemically as important for the protein's interaction with Vif but extended across its whole length, making it one of the fastest evolving of all known human proteins (*PLoS Biology* **2**, 275, 2004). This suggests that in its long history every piece of APOBEC3G has been used to engage some attacker. "The analogy of a Swiss army knife is pretty good," says Malik. "Think of it as having different blades that have been honed to different purposes."

Work by Olivier Schwartz at the Institut Pasteur in Paris suggests that viruses alone may not have driven APOBEC to develop these diverse set of skills. Instead the protein may have been shaped by fighting a danger from within, endogenous retroviruses. Endogenous retroviruses have a life cycle similar to HIV, including the reverse transcription of their genome and incorporation of a DNA copy into a new chromosome. The only difference is that endogenous retroviruses never leave a host's cells. They reside within a genome and their *raison d'être* is simply to spread more copies of themselves. But because these retroviruses can splice themselves into functioning genes, cells have mechanisms to suppress this activity. Now Schwartz's team has shown that human APOBEC3G can provide another layer of protection against some of these retroviruses (*Nature* **433**, 430, 2005).

Retroviruses would fit the bill of a constant and rapidly shape-shifting adversary for APOBEC3G to combat, and this enzyme is expressed in reproductive tissues where the threat of retroviruses to species survival is most serious. But even if fighting HIV isn't APOBEC's day job, some new data suggests that variations in how it works from individual to individual could partly explain why an HIV infection progresses rapidly to disease in some people but causes disease slowly in others.

One study of more than 3000 HIV-infected people found that a variant of APOBEC3G common in the African American population is associated with acceleration to the development of AIDS symptoms (*J. Virology* **78**, 11070, 2004). However, a French study of APOBEC3G gene variants in 245 HIV-infected Caucasians with slow disease progression and 82 with rapid progression found no significant association between gene variants and disease (*J. Infect. Disease* **191**, 159, 2005), suggesting the influence of APOBEC genetic background could differ between populations.


Genetic differences in the infecting virus may also have an effect on disease progression. At the recent Conference on Retroviruses and Opportunistic Infection in Boston, Australian researchers reported that in a group of 227 HIV patients they found that virus from about 3% showed evidence of G to A hypermutation and, in all these cases, the integrated proviruses showed evidence of defec-

tive *vif* genes. Importantly, these patients also showed a lower viral load before receiving antiretroviral drugs (CROI, [www.retroconference.org](http://www.retroconference.org), Paper #242).

While the clinical picture is still developing, any evidence of APOBEC's influence on the course of disease fuels the hope that boosting APOBEC activity could one day be a powerful therapeutic. "Ideally, you'd want to stabilize APOBEC enzymes, raise their level or make them resistant to degradation," says Stevenson. The most actively pursued strategy is to screen chemical libraries for small molecules that block Vif's ability to mark APOBEC for destruction. Dana Gabuzda at the Dana-Farber Cancer Institute in Boston has been pursuing this approach and now Stevenson and Greene say they are independently joining the hunt.

But some of the recent research into APOBECs suggests that other types of therapeutics could also prove effective. One human APOBEC family member, APOBEC3B, appears naturally impervious to Vif but is not normally expressed in cells which HIV infects (*Current Biology* **14**, 1392, 2004). Drugs that upregulate the *APOBEC3B* gene in these cells could therefore erect a new barrier to viral replication. A completely different strategy would be to promote the formation of the low molecular mass version of the APOBEC3G protein in more cells that, as Greene's team has shown, could freeze the virus early after its invasion into the cell.

No one is under the illusion that developing a drug to target the APOBEC pathway will be easy. The road to developing a new drug against HIV is a hard, long one with no guarantee of success—witness the 20-year struggle to get an effective and safe HIV integrase inhibitor to market. And until further research nails down the normal physiological role of these enzymes, worries about possible toxic side effects of APOBEC drugs are likely to make companies cautious about trying to exploit their potential. "Pharmaceutical companies don't embrace novel approaches and they haven't embraced APOBEC," says Stevenson. But he adds that companies will be more likely to pay attention if academic labs come up with some promising compounds.

If such compounds can be found, Sheehy speculates that targeting the APOBEC pathway could give medications unusual bang. After all, current drugs simply block critical functions of the virus. She compares APOBEC-based pharmaceuticals to vaccination. "Vaccines tend to work so well because they harness the strength of your own body to fight the virus," she says. "Similarly a drug that can keep APOBEC super active would help one of our own proteins fight the virus for us." In the end, the APOBEC enzymes may not be the type of heroes that Hollywood rushes to immortalize in a big budget movie. But researchers seem convinced that a drug which boosts the ability of these enzymes to fight HIV may still be worthy of the term "blockbuster." 



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As a result researchers have focused on developing vaccines that elicit potent cellular immune responses. These vaccines are comprised of recombinant virus vectors to deliver HIV genes or gene segments. Unfortunately, recent large-scale trials of recombinant virus vector-based vaccines have also proven disappointing as the regimens tested have not induced potent T cell responses in many vaccinees. Recent evidence also indicates that HIV infections have occurred even in vaccinees who did make detectable responses.<sup>3,4</sup>

The disappointing results to date of antibody- and T cell-based vaccine strategies may seem to add up to a bleak picture for AIDS vaccines. But in fact we are still ignorant of the correlates of protection, so it remains unclear exactly what a successful vaccine needs to do. Why do a few rare individuals seem to make broadly-neutralizing antibodies, while most antibodies do not neutralize? Do antibodies need to recognize intact envelope multimers? What titers of antibodies would be protective? Similarly, what defines effective T cell responses? Must they be of particularly high avidity? Should they traffic to the right anatomical compartments? Should they recognize a broad array of viral epitopes? Or epitopes that are under functional constraints, and thus poorly tolerant of mutation? The answers to these and other questions about effective immunity to immunodeficiency viruses can be informed by experiments in the macaque model.

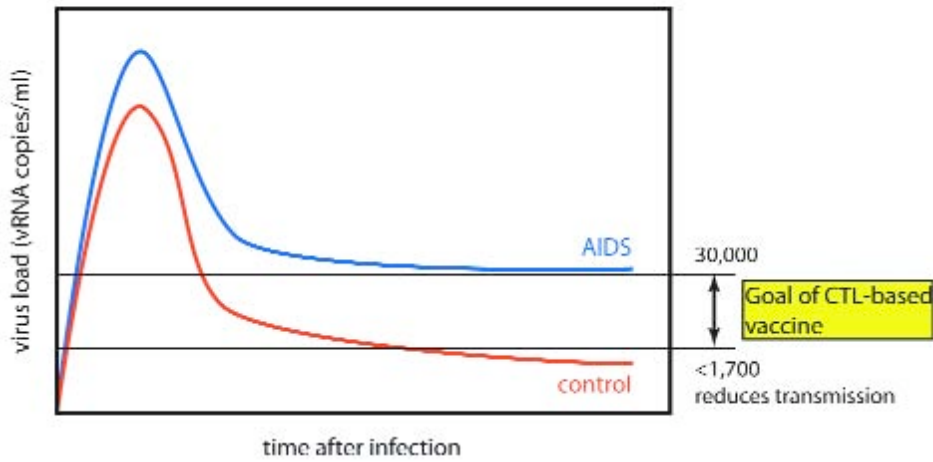
#### **Cellular immunity to HIV and SIV**

As T cell biologists, we will be somewhat parochial here and focus on T-cell responses to HIV and SIV. In fact there are practical reasons for this T cell-centrism. The enormous difficulty in generating NAbs encourages us to focus on T-cell responses. Unlike NAbs, these responses are detected in most HIV-infected individuals. This may on first glance seem to argue against the wisdom of inducing T-cell responses through vaccination. After all, vigorous T-cell responses can be detected even in progressive infection, so what use could T cells be in controlling virus replication, let alone in preventing infection? We believe that these responses could provide the "raw material" of immune responses that could be augmented and focused by cleverly designed AIDS vaccines to ameliorate disease and help prevent HIV transmission. Several lines of evidence suggest that CD8<sup>+</sup> T cell responses are important in con-

trolling acute infection and maintaining the chronic phase viral set point. CD8<sup>+</sup> T cells were first implicated in suppressing HIV replication in 1994 in two studies demonstrating that the reduction in viremia in acute infection was temporally associated with the appearance of HIV-specific CD8<sup>+</sup> T cells<sup>5,6</sup>. A vigorous antibody response occurs subsequent to this initial CD8<sup>+</sup> T cell response, after viremia has been controlled. Moreover, vigorous CD8<sup>+</sup> T cell responses have been reported in some subjects with long term non-progressive infection<sup>7,8</sup>. Certain HLA alleles, such as B27 and B57, are also associated with long term non-progression<sup>9-12</sup>, and some CD8<sup>+</sup> T cell responses exert selective pressure on replicating viruses, resulting in the outgrowth of escape mutant viruses.

The relative contributions of cellular immune responses to control of virus replication and evolution have been difficult to discern from human studies alone. The existence of viral escape from CD8<sup>+</sup> T cell responses remained controversial until evidence from the macaque model showed that these responses did indeed select for *de novo* escape variants<sup>13</sup>. This is a particularly apt illustration of the utility of the animal model: Infection of macaques with a molecularly-cloned virus, SIVmac239, allows for the unambiguous identification of mutations that were not present during initial infection. Subsequently, cloned viruses derived from SIVmac239 have been used to show that escape from CD8<sup>+</sup> T cell responses can exact a cost to viral fitness, an idea that may turn out to be important in the design of successful vaccines<sup>14</sup>.

These studies used the animal model to complement investigations of HIV-infected humans but the macaque model also allows experiments that would be impossible in humans. For example, crucial evidence for the role of CD8<sup>+</sup> cells in controlling immunodeficiency virus replication came from studies of transient depletion of CD8<sup>+</sup> cells in SIV-infected macaques<sup>15-17</sup>. In these studies, anti-CD8 monoclonal antibodies (MAbs) were used to transiently deplete circulating CD8<sup>+</sup> cells, which resulted in an increase in viremia lasting until these cells repopulated the periphery. In some cases administration of these MAbs during acute infection seems to prevent control of virus replication, leading to rapid disease progression.



**Fig. 1. The goal of a CTL-based vaccine is to reduce chronic phase transmission of HIV.** Recent evidence suggests that transmission of HIV is unlikely when the infected individual's virus load is less than 1,700 vRNA copies/ml plasma. This represents a reduction of about 1.5 logs below the median virus load of 30,000 vRNA copies/ml in untreated subjects. We therefore suggest that the goal of a CTL-based vaccine candidate in macaques should be to reduce virus load by 1.5 logs.

### Antibodies and T cells

Although we have chosen to focus on cellular immune responses here we will admit to some degree of antibody envy. In many ways the antibody field is years ahead of the T-cell field. A few broadly-neutralizing antibodies have been identified and investigators are now beginning to understand the mechanisms that confer their neutralizing activity. There is also a straightforward conceptual model guiding our thinking: NAbs must be broadly reactive and bind the viral envelope protein in such a way as to prevent infection of target cells. Although such antibodies seem to be extremely rare, at least we think we know what they should do<sup>18</sup>.

In contrast we have not yet been able to define what an effective CD8<sup>+</sup> T-cell response might be. Many investigators have speculated that not all CD8<sup>+</sup> T cells will be equally effective in eliminating virus-infected cells, and it has recently been shown in an *in vitro* system that HIV-specific CD8<sup>+</sup> T-cell clones have differential abilities to select for escape variant viruses<sup>19</sup>. There are several viral and host parameters that could affect CTL efficacy, including (i) avidity of the T cell receptor (TCR) for the MHC/peptide complex; (ii) CTL frequency and anatomical localization; (iii) the kinetics of expression of the viral protein from which the epitope is derived; (iv) fitness constraints on the epitope region; (v) immunodominance relationships among CTL specificities. We cannot yet evaluate the contributions of each of these

factors to T-cell efficacy but a rational approach to AIDS vaccine design will depend on our ability to understand them.

### The role of the SIV model in vaccine research

In twenty years only one AIDS vaccine candidate has progressed all the way through clinical trials and it failed to protect people from infection. In the immediate aftermath of those results the feeling of disappointment in the field was palpable. But we believe that the AIDS VAX trial should be a call back to the drawing board. In order to find a safe and effective vaccine, first we need to know what we're looking for. Sterilizing immunity is an ideal but, perhaps for now, unrealistic goal. We find reason for hope in studies of discordant couples, in which one partner is HIV-infected but the other is not. Longitudinal studies of such couples suggest that there is a threshold of viremia, about 1,700 vRNA copies/ml plasma, below which transmission of HIV is extremely unlikely<sup>20</sup>. Furthermore, a study of over 550 mother-child pairs found no cases of mother-to-child transmission when the mother's viral load was less than 1,000 vRNA copies/ml<sup>21</sup>.

We therefore propose that the immediate goal of an effective AIDS vaccine should be to reduce transmission of the virus (Fig. 1). This may be a more realistic goal for a CTL-based vaccine since cellular responses would need to allow infection of at least some host cells and could not provide

absolutely sterilizing immunity. Such a vaccine would be a truly positive development for public health since a reduction in transmission rates would roll back the epidemic. Furthermore, lower chronic phase virus loads are associated with slower progression to AIDS, so a vaccine that reduces transmission should also prolong survival in vaccinated individuals who become infected. A CTL-based AIDS vaccine should therefore reduce chronic phase viremia from its present level of ~30,000 vRNA copies/ml in untreated subjects to 1,700 vRNA copies/ml, about a 1.5-log reduction. When designing and evaluating experiments in the macaque model, we must keep this goal in mind. To be labelled promising, then, an experimental vaccine should reduce chronic phase replication of a pathogenic SIV by at least 1.5 logs below controls. Given this goal, how can we evaluate vaccine candidates in the monkey model?

1. Define the parameters of effective CD8<sup>+</sup> T cells. The characteristics of "effective" CD8<sup>+</sup> T cell responses remain a mystery. Since the cellular field lags behind the antibody field in defining the characteristics of "good" responses, the SIV model will be instrumental in testing hypotheses about what defines a desirable T-cell response. Let us consider two examples of the ways in which such hypotheses could be addressed in the animal model. First, CD8<sup>+</sup> T cell-based vaccines must deal effectively with the ability of the virus to escape. As we mentioned above, it may even be possible to use the plasticity of the viral genome to our advantage by targeting epitopes that are under functional constraints and poorly tolerant of variation. These epitopes should be less likely to accumulate escape mutations in individual patients or in the population. If escape variants are selected, they may reduce viral fitness, resulting in a mutant virus that is easier to control. Indeed it seems likely that vaccine-induced CD8<sup>+</sup> T cells specific for an epitope in SIV Gag have done just that in a recent trial<sup>22</sup>. In that study, 5 of 8 vaccinated Burmese rhesus macaques rapidly controlled an intravenous SIV challenge. Control in each case was associated with sequence variation in *gag*. One mutation appeared in each of three animals that shared an MHC haplotype, and appeared to have a detrimental effect on viral fitness. Unfortunately, though, CD8<sup>+</sup> T cells that target constrained epitopes may not be immun-

odominant in natural infection. Indeed the eventual progression of most infected individuals to AIDS indicates that the natural hierarchy of T-cell responses, that is, their relative frequencies in natural infection, is usually not sufficient to control virus replication. A vaccine, therefore, should not simply aim to induce as many CD8<sup>+</sup> lymphocyte responses as possible. In many cases, it will be necessary to alter the natural immunodominance of the HIV- or SIV-specific CD8<sup>+</sup> T-cell response since immunodominant responses may limit the development of potentially more effective sub-dominant responses<sup>23,24</sup>. For a vaccine, it may be important to force escape at a price to the virus.

2. The challenge SIV strain must be appropriate to the experiment. The identification of escape mutations associated with reduced fitness was facilitated by the use of a cloned challenge virus, SIVmac239. This illustrates another important consideration in the monkey model: which challenge virus is the most appropriate? Few CD8<sup>+</sup> T cell-based vaccine regimens have significantly lowered viral load or affected disease course in macaques using the most stringent SIV challenge models available. Recently several vaccines have claimed success in control of the chimeric virus SHIV89.6P<sup>25-28</sup>. However, these results have the potential to be misleading for two reasons. First, all but one of these recent vaccine regimens includes a similar Env in the vaccine. The only regimen that did not use Env, a DNA prime/recombinant adenovirus (rAd) boost encoding only Gag, was ineffective against a SIVmac239 challenge despite its success against SHIV89.6P (Casimiro *et al. J. Virol.* in press). Second, SHIV viruses were developed to test vaccines designed to elicit Env-specific antibodies in the macaque model. While they have had utility in this regard, the biology of these chimeric viruses has raised some doubts about their relevance for use in evaluation of CTL-based vaccines<sup>29</sup>. SHIV89.6P, for example, causes a dramatic, acute loss of virtually all circulating CD4<sup>+</sup> T cells, which is thought to be a main reason for its pathogenicity. Such a dramatic loss of CD4<sup>+</sup> T cells from the periphery is rare in HIV infection. In contrast, pathogenic SIV strains such as the molecular clone SIVmac239, or the biological isolates SIVmac251 or SIVsmE660, replicate to high titer in the chronic phase but do not cause as dramatic a depletion of CD4<sup>+</sup> T cells<sup>30,31</sup>. Uncloned SIV isolates that


are difficult to neutralize may be the most relevant challenge stocks since they mimic the diversity of quasispecies that are likely to be present in transmission of HIV.

3. Repeated low-dose challenge may help the SIV model more closely mimic natural HIV infection. It is possible that high-dose SIV challenge is too stringent a test for our vaccine regimens. In most SIV experiments animals are challenged with a single very high dose of virus, equivalent to thousands of infectious particles. This approach has a sound rationale: in order to detect a vaccine effect when animal numbers are low, it is essential that all animals in the control group become infected. But it is possible that an extremely high dose of infectious virus will overwhelm any vaccine-induced immune responses, causing us to reject what may actually be promising approaches. Several laboratories have recently begun developing lower-dose challenge models in which animals are exposed to 10- or 100-fold less infectious virus than in conventional high-dose experiments<sup>32</sup>. Challenges must be repeated multiple times in order for animals to become infected, complicating experiment schedules. Low-dose challenge approaches must therefore be optimized to deliver as small a dose of virus as possible while maintaining experimental tractability.

### Future directions in vaccine research

Reviewing the recent state of AIDS vaccine research could understandably be a depressing exercise. Like the stage at the end of a Shakespearean tragedy, the field is littered with approaches that, noble attempts though they were, were tragically brought low. We believe, however, that recent results from the SIV model may offer a glimmer of hope. We need to view these results as critically and honestly as possible to avoid future disappointment and the prolongation of an epidemic whose human toll is already disastrous. Antibodies and T cells alike need to deal with the prodigious ability of the virus to spawn variants that evade immune responses. But there may be chinks in this armor of variability. Constrained epitopes may persist in the virus population and represent attractive targets for CD8<sup>+</sup> T-cell responses. New generations of vaccine vectors may be able both to induce more potent mucosal responses and to refocus CD8<sup>+</sup> T cells on epitopes in which variation results in a loss of viral fitness. Indeed, we

may already be on the trail of successful vaccine approaches whose tracks are currently obscured by too-high doses of challenge virus.

We hope that we are experiencing a new era of innovation in AIDS vaccine research in which many exciting new ideas are being put forward from many investigators. Not all of these ideas can be tested in human trials. The SIV model can provide some road signs to the clinic by allowing us to evaluate hypotheses in ways that are impossible in human subjects. Immunogenicity is not enough. We must challenge our new ideas, figuratively by refining our hypotheses and experimental approaches, and literally by infecting vaccinated animals with appropriate pathogenic viruses. 

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1. T. Ready, *Nat. Med.* **9**, 376 (2003)
2. WHO. HIV Vaccines, <http://www.who.int/hiv/topics/vaccines/Vaccines/en/> (2005)
3. D. Lee, B. S. Graham, *et al. J. Infect. Dis.* **190**, 903 (2004)
4. M. R. Betts, B. Exley, *et al. Proc. Natl. Acad. Sci. USA* **102**, 4512 (2005)
5. P. Borrow, H. Lewicki, *et al. J. Virol.* **68**, 6103 (1994)
6. R. A. Koup, J. T. Safrin, *et al. J. Virol.* **68**, 4650 (1994)
7. T. Harrer, E. Harrer, *et al. AIDS Res. Hum. Retroviruses* **12**, 585 (1996)
8. T. Harrer, E. Harrer, *et al. J. Immunol.* **156**, 2616 (1996)
9. S. J. O'Brien, X. Gao, and M. Carrington, *Trends Mol. Med.* **7**, 379 (2001)
10. A. D. Kelleher, C. Long, *et al. J. Exp. Med.* **193**, 375 (2001)
11. M. Altfeld, M. M. Addo, *et al. Aids* **17**, 2581 (2003)
12. S. A. Migueles, M. S. Sabbaghian, *et al. Proc. Natl. Acad. Sci. USA* **97**, 2709 (2000)
13. D. T. Evans, D. H. O'Connor, *et al. Nat. Med.* **5**, 1270 (1999)
14. T. C. Friedrich, E. J. Dodds, *et al. Nat. Med.* **10**, 275 (2004)
15. X. Jin, D. E. Bauer, *et al. J. Exp. Med.* **189**, 991 (1999)
16. T. Matano, R. Shibata, *et al. J. Virol.* **72**, 164 (1998)
17. J. E. Schmitz, M. J. Kuroda, *et al. Science* **283**, 857 (1999)
18. D. R. Burton, R. C. Desrosiers, *et al. Nat. Immunol.* **5**, 233 (2004)
19. O. O. Yang, P. T. Sarkis, *et al. J. Exp. Med.* **197**, 1365 (2003)
20. R. H. Gray, M. J. Wawer, *et al. Lancet* **357**, 1149 (2001)
21. P. M. Garcia, L. A. Kalish, *et al. N. Engl. J. Med.* **341**, 394 (1999)
22. T. Matano, M. Kobayashi, *et al. J. Exp. Med.* **199**, 1709 (2004)
23. W. Chen, L. C. Anton, *et al. Immunity* **12**, 83 (2000)
24. M. J. Palmowski, E. M. Choi, *et al. J. Immunol.* **168**, 4391 (2002)
25. J. W. Shiver, T. M. Fu, *et al. Nature* **415**, 331 (2002)
26. N. F. Rose, P. A. Marx, *et al. Cell* **106**, 539 (2001)
27. D. H. Barouch, S. Santra, *et al. Science* **290**, 486 (2000)
28. R. R. Amara, F. Villinger, *et al. Science* **292**, 69 (2001)
29. M. B. Feinberg and J. P. Moore, *Nat. Med.* **8**, 207 (2002)
30. Y. Nishimura, T. Igarashi, *et al. Proc. Natl. Acad. Sci.* **101**, 12324 (2004)
31. Y. Nishimura, C. R. Brown, *et al. Proc. Natl. Acad. Sci.* **102**, 8000 (2005)
32. A. B. McDermott, J. Mitchen, *et al. J. Virol.* **78**, 3140 (2004)



# New strides in protecting infants from HIV

*Researchers continue quest to give pregnant women better options to prevent mother-to-child transmission*

by **Kristen Jill Kresge**

**M**ore than a decade after researchers first found that antiretroviral (ARV) drugs given to women during childbirth could greatly reduce the risk of HIV transmission to their infants, children are still acquiring HIV at an alarming rate. The most recent data from UNAIDS, the Joint United Nations Programme on HIV/AIDS, estimated that 630,000 children worldwide were newly HIV infected in 2003.

The transmission of HIV from mother to child can occur at three points: during pregnancy while the baby is still in the womb (*in utero*), during childbirth (intrapartum), or after birth through exposure to HIV-infected breast milk. Without any treatment intervention, about 30% of infants become infected through one of these routes. The first study that attempted to lower the mother-to-child transmission (MTCT) of HIV in 1994 showed that AZT—the first ARV approved by the US Food and Drug Administration—reduced the transmission rate to 8% through 18 months post-delivery in breast-feeding women. This strategy was immediately adopted throughout the US and Europe and the number of HIV-infected infants quickly began to decline. Now most HIV-infected pregnant women in wealthy countries with low CD4<sup>+</sup> T-cell counts receive highly active antiretroviral therapy (HAART), and transmission rates now hover between 1 and 2%. In the US, only around 200 babies are born HIV infected each year.

But using AZT to prevent transmission requires a lengthy course of treatment. Mothers have to receive the drug from early in pregnancy through childbirth and infants must receive treatment during their first six weeks, so women must be diagnosed with HIV infection and have access to prenatal care early during their pregnancy. This is often not possible in developing countries and therefore led researchers to explore alternative ways to halt MTCT.

In 1999 a subsequent study showed that nevirapine, a non-nucleoside reverse transcriptase inhibitor (NNRTI), was easier to administer—only a single pill at the onset of labor and a single dose to the baby—and effective at cutting transmission rates by more than half. This was a breakthrough discovery that didn't require extensive healthcare infrastructure. "Nevirapine is very safe, very simple, and very cheap," says Marc Lallemand of the Perinatal HIV Prevention Trial Group that works in Thailand. The implementation of single-dose nevirapine programs drastically altered the landscape of MTCT programs, bringing women in developing countries a simple way to protect their newborns that has, to date, saved countless infants from the fate of HIV infection.

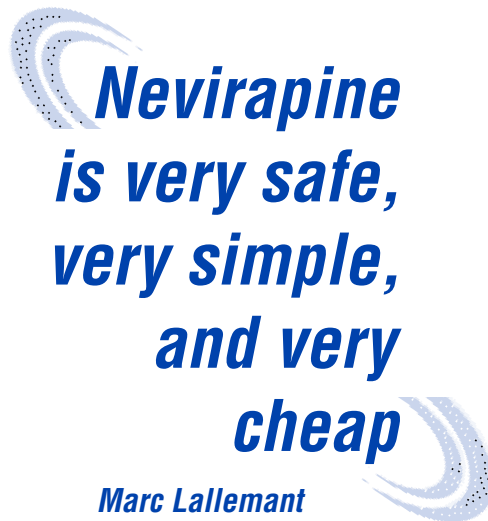
Single-dose nevirapine still remains the most feasible, effective, and simple regimen for preventing MTCT in many countries. The

potential side effects associated with its chronic use, severe and occasionally fatal liver toxicity, have not been reported with short-term use. But the single-dose regimen brings its own complications—the prospect of the emergence of drug-resistant virus. Pregnant women given nevirapine may develop virus resistant to nevirapine that could potentially compromise their future response to ARV treatment with either nevirapine or other NNRTIs like efavirenz. Currently there is sparse clinical evidence to show just how nevirapine resistance might affect treatment outcomes. The World Health Organization (WHO) reviewed the MTCT guidelines last year and decided to maintain single-dose nevirapine as an option based on insufficient evidence on the clinical ramifications of nevirapine resistance.

Meanwhile researchers are focused on balancing the need to preserve nevirapine use in areas where it is the only feasible and effective intervention with finding other regimens that reduce MTCT rates even further and avoid the emergence of viral resistance, maximizing the benefits to the newborn while reducing potential drawbacks for the mother. "If we can minimize resistance we should do that, but it's not the end of the world. I wouldn't throw out the baby with the bathwater," says Lallemand. "We are trying to balance efficacy of the intervention with efficacy of future ARV treatment."

Recent studies show that administering a combination of ARVs, including nevirapine, can reduce mother-to-child (vertical) transmission rates to below 5%, as well as limit development of nevirapine resistance. Such results inspired the WHO to consider changing its international MTCT guidelines again this year. Researchers in the field hope that the rollout of ARVs and access to newer regimens will help curb the number of pediatric AIDS cases throughout the world. "For us, nevirapine was a good place to start," says Glenda Gray, director of the perinatal HIV research unit in Soweto. "But we should accept nothing less than complete eradication. Anything else is a compromise."

Lallemand emphasizes that regardless of which treatment regimen is available, the most pressing crisis in the field is the failure to get women into MTCT programs at all. Estimates are that only 3 to 10% of women who are in need are accessing MTCT programs. This is due in part to the paucity of available services but, even where there are ARVs and prenatal clinics available, uptake is low because pregnant women often fear the stigma associated with HIV infection and will refuse HIV tests. Eradication of pediatric AIDS will require improved access to HIV testing and MTCT programs, not just newer regimens, and this remains one of the most substantial obstacles in many countries.



**Nevirapine**  
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**Marc Lallemand**

### **Pièce de résistance**

The study that elucidated nevirapine's role in preventing MTCT was the HIVNET 012 trial (*Lancet* **354**, 795, 1999) in Uganda, which reported a 13% rate of transmission through 14-16 weeks in a breast-feeding population when both mother and infant received a single-dose of nevirapine. Soon after this trial new programs sprang up in other developing countries. Numerous subsequent follow-up studies have confirmed nevirapine's efficacy at reducing MTCT to around this level (*JAMA* **292**, 202, 2004; *J. Acquir. Immune Defic. Syndr.* **34**, 274, 2003; *J. Infect. Dis.* **187**, 725, 2003).

Recently the US National Institutes of Health (NIH), which ran the HIVNET 012 trial through its HIV Prevention Trials Network (HPTN), was accused of poor record keeping during the study. Although the Institute of Medicine and other independent groups have discredited these claims, the message reverberated throughout countries where nevirapine forms the basis of MTCT programs. Researchers who gathered at the Conference on Retroviruses and Opportunistic Infection (CROI) earlier this year attempted to set the record straight by highlighting the subject of MTCT and nevirapine's crucial role in the field.

In addition to sparing many thousands of infants from HIV infection, the introduction of nevirapine-based MTCT programs have also served as the impetus for expanded treatment programs that benefit whole communities. According to Lallemand, women who access MTCT programs are more likely to receive ARV therapy when they become immunologically compromised. "Single-dose nevirapine gave countries overwhelmed by the problem of mother-to-child transmission the ability to do something and to start services," said James McIntyre of the University of Witwatersrand in Johannesburg at CROI. "These services have provided the foundation for treatment access."


But this year's CROI meeting also continued the debate over nevirapine resistance. There is now mounting evidence that a significant proportion of women who receive single-dose nevirapine during pregnancy will develop drug-resistant HIV. The development of resistance is problematic because both of the available NNRTIs (nevirapine and efavirenz) can be rendered ineffective by just a single signature point mutation in the virus, a lysine to asparagine substitution

at position 103 in HIV's reverse transcriptase (referred to as the K103N mutation). Many generic ARV combinations that are gradually rolling out in the developing world contain nevirapine.

The development of resistance from a single dose of nevirapine is not surprising. While the drug was an obvious choice for study in MTCT due to its antiviral and pharmacokinetic properties, its prolonged clearance does not come without complications. The drug's 60-hour half-life translates to gradually declining plasma concentrations for extended periods after dosing, and effective concentrations (10 times the minimum inhibitory concentration) persist for 7 days after the single dose given to neonates. But this means that as nevirapine is slowly cleared thereafter, there is a lengthy widow of opportunity when HIV can develop resistance as doses fall below viral inhibitory concentrations.

Debate has been simmering among researchers for several years over how common and clinically significant this viral resistance will be in women who receive an intrapartum dose of nevirapine. A quarter of the women in the original HIVNET 012 trial had virus with detectable resistance after a single dose of nevirapine. Two other studies at CROI tackled the prevalence of nevirapine resistance. Both groups compared the detection of viral resistance using standard genotypic resistance tests and highly sensitive PCR assays. The sensitivity limitations of conventional genotypic sequencing require a resistant variant to comprise at least 20% of the virus population being tested. The real-time PCR assay has a lower limit of detection of 0.2%. With this approach, researchers found a higher percentage of the K103N mutation in samples from women given single-dose nevirapine during MTCT trials. In a presentation by Jeffrey Johnson, from the US Centers for Disease Control and Prevention, viral resistance was reported in an additional 40% of women with undetectable resistance by traditional genotyping (*J. Infect. Dis.* **192**, 16, 2005). In total, 65% of women in this study had detectable resistance mutations after single-dose nevirapine.

"This study provides new insights, but as expected when you use a more sensitive technique you will find more resistance," says Francois Dabis, a pioneer of MTCT research from the University Victor Segalen in Bordeaux, France.



# **Single-dose nevirapine gave countries overwhelmed by the problem of mother-to-child transmission the ability to do something and to start services**



**James McIntyre**

In another presentation, Sarah Palmer of the US NIH found that this more sensitive detection technique also revealed that NNRTI-resistance persists longer than originally thought—for at least one year following receipt of intrapartum nevirapine (*J. Infect. Dis.* **192**, 24, 2005).

“Resistance is more frequent and lasts longer when you use more sophisticated assays, but what this means is very unclear,” adds Dabis. “This resistance pattern may have some immunological and clinical consequences but no one knows how important it will be.”

Another study at CROI demonstrated a correlation between the emergence of viral resistance and HIV subtype. Susan Eshleman from Johns Hopkins Medical Institutions in Baltimore presented data indicating that individuals with subtype C HIV infection are more likely to develop nevirapine resistance after a single dose—69% of women with subtype C HIV developed resistance compared to only 19% with subtype A and 36% with subtype D (*J. Infect. Dis.* **192**, 30, 2005).

But to date only one published study has evaluated the clinical relevance of nevirapine resistance in women subsequently treated with a combination ARV regimen containing nevirapine. The authors of this study (*NEJM* **351**, 229, 2004) concluded that women with nevirapine-resistant virus were less likely to achieve viral suppression below detectable limits after six months of treatment with combination therapy containing an NNRTI. Although the treatment was less successful in women previously exposed to nevirapine, the regimen was not rendered completely ineffective.

The six-month interim results indicated that 49% of women in the nevirapine group had undetectable viral loads (<50 vRNA copies/ml), compared to 68% in the control group. “It’s nothing new that resistance has an impact on success of therapy, so this isn’t exactly a surprise,” says Lallemand, an author on the study. “The important question is how long these mutations remain biologically significant.”


He is currently collecting the one-year data from this study and will only hint at the expected results with guarded optimism. “We were concerned that women who did not reach viral suppression at six months would have a worse response at one year, but things do not seem to be getting worse,” he says.

Another presentation at CROI from Shayne Loubser of the National Institute for Communicable Diseases in Johannesburg gave researchers more reason to be cautiously optimistic. The K103N resistance mutation does not appear to be archived in viral DNA in the blood for longer than one year after single-dose nevirapine. However, the researchers did not look at virus that might be archived in other compartments and reservoirs.


Scott Hammer of Columbia University wrote in a recent editorial that there is a “moral imperative” to continue efforts to reduce the rate of MTCT while trying to limit the emergence of drug resistance (*J. Infect. Dis.* **192**, 1, 2005) and there are now several options for doing just that. All public hospitals in Thailand offer women either AZT from the 28th week of pregnancy and a dose of nevirapine during labor or HAART if their immunological status mandates. This comprehensive program, which prevents 2,600 pediatric HIV infections every year, has been effective at lowering transmission rates in that country to almost 2%. This approach can be combined with an extended course of AZT to further protect women from nevirapine resistance and is known as adding a “tail” to MTCT therapy. If mothers continue taking AZT during the three-week period after receiving single-dose nevirapine, they are less likely to develop resistance mutations.

Dabis and his colleagues in Cote d'Ivoire presented another innovative, albeit more complicated, approach at CROI from a clinical trial that provided 320 pregnant women in Abidjan with AZT and 3TC, two nucleoside reverse transcriptase inhibitors, starting at 32 weeks gestation. The women also received an extra dose of AZT and 3TC along with a single dose of nevirapine during labor and 3 days of AZT/3TC postpartum. Infants received AZT for their first 7 days, with a single dose of nevirapine on day 2. The transmission rate through 6 weeks was just under 5%. Most importantly, the frequency of nevirapine-associated mutations was less than 2% in mothers. “Using nevirapine in a different way allows us to lower transmission rates and limit resistance,” says Dabis. “Single-dose nevirapine is not dead, there is just more variability in the way it’s used.”

According to Gray, this course of treatment is the next best thing to offering moth-



***This resistance pattern may have some immunological and clinical consequences but no one knows how important it will be***



***Francois Dabis***

ers HAART. The WHO is considering revising its guidelines to include this regimen in the menu of options available for preventing MTCT and Gray hopes the South African government will adopt this regimen for nationally-supported MTCT programs. But a combination regimen is obviously more expensive than single-dose nevirapine and will require the expansion of healthcare infrastructure, especially in rural areas. Nevirapine can be taken at home at the onset of labor and few interventions can aim to be that simple. "The only medical requirement for nevirapine is getting tested for HIV. The rest can be done at home," says Lallemand.

For this reason, Gray is also emphasizing the need for research into newer ARVs that retain nevirapine's simplicity. Tenofovir is a promising candidate for MTCT because it is unlikely to cause resistance and is available as a single pill, making it easier to administer than a combination of drugs. Trials to test its efficacy in preventing vertical transmission have been stuck in the planning

stages. "It's criminal that it is taking so long to develop these protocols," laments Gray.


### ***Limiting breast-feeding transmission***

Researchers are also looking at interventions that can protect infants from becoming HIV infected through breast feeding. It is estimated that half of all new pediatric HIV cases in 2003 occurred by this route since the drugs administered during labor only offer partial protection during the breast-feeding period. The quantity of HIV in breast milk is dependent on plasma viral load, but about 80% of breast milk samples from HIV-infected mothers will contain the virus.

The most effective approach to avoid breast-feeding transmission is to use bottle feeding with a powdered milk substitute, and in more urban areas women are likely to accept this as an alternative. The South African government provides milk alternatives free to HIV-infected mothers for the first 6 months, which is a relatively short time to transition to solid food. The government of Thailand provides new mothers

with enough bottled milk substitute for the infant's first year.

But this approach has its limitations. Clean water is necessary to prepare the bottles and in some more rural areas potable water is not available. Some women may also choose not to use breast milk alternatives to avoid being stigmatized as HIV-infected within communities where breast feeding is more common. For women who do breast feed, extended treatment with ARVs and early weaning can help lower the baby's risk of acquiring HIV. "Opportunities exist to stop transmission through breast feeding and should be used," says Gray.

Again the biggest challenges for researchers and physicians are improving access to proven interventions where they are not available and convincing women to be tested and to access prenatal care where they are. "The real headline should be that only 5% of women are accessing mother-to-child transmission programs," warns Lallemand. 

# Obituary: Maurice Hilleman

*Scientist who developed a multitude of vaccines and saved countless lives*

by Kristen Jill Kresge

**M**aurice Hilleman was without doubt one of the towering figures of 20th Century science. Since his death on April 11, tributes have consistently cited him as the medical scientist responsible for saving the most human lives.

Hilleman let no obstacles stand in his way and this led him through one of the most distinguished careers in the history of vaccinology. Developing one successful vaccine is certainly a milestone, but Hilleman had a career littered with milestones. In total he developed more than 40 vaccines, including 5 of the 14 immunizations routinely given to children and adults today. Vaccines against measles, mumps, rubella, *Haemophilus influenzae* type B, and hepatitis B are just some of Hilleman's contributions to the field. He also pioneered the combination of the measles-mumps-rubella (MMR) vaccine as a single immunization.

Hilleman was raised on a farm in Montana, an environment he considered "a crucible for learning science", and he maintained his rural demeanor throughout his life. "Maurice believed there were very simple, profound truths to the way you do science and develop vaccines. Even though it is very complicated work, he always had a clear path," recalls Mike Goldrich of the International AIDS Vaccine Initiative (IAVI). At a public memorial service held in his honor on April 25, Hilleman was described as "an affable curmudgeon". "At times you have to be cantankerous. It may not get you prizes, but it is what is needed for you to develop more vaccines than any other person," adds Goldrich. "He was like the Yogi Berra of science," referring to the baseball legend known for his pithy insights. Goldrich remembers Hilleman as a steadfast—maybe even stubborn—scientist and humanist who dedicated his life to protecting people from disease.

Hilleman was awarded the Lasker Public Service Award and the US National Medal of Science. Yet even among other scientists, the number and importance of Hilleman's contributions to the field were underappreciated, perhaps because he was known best for his applied science rather than basic research. But his contribution to new fundamental knowledge was still immense; he was the first to work out how to serotype microorganisms (*Chlamydia* spp.), he discovered SV40 and the adenoviruses, he was the first to purify interferon and show that it was induced by double-stranded RNA. He was also the first to describe the phenomenon of antigenic shift in influenza virus, the genetic reassortment that occasionally throws up new pandemic strains.


This latter understanding led to him almost single-handedly averting an influenza epidemic in the US. In 1957 he noticed a report of a new outbreak of influenza in Asia and realized a new pandemic was on its way. He obtained samples and worked nine 14-hour days with his team to confirm that a new influenza virus strain was responsible for this 'Hong Kong flu'. Knowing that the US population would have no immunity to this strain, he convinced manufacturers that production of a new vaccine was imperative, even providing the virus stock samples. As a result the death toll in the US from Hong Kong flu was a relatively modest 69,000 people rather than the predicted one million.

Many of his outstanding accomplishments came during the subsequent 28 years he spent at the Merck Research Laboratories in West Point, PA, an association he maintained in an advisory capacity right up until his death. Emilio Emini of IAVI spent 20 years at Merck and recalls that "Hilleman was my hero. He was the scientist who set the standard for what my colleagues and I wanted to accomplish. Of course, the standard proved to be too high for any of us."

Occasionally, Hilleman seemed to have even more gravitas than that usually accorded to such an exceptional scientist. Pat Fast of IAVI recalls a high-level advisory council meeting at the National Institutes of Allergies and Infectious Diseases where Hilleman was advocating decisive action. A storm was brewing outside and each proclamation from Hilleman was met with a huge clap of thunder. "It was as if there was endorsement from on high," remembers Fast. "No one seemed very surprised."

Even well into his retirement, Hilleman remained active in the field. When it came to AIDS vaccines, Hilleman recommended a Manhattan Project-style approach, suggesting that the only way to tackle the extensive scientific difficulties was to get the best people, whether from academia, industry, or government, together in the same building where they could work on solving each problem.

At his memorial service, a quote from Hilleman summed up his ethos. "Well, looking back on one's lifetime, you say, 'Gee, what have I done—have I done enough for the world to justify having been here?' That's a big worry—to people from Montana, at least. And I would say I'm kind of pleased about all this. I would do it over again because there's great joy in being useful."

The number of lives that Hilleman's vaccines have saved is immeasurable—one estimate has put it at 8 million lives each year. His passing marks the end of a life that saved so many millions of others. 



An Interview with

Frances  
Gotch



## HIV/AIDS cohorts in Uganda

*Frances Gotch, D.Phil has been working with cohorts of people affected by HIV/AIDS in the UK and in Entebbe, Uganda for more than 10 years. She was instrumental in setting up the immunology laboratories at the Uganda Virus Research Institute in Entebbe with funding from the Wellcome Trust, and in close collaboration with the MRC-funded Programme on AIDS in Uganda. Epidemiological studies have helped define progression rates and risk factors, and virological, statistical, and immunological studies within the cohorts have led to a greater understanding of the pathology of HIV infection in developing countries. The AIDS Support Organisation (TASO) has provided a backdrop for all the work, providing care, support, and counseling for people living with AIDS in Uganda.*

*Gotch started her working life as an immunologist with Professor John Humphrey—a founding figure in the immunological world—at the National Institute for Medical Research, Mill Hill, UK. She then spent 20 years at Oxford University—most latterly in a productive partnership with Andrew McMichael and Alain Townsend. The possibility of working at the Chelsea and Westminster Hospital, which houses the largest clinic for HIV-infected persons in Europe, brought her to London in 1996. She is now Professor of Immunology at Imperial College London, based at the Chelsea and Westminster Hospital, and a fellow of the Royal College of Pathologists. Her research interests center around cellular immunology, particu-*

*larly the mechanisms of T cell activity in disease associated with HIV-1, HIV-2, SIV, influenza virus, cytomegalovirus, Epstein-Barr virus, human herpes virus 8, and malaria. Her current studies focus on identifying HIV-specific immunological functions in vaccinated, infected, and high-risk non-infected individuals, trying to tease out the immunological parameters that might confer benefit in HIV-associated disease. Gotch has been intimately involved in efforts to standardize assays across the various AIDS vaccine trial sponsor organizations and is the Principal Investigator at IAVI's state of the art, GCLP-accredited core laboratory facilities at Imperial College London. She recently spoke with IAVI Report Editor Simon Noble about her Ugandan studies and efforts to define immunological parameters relevant to AIDS vaccine development.*

### **Could you just talk a little bit about the kind of individuals who make up the Ugandan cohorts you've been working with?**

I've been working in Uganda for 10 years now, primarily with two cohorts. One is what we call the Progression cohort, the other is the Exposed Seronegative (ESN) cohort. The Progression cohort is a group of HIV-infected individuals living in and around Entebbe who attend the local AIDS Support Organization (TASO), and we've been following these individuals every six months for about eight years now. The principal investigators in Uganda are Pontiano Kaleebu, Heiner Grosskurth, Tony Kebba and Jennifer Serwanga.

We originally tried to define people as rapid or slow progressors, simply on their CD4<sup>+</sup> T cell count and the length of time that they had been infected, or at least length of time since diagnosis—we very rarely know the time of seroconversion.

Now we've been able to get the statisticians involved and they've carefully assigned these people as rapid or slow progressors, and everything in between. Of course there's only a small proportion of people who by these very stringent statistical criteria are true long term non-progressors (LTNPs), only about 3 or 4% of the total.

We also now have our viral load assays in Uganda, and also the patients are staged according to the World Health Organization clinical staging of HIV disease: Stages One, Two, Three, Four.

### **Is this designed to get a clearer picture of the natural history of HIV infection in a developing country setting?**

Yes, but personally I'm also interested in this group of patients in terms of potential immunotherapy, and the kind of responses that are made by the LTNPs in this group, compared to those individuals who are progressing extremely rapidly, so that we know what we're trying to reconstitute when we treat people with immunotherapy or with drugs or whatever.

Many people think that the kinds of responses in LTNPs are those that should be induced by a prophylactic vaccine, but it should be remembered that although these individuals are keeping the virus under control quite well, eventually most of them will get sick. So we're not certain that these responses, if they were there in the first place, would actually protect from infection.

We now have a very clearly defined group of LTNPs and we're able to look at both CD4<sup>+</sup> and CD8<sup>+</sup> T cell

responses, using conventional methods—ELISPOT assays and intracellular cytokine staining analysis. We've looked at responses to all HIV proteins—not just Gag and Pol but also Nef, Vif, Vpr and Vpu. Everybody has been HLA typed and we can sequence virus and see if escape has taken place. We've identified new epitopes because the HLA types are slightly unusual in some cases, and we've looked at other kinds of genetic polymorphisms, like the CCR5 polymorphisms. So this is a very well characterized progression cohort with a group of true LTNPs that we follow over time.

### ***So what correlates of protection have you been able to identify in these LTNPs?***

Probably the biggest hint is in the CD4<sup>+</sup> T cell responses, which seem to be broader and more robust in the LTNPs. We see quite good CD8<sup>+</sup> T cell responses even in some of the rapid progressors, so there's not much difference there. Truthfully, I can't really say that we've had any very groundbreaking findings in Africa, similar to what we've seen in the UK.

One thing that always fascinates me is the fact that in Uganda the average time from seroconversion to AIDS is nine or ten years, which is not vastly different from the time in the UK or the US before HAART was introduced. If you consider all the other bombardments that people in Africa get—malaria, diarrhea, lack of clean water, typhoid, etc.—you would think that having a disease that makes you immunosuppressed would be very detrimental in such a setting. But it's almost as if there's something compensating.

So we're very interested in concurrent infections—we've looked at helminth infection, and a little bit at malaria.

### ***Could this be related to the 'hygiene hypothesis'?***

Well, it could be, who knows? We, along with Allison Elliott and Mike Brown, thought that intestinal helminth infection, which is known to induce Th2 type responses, would be rather detrimental if you were also HIV infected—we were proposing to treat all our vaccinees before vaccination.


But when you treat the helminth infection, if you're HIV infected your viral load actually goes up and your CD4 cell count drops. So it seems to be a balance between Th1 and Th2 that is so important.

One of the most interesting studies we're doing is looking at links between

hepatitis G virus infection and HIV progression. There is no doubt that infection, or at least the presence of hepatitis G antigen, has been shown to be associated with non-progression, so it looks as if there might be some kind of immune response to hepatitis G virus that is somehow keeping the HIV better under control. I think some very interesting data will come from these studies of intercurrent infections in our cohorts.

### ***So do you see hepatitis G virus as a potential immune therapy?***

My colleague in Uganda, Edward Wright, and I have discussed it. The data would suggest yes, but we actually don't know what this virus does, it hasn't ever



***They [ESNs] must surely hold the key to the prophylactic vaccine, if you believe that such individuals exist***

been shown to have any pathogenic effect. One could imagine perhaps administering heat-killed virus. It depends on what data he gets in the next couple of years, whether we actually can see any kind of immune responses to hepatitis G virus that perhaps are cross-reactive with HIV, or maybe it could be some kind of innate immune response, who knows? The goal now is to identify a mechanism of this non-progression.

### ***So what about the ESN cohort you work with in Uganda?***

In my opinion, they must surely hold the key to the prophylactic vaccine, if you believe that such individuals exist. We really do think there are people who are in some way protected from infection. In Uganda we've chosen to go for a rather different type of cohort—discordant couples. These are heterosexual couples who've been living together in stable relationships for long periods of time, with documented frequent unprotected sex, with one partner HIV infected and the other not. We've recruited quite a large cohort, about 40 couples, and we're hoping to expand the cohort to 200 couples, because we think some of the trouble with research from ESNs, I think it's true to say, is that there are a lot of people who don't believe the data, so you have to have large numbers of these people because if you're looking at quite small responses you've got to see them in an awful lot of people.

This is largely the work of Tony Kebba, a Ugandan clinician, and he has a very stringent questionnaire to make sure, in his estimation, that these people genuinely are being exposed to virus—lack of condom use and so on. We have some evidence that they are exposed to virus because in about four years in the entire cohort we've had three seroconversions. We've also had several pregnancies, which also indicate that people are having unprotected sex.

A very important asset of this cohort where we know both partners is that we can look at the HIV-infected partner as well to characterize the quality and the quantity of the virus that the seronegative partner is exposed to. With a commercial sex worker, you really do not know that.

This is very much ongoing work but we've looked at lots of different things—humoral and cellular responses in the peripheral blood, as well as on mucosal surfaces, and in urine and semen. We also want to look at innate immune responses, natural killer cells in particular. We're beginning to get some kind of handle on what might be protecting these individuals. We do see HIV-specific IgA on mucosal surfaces in these ESNs, and we're trying to map the epitopes on Env that these IgA's actually bind to.

We also see CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses, always of narrower breadth and quantitatively lower than in the seropositives. These responses would be defined as positive according to the most stringent cri-



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epidemic***



teria used in vaccine trials but they're not hugely impressive data, I would say. With the CD8<sup>+</sup> T cell responses we tend to see responses to slightly different parts of HIV—we rarely see responses to Gag in the ESNs, which is contrary to what's seen in the seropositives, and we see responses in the ESNs to some of the non-structural regulatory genes, which perhaps are seen less frequently in the seropositives.

What I must say is that although we have never been able to show any virus by sensitive PCR in these ESNs, that's only sensitive down to a certain level and we can't say that there is absolutely no virus, for instance in the lymph nodes, because biopsies wouldn't be acceptable.

***Have you followed any of these ESNs over time and looked at the evolution of the immune response and compared that to the virus in their infected partner?***

Yes. The three patients that we're following in greatest depth at the moment, the evolution of their virus, are the three seroconverters because if these people genuinely were ESNs, and they truly did have some response before they seroconverted, maybe their course of disease is going to be different because perhaps it will be as if they were vaccinated. We're also interested in transmission of escape mutants, and HLA sharing between the partners. Was Frank Plummer right, if people are very disparate in their HLA types, are they less likely to transmit than otherwise?

ARVs are now being rolled out in Uganda, which might mean that the non-infected partner will be exposed to very much less virus. If viral resistance then emerges in the HIV-infected partner, as it very often does, their viral load will increase again, and then the ESN may be more susceptible. Perhaps it will be similar to Sarah Rowland-Jones' sex worker cohort in Nairobi who stopped working for a while, then when they went back to sex work, some became infected—it was as if they had to keep on being exposed to the virus, keep on being vaccinated as it were. So we're going to be looking out for that.

***Do you think there's any mileage in mimicking this continuous exposure idea using a microbicide containing antigen, as a kind of topical vaccine?***

It seems clear that providing continual exposure to a vaccine antigen may be useful, and that immune responses on mucosal surfaces may be very important. Sometimes I

find it a little bit hard to think about true vaccine protection on mucosal surfaces. Whether or not this could be provided by a microbicide containing antigen remains to be seen, but a microbicide with an ARV and a monoclonal neutralizing antibody could, I think, have a huge and profound effect upon the epidemic. I'm very, very keen on microbicides in principle. They empower women to protect themselves, which is sometimes very difficult, and studies in Uganda suggest that they will be acceptable. They will have to be relatively easily applied, but it makes absolute sense.

***To switch to Uganda more generally, it's been hailed as the success story of Africa time and time again.***

I do think Uganda has been a success story, although I think we mustn't get overwhelmed, there's still a higher than acceptable prevalence of HIV in some parts of Uganda. I think above all it's been the openness in Uganda that has helped to combat the epidemic to an extent. It's not a huge country and it has had a relatively stable government ever since Museveni has been in power. They have a fairly open press, including television, and HIV comes up in almost all of the soap operas and consequently people are not scared. Prostitution is illegal in Uganda, as it is in many places in the world, but they don't throw anybody in prison, they try treating STIs.

Almost everybody in Uganda, I would think, has been touched by HIV in some way. There's a Member of Parliament in Uganda who has stood up and said "I am HIV positive", and education has been taken extremely seriously, public health messages on hoardings (billboards). People in developed countries sometimes don't realize what profound effects such public health measures can have.

There's always been the admission in Uganda that HIV causes AIDS. There's also been the admission that hunger and poverty may have exacerbated the epidemic, and I think nobody would argue with that, but they do not say that hunger and poverty causes AIDS. They have a serious wish to understand what HIV is all about.

I don't think the prevalence is dropping, not vastly, it seems to have plateaued. Now ARVs are being introduced, so we might even see an apparent increased prevalence. Imagine 10 years ago, if you were a Ugandan, perhaps you might have put yourself at risk a couple of times, you might think to yourself, "I wonder?" What really would have been the



point of going to be tested? Because there were no drugs. But now there is a point because now the drugs are beginning to be available. I can imagine now, if you were that Ugandan, you might think, "Well, there's a clinic just up the road, I've got a couple of friends who are HIV infected, they're getting these drugs, I'll go and be tested, and if I'm infected then maybe I'll be eligible for these drugs." So I think we might see an increase, but it won't be a real increase.

***Recently there's been good progress on standardizing assays, but the field now seems to be taking a step back and looking at it again as a real research endeavor, using more sophisticated, multi-parameter flow cytometry to identify a marker that better reflects an effective immune response. What do you think are currently the most promising markers that will better inform us about the functionality of any induced response?***

Flow cytometry is extremely powerful technology and I think we need combinations of techniques to get complementary information. Really, what we're interested in are effector functions, the way these cells might actually work, not just the markers they have—it's now accepted that mere detection of virus-specific T cells is not enough. We could measure CFSE staining for cell division and simultaneously characterize the phenotype and proliferative ability of T cells—it won't necessarily tell you their function, but it's this simultaneous analysis of different characteristics that flow cytometry allows.


I think the Holy Grail would be the ability to measure two functions, to measure recognition of a virally-infected cell and then destruction of that cell. Or with humoral immune responses, neutralization of the virus. We've got to have assays that really do reflect functionality. You can measure cytokines, such as TNF $\alpha$  or IFN $\gamma$ , but you can almost as easily in the same kind of assay measure secretion of cytotoxic granules, the perforins and the granzymes, for example. With perforins in a CD8<sup>+</sup> T cell you are actually looking at its cytolytic potential, but you might also want to look at the suppression of HIV replication by those cells, which is, ultimately, what you want to measure.

But it's very, very important that, whatever we decide, we've got to have standard operating procedures and work according to

good laboratory practice, and we've got to then use the same reagents and equipment. The culture of working to good laboratory practice with standard operating procedures is spreading.

***What's your current picture of the immune response against HIV? What do you think are the relative contributions of neutralizing antibody and cellular immunity, and within that, CD8 versus CD4.***

I think that high titer neutralizing antibodies are of extreme importance. Just because I describe myself as a cellular immunologist doesn't mean I don't recognize the importance of antibodies. I think that the AIDS vaccine field, and the HIV field generally, has become too compartmentalized—you're either an antibody person, or you're a T cell person, or sometimes you're just a CTL person—and we're not thinking in a broad enough fashion. The immune response is a concerted, beautifully designed series of interdependent events, and you shouldn't study one part of this response divorced from another because the interplay between them is obvious to anybody who learns about immunology.

However, having said that, I think central to all this is the CD4<sup>+</sup> T cell, which, by its very name, the helper T cell, is central to the successful immune response to HIV. That the loss of function of HIV-specific CD4<sup>+</sup> T cells happens from such an early stage of infection sets the scene for what is to come. That's what we've got to induce, to de-energize or reconstitute in the HIV-infected individual. 



***The immune response is a concerted, beautifully designed series of interdependent events... the interplay between them is obvious to anybody who learns about immunology***



# Research at the extremes

*Presentations at Keystone 2005 focused on both very early and prolonged infection to try to discern what an effective vaccine might need to do*

by Philip Cohen

This year's Keystone joint symposia on "HIV Vaccines: Current Challenges and Future Prospects" and "HIV Pathogenesis" were held in Banff amid the soaring Canadian Rockies. The seven-day symposia are perhaps the highlight of the HIV conference calendar, a forum for the world's top researchers to air new data and put forward new hypotheses, as well as get together to share ideas and spark collaborations.

Much of the talk at the social events this year centered on the recently announced funding streams and organizational mechanisms—the National Institutes of Health's Center for HIV/AIDS Vaccine Immunology (CHAVI) initiative generated plenty of discussion and opinion, as did the Bill and Melinda Gates Foundation's call for new research proposals. A more somber topic of discussion was the passing of the remarkable vaccinologist Maurice Hilleman, and a number of scientists took time in their presentations to pay him personal tribute (see Obituary, page 13).

The presentations at the Keystone symposia covered much of the diversity of topics within HIV research. A recurrent theme of many talks, though, took HIV or SIV infection at either extreme as their model system in which to look for immunological clues towards an effective vaccine—immediate early infection or established infection in humans and monkeys able to control their virus.

## **Early events**

The course of HIV infection and its progression to AIDS may take decades, but the early events in infection have always been of interest to researchers since the goal of any preventive vaccine is to stop the incoming virus from establishing a firm foothold. A quartet of recent publications has highlighted just how narrow this early window of opportunity might be, and they set the stage for much of the Keystone meeting. The critical battle between HIV and the immune system is now envisioned as taking place within the first few weeks of infection, during which virus irreversibly destroys the vast majority of CD4<sup>+</sup> memory T cells in mucosal tissues, in particular in the gut-associated lymphoid tissue (GALT) (see Research Briefs, page 30).

One key question is the nature of the virus that establishes early infection. Eric Hunter of Emory University in Atlanta focused on the virus that is transmitted in a cohort of heterosexual couples initially discordant in their HIV infection status. Previous work by his team involving 8 transmission pairs (*Science* **303**, 2019, 2004) suggested

that sexual transmission of HIV is essentially a severe bottleneck where new infection is established by a small population of founding viruses with particular characteristics. They also found that the resulting population of sexually-transmitted virus had shorter V1-V4 regions in their envelope, fewer glycans and were unusually sensitive to neutralization by antibodies from the chronically-infected partner. Hunter and his colleagues propose that these properties reflect different selective pressures on the virus during the long course of an infection and during transmission. During chronic infection the virus adapts its envelope to evade neutralizing antibodies. In contrast, during transmission they speculate the virus is forced to adopt a more compact structure, in effect throwing off its protective shield against antibodies.


It was possible, however, that the bottleneck was not driven by transmission but by the recipient's early antibody response. To address this issue Hunter described five new transmission cases in which the researchers sampled virus in the first few weeks of infection, before antibodies were detectable. Consistent with his hypothesis these early viruses were still subject to a severe population bottleneck.

In collaboration with Bette Korber's team at Los Alamos National Laboratory in New Mexico, the Emory group has analysed more closely clade C viral features that correlate with neutralization sensitivity, using their database of donor and recipient viruses. Again, the researchers found evidence for a correlation between shorter V1-V4 regions of gp120 and better neutralization. But in this new analysis amino acids in a region adjacent to the V3 loop, rather than in V3 itself, appeared to influence neutral-


ization sensitivity, suggesting a novel mechanism for antibody escape in clade C viruses.

Ashley Haase of the University of Minnesota Medical School, Minneapolis discussed data from his study on a rhesus macaque model characterizing the first critical days of SIV infection, which was published shortly after the meeting (*Nature* **434**, 1148, 2005). Haase's group and another team have independently reported on the massive depletion of CD4<sup>+</sup> memory T cells in the first few weeks of infection (see Research Briefs, page 30).

Haase noted that while the virus was making short work of CD4<sup>+</sup> memory T cells in the GALT, the response of the immune system to this onslaught was "too little and too late." In gut tissues that were



***The critical battle between HIV and the immune system is now envisioned as taking place within the first few weeks***



awash with viral particles at 10 days, the researchers never detected significant numbers of CD8<sup>+</sup> T cells. A robust CD8<sup>+</sup> T cell response in vaginal mucosa only came up after the virus had done its damage, raising the possibility that prophylactic immunity induced by a vaccine could beat the virus. “Immediately following infection, the virus is vulnerable,” says Haase. “You have a small founder population of infected cells fighting to keep the infection going.”

Two other talks focused on the potential importance of cell-cell contacts in spreading HIV. Dendritic cells (DCs) are among the first cells the virus encounters in mucosal tissue. DCs normally act as antigen presenting cells, processing viral proteins and presenting them to T cells to fire up an immune response. But DCs can also enhance HIV infection in two ways: they can host HIV replication and transfer viruses from their surface directly to T cells without replication.

Yvette van Kooyk of the Vrije University Medical Center, Amsterdam discussed the complex interplay of HIV with receptors on the DC surface that govern the path the virus takes inside the cell. HIV envelope glycoprotein gp120 binds the C-type lectin receptor (CLR) DC-SIGN leading to rapid internalization of the whole viral particle and its subsequent transmission to CD4<sup>+</sup> T cells. So the DC-SIGN pathway is possibly one way in which HIV manages to avoid being processed to antigen or inducing DC maturation and inflammatory responses. But van Kooyk's team showed that when DC-SIGN was blocked with antibodies, presentation of gp120 on MHC class II and activation of gp120-specific CD4<sup>+</sup> T cells was reduced. This suggests that after HIV engages DC-SIGN, the virus may simultaneously proceed on two intracellular routes that work together to boost infection: antigen presentation to enhance proliferative responses of CD4<sup>+</sup> T cells and transmission of whole virus to these cells.

Clare Jolly, a postdoctoral researcher in Quentin Sattentau's laboratory at the University of Oxford, described her work on the virological synapse (VS), a tight union between the membrane of an infected and uninfected T cell. It's been proposed that HIV uses the VS to rapidly colonize closely-packed T cell populations, as is the case in lymph nodes, but recently-transmitted virus may also encounter clusters of T cells in the genital tract, especially in the inflammatory context of a secondary sexually-transmitted


disease. Jolly used confocal and electron microscopy to visualize the synapses and fluorescent antibodies to probe their protein content in primary CD4<sup>+</sup> T cells. Her data support a model where proteins are actively recruited to the VS since inhibitors of cytoskeleton or microtubule function stopped the accumulation of viral and cellular proteins there. Jolly found that cholesterol-rich lipid rafts—which HIV favors as a budding site—were required for VS formation and were clustered preferentially at the synapse. Jolly pointed out that the tight VS may shield emerging viruses from antibodies or recognition by immune cells, making these structures of interest to vaccine designers. “The question is whether neutralizing antibodies gain access to the synapse and can they block cell to cell transmission?” she says. Her team is now characterizing the ability of antibodies, peptides and small molecules to block HIV budding through the VS.

#### **Long-term lessons**

Even twenty years into studying HIV, researchers continue to learn about the complex interaction between the virus, its host, and its environment. By exploring those interactions researchers are gaining insights into why some rare people are able to control infection indefinitely, what factors determine and predict the immune response to particular viruses, and how antiretrovirals (ARVs) shape viral evolution and host immunity.

One longstanding area of interest are long term nonprogressors (LTNPs), patients who have never received ARV therapy yet maintain normal CD4<sup>+</sup> T cell counts and nearly undetectable levels of virus for years. “We have this excellent example in humans of immunologic control of HIV and it's incumbent upon us to develop a deeper understanding of it,” says Mark Connors of the National Institute of Allergy and Infectious Diseases.

Connors' team has found that in many ways the HIV-specific CD8<sup>+</sup> T cell response of their LTNP and progressor cohorts are indistinguishable—similar quantitatively and in clonality, surface protein markers, and microarray expression patterns—and there was no correlation between mutations allowing the virus to evade T cell responses and the ability of the immune system to control viral replication. But they did find one striking difference in CD8<sup>+</sup> T cells taken from LTNPs: these cells were much more likely to proliferate after restimulation with HIV-infect-



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**Mark Connors**



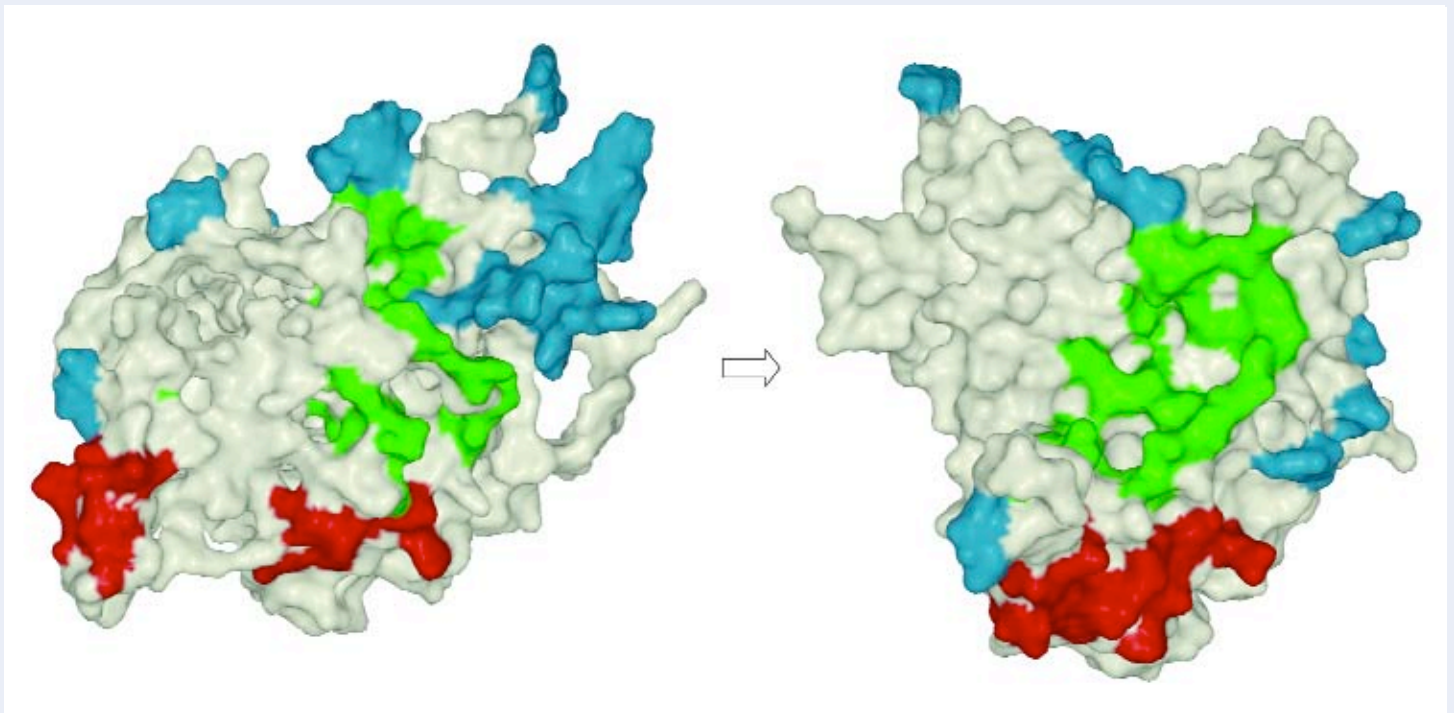


Figure 1.

## HIV up close

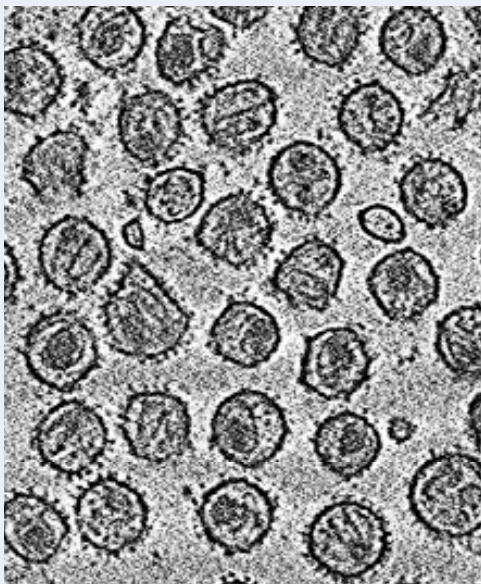


Figure 2.

**R**esearchers have recently determined some important structures in HIV biology as they try to better understand virion formation, virus-host interactions, and gain clues about possible mechanisms of virus neutralization to aid in the rational design of potent immunogens. Some of these were the centerpieces of presentations at the joint Keystone symposia.

One of the most important of these recent structures is the native structure of part of the HIV Env protein, gp120, before it attaches to its host cell receptor, CD4. Researchers have been after the unliganded (not receptor-bound) gp120 for almost 20 years because this form of the molecule is present on cell-free virions, the stage of HIV's replication cycle where it may be most vulnerable as an accessible target for neutralizing antibody. But some of the properties of gp120 that make it so elusive to the human immune system have also presented significant technical hurdles to X-ray crystallography.

Bing Chen, Steve Harrison and colleagues at Harvard Medical School and the National Institute of Medical Research, UK have recently managed to partly solve the conundrum by determining the structure of fully-glycosylated monomeric gp120 cores from SIV (*Nature* **433**, 834, 2005).

Comparison of the already known CD4-bound (liganded) HIV gp120 with the unliganded SIV gp120 core structures shows that the molecule undergoes very large structural rearrangements on binding its receptor—about half of the protein refolds resulting in one of the largest changes ever observed in any protein. Molecular surface representations of the two structures (Figure 1) depicts the amino acid residues that are in direct contact with CD4 (green), human monoclonal antibody 17b (red), or carbohydrate (blue), and the difference in the distribution of these residues before (left) and after (right) CD4 binding gives some idea of the dramatic rearrangements that gp120 undergoes as it attaches to human cells. This extensive refolding enables the viral and cellular membranes to fuse but also appears to conceal or disrupt

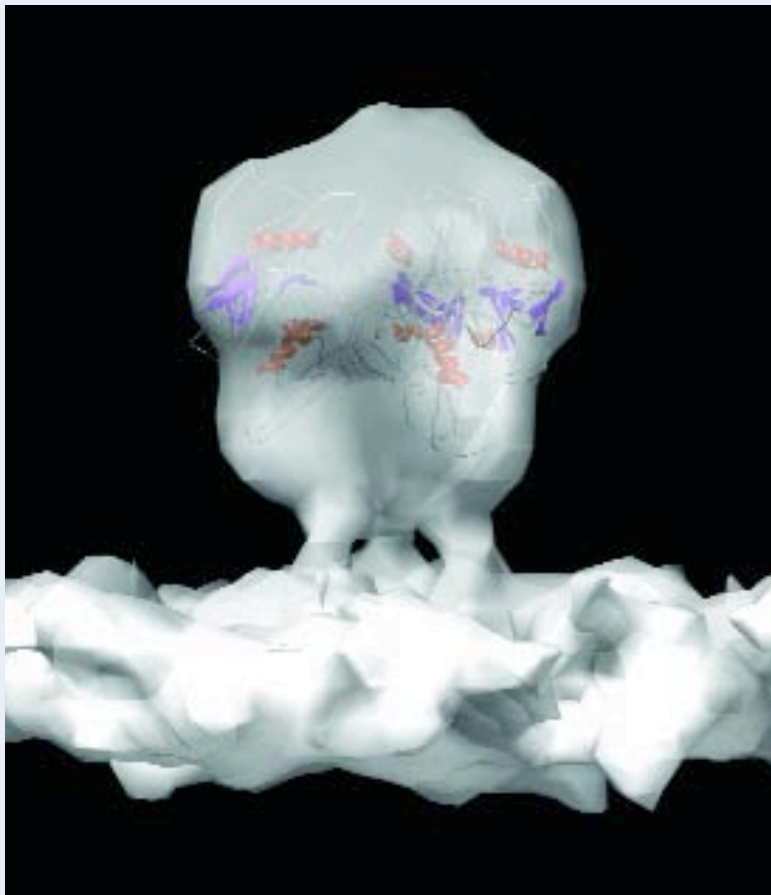


Figure 3.

potentially antigenic sites. The elucidation of this structure is an important step forward and provides researchers a platform from which to now go on to solve the unliganded trimeric HIV gp120 to give further insight into immunogen design.

Ping Zhu and colleagues at Florida State University and the US National Cancer Institute demonstrated a couple of years ago (*Proc. Natl Acad. Sci.* **100**, 15812, 2003) using negative stain electron tomography that wild-type HIV and SIV particles have an average of only 8-10 Env trimers on their surface. Working with a SIVmac239 mutant that has a truncated Env transmembrane domain and therefore an increased Env content (70-79 Env trimers per particle), they found no obvious geometric distribution pattern or orientation of these molecules.

But the negative stain preparation could be structurally distorting the particles so they have now used non-distortive stain-free cryo-electron microscopy techniques to analyze virions. Images of particles (Figure 2) show the gp120 trimer spikes a round the surface of the particles and the distinctive conical core structure within. Three-dimensional average mapping of these spikes (Figure 3) shows the trimeric gp120 structure protruding upwards from the viral membrane and three ribbon diagram versions of Chen's SIV gp120 cores superimposed. The team have found that the Env trimers on the mutant SIV particles are distributed uniformly and hints that they are regularly spaced, sug-

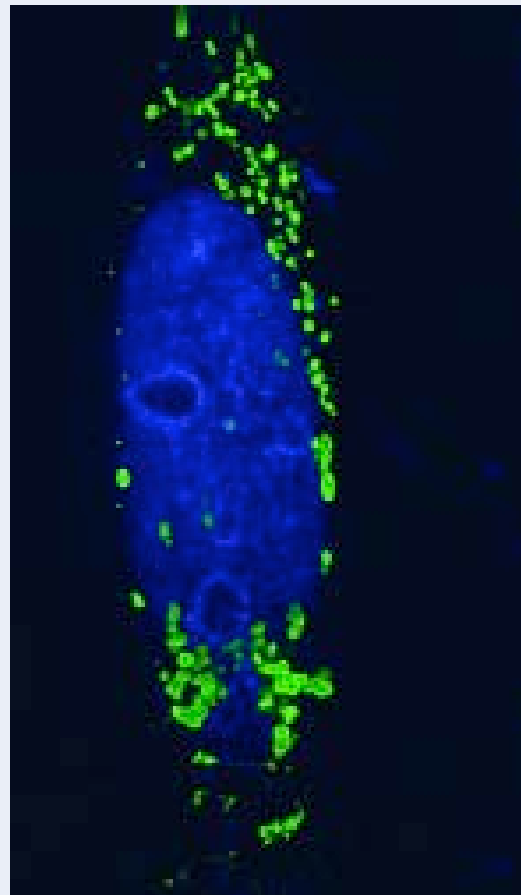



Figure 4.

gesting sequestration by the underlying HIV matrix protein. They are currently looking at the distribution of the Env spikes on wild-type HIV and SIV particles.

Tom Hope and colleagues from the University of Illinois at Chicago have captured movies of cytoplasmic bodies in living cells using high tech microscopy and genetically engineered versions of the antiviral protein TRIM5 $\alpha$  (see *Getting acquainted with the host factors*, page 23) containing green fluorescent protein (GFP) (Figure 4). One theory about cytoplasmic bodies is that they don't represent an active form of the protein but inactive aggregates of insoluble molecules at a dead-end. But Hope's films demonstrated the hollow, roughly spherical bodies are dynamic structures which race around the cell, with small bodies joining to make large ones. He then fused TRIM5 $\alpha$  to a different form of GFP that can be activated by laser light. When the researchers used a laser to paint a single cytoplasmic body fluorescent, the signal was quickly distributed to cytoplasmic bodies throughout the cell, showing protein molecules in the bodies are rapidly exchanged. One suggested function of the bodies is to engulf incoming viruses. Hope showed preliminary data of a viral particle overlapping with a cytoplasmic body. While intriguing, he points out it is still unclear whether this observed association would block viral replication. "This just sets us up for the real work of figuring out what this factor does," he says. 

ed cells or HIV-epitopes *in vitro*. This proliferation was accompanied by the production of perforin and granzyme B, indicating cytolytic function. In contrast restimulation of CD8<sup>+</sup> T cells from progressors triggered the production of cytokines and degranulation but did not induce proliferation.

What this means for the function of these cells *in vivo* is still unclear, but Connors discussed three possible explanations for this proliferation defect in CD8<sup>+</sup> T cells from progressors: either their cells have already been pushed to the point of replicative senescence, they are being restricted in their replication (anergy), or their developmental program has halted at an immature state. If the cells are senescent Connors thinks there are few options for therapy to rejuvenate them. However if the cells are anergic or immature then therapies may be devised to jump start them. "We're going to be looking at this in much greater detail and testing the likely actors, including factors involved with intracellular signaling and protein translocation," says Connors. His team has already tried treating CD8<sup>+</sup> T cells from progressors with IL-2, a cytokine that is able to stimulate some types of anergic cells, so far without success.

While many aspects of how an immune response shapes an HIV infection remain a mystery, Bruce Walker of Harvard Medical School in Boston pointed out that some examples of virus-host interactions are highly predictable and could possibly be exploited for vaccine design.

In a study of 375 Zulus infected with HIV clade C, Walker's team found a strong association between the HLA MHC type and IFN $\gamma$  ELISPOT assay readout. "Our technicians got to the point where they could even predict a person's HLA based on the IFN $\gamma$  readouts they were seeing," said Walker. The researchers found that 111 of 410 overlapping peptides, representing epitopes from a reference strain, showed strong allele-specific HLA associations. In addition, his team has reported that HLA-B alleles have a powerful influence on viral load (*Nature* **432**, 769, 2005), providing strong evidence that the cellular immune response does indeed influence virus replication in humans.


Walker also presented a remarkable example of how host and viral genetics determine the course of infection, involving a rare incidence of identical male twins infected by HIV through intravenous drug use. Sequence analysis demonstrated that both twins were

infected by the same virus, suggesting they were probably infected on the same day. Walker and his colleagues found that the subsequent course of infection appeared to proceed in parallel in the two brothers. The time course of their viral load and CD4<sup>+</sup> T cell counts were almost indistinguishable and antibody sera from one twin were able to neutralize virus from the other. A very similar pattern of response to different HIV epitopes was also found in IFN $\gamma$  assays of both twins. In addition, the same viral escape mutants from the initial immunodominant epitope arose around 29 months after infection in both men, a clear indication that the virus and host genetic interplay rather than stochastic events determine the course of HIV infection.

ARV therapy can also play a role in shaping the course of infection. In the best case scenario, drugs beat down viral replication to undetectable levels in some HIV-infected people, halting disease progression indefinitely. But in an intriguing talk, Steven Deeks of the University of California, San Francisco, argued that even in cases where drug therapy "fails"—that is, drug-resistant virus emerges—there still may be therapeutic benefit.

Deeks and his colleagues have defined an HIV-infected group they refer to as partial controllers on ARV therapy (PCATs), which comprise 35% of people who fail drug therapy. These individuals show a persistent 10-fold or greater reduction of viral load from their pretreatment viremia (consistently <10,000 vRNA copies/ml) despite the emergence of highly drug-resistant virus.

This suggested that the selection of some types of HIV drug resistance may result in the evolution of less fit viruses which are more likely to achieve a balance with the immune system. Indeed, the researchers found that the immune response of PCATs in many ways resembles that of LTNPs. CD4<sup>+</sup> T cells in PCATs and LTNPs produced high levels of both IL-2 and IFN $\gamma$  in response to Gag, while this was not true for patients whose viral load was reduced below detection by ARVs or in patients where ARVs had no lasting benefit. Deeks' team also showed that non-specific activation of CD8<sup>+</sup> memory T cells was significantly lower and similar in PCATs and LTNPs compared to progressors. In LTNPs and PCATs, Deeks concluded, the host generates a strong, multifunctional HIV-specific CD4<sup>+</sup> memory T cell response in the context of relatively low inflammatory response.



**Our technicians got to the point where they could even predict a person's HLA based on the IFN $\gamma$  readouts**

**Bruce Walker**



# Getting acquainted with the host factors

A series of talks at Keystone focused on host intracellular factors that HIV engages in order to facilitate its replication or that the virus must overcome to avoid destruction. Either way, the hope is that therapies which target these factors may some day offer novel ways to attack the virus.

All viruses must subvert human proteins and cellular machinery to their own ends, but for HIV the list of these proteins continues to grow. Mario Stevenson of the University of Massachusetts Medical School tracked down two more HIV collaborators while investigating the role of the nuclear envelope in infection. HIV's ability to infect some non-dividing cells has been attributed to its ability to transverse the nuclear envelope. In contrast, murine leukemia virus (MLV) can only infect dividing cells that go through mitosis, during which the nuclear envelope dissolves. But, unexpectedly, when Stevenson's group froze cells in mitosis using drugs like taxol, they discovered that MLV could integrate DNA into these cells but HIV could not, suggesting that HIV is actually dependent on nuclear factors to replicate.

Using RNA interference, Stevenson's team knocked down expression of some 20 known nuclear envelope-associated proteins and discovered two that were necessary for the viral integration: BAF and Emerin. Emerin is a protein inserted into the inner portion of the nuclear membrane, while BAF is its DNA-binding partner. Together the pair serve as a scaffold for chromosomal DNA. When either factor was knocked down, HIV DNA did not integrate but instead accumulated as a circular molecule of one or two copies of its genome—a dead end for the virus. Stevenson proposed that HIV uses BAF and Emerin to navigate quickly to chromosomes. “The virus is much smaller than the cell and needs sign posts,” he says. The virus may end up circularized because chromosome repair enzymes mistake the linear virus for a broken chro-


mosome and “heal” it by joining the ends.

While HIV benefits from its association with BAF/Emerin, it has a different and complex relationship with Murr1. Two years ago, Gary Nabel of the Vaccine Research Center at the NIH and his colleagues found that Murr1—a protein formerly known for its influence on copper metabolism—contributes to the restriction of HIV replication in resting T cells (*Nature* **426**, 853, 2003). Part of this effect was attributed to Murr1's ability to inhibit the activity of NFκB, a transcription factor that HIV requires to amplify its replication. Murr1 influences NFκB indirectly: it stops E3 ubiquitin ligase from tagging a protein called IκB for destruction by the proteasome. IκB is a natural inhibitor of NFκB, so the increase in IκB concentration drives down the transcription factor's activity and cripples the replication of HIV.

Since Murr1 has little homology to other known proteins, Nabel's team first assumed its relationship to HIV was probably unique. But at Keystone he reported that searching databases for human proteins with a similar predicted structure revealed that Murr1 is probably just one of 10 genes in a new family (dubbed COMMD for Copper Metabolism Murr1 Domain), which seem to pair off to produce dimers with different biological properties. So while the Murr1 (now called COMMD1) homodimers can inhibit HIV replication, COMMD6 disrupts this inhibition by forming an inactive heterodimer with COMMD1.

The function of all these proteins is still unclear but based on preliminary data Nabel proposed that these proteins might be more generally “gatekeepers to the proteasome,” guiding proteins to or away from this disposal pathway. “HIV-1 may need to find its way around these elements to survive its journey through the cell,” he says. One intriguing connection is that HIV inactivates the human protein APOBEC3G by misdirecting it to the proteasome. APOBEC3G is an antiviral pro-

tein that is currently a hot topic of HIV research (see *Guardian of the genome*, page 1). Nabel's team has shown that COMMD1 can bind APOBEC3G, but appears not to play a role in APOBEC3G's degradation. Now Nabel's team is collaborating with Michael Malim's laboratory at King's College London, where APOBEC3G was discovered, to see if any of the other COMMD members play a role in this pathway.

Greg Towers of University College London revealed unexpected connections between two other known antiviral proteins, the murine Fv1 and TRIM5α. Fv1 was first described in the late 1970s and restricts the replication of MLV. TRIM5α was recently uncovered as a human protein that blocks MLV in human cells but also appears to be a major contributor to species-specific barriers to retroviral replication—including that of HIV—in many primates. TRIM5α and Fv1 are unrelated by sequence and seem to differ in their ability to restrict virus in a fundamental way: TRIM5α, as a rule, erects a replication block prior to reverse transcription while Fv1 blocks after DNA synthesis. However the same amino acid residue in viral capsid, 110, controls sensitivity to both factors. Now Towers' team has demonstrated some mechanistic links between the two factors. When Fv1 and human TRIM5α are coexpressed in the same cell there is less viral suppression before reverse transcription than when TRIM5α is expressed alone. This would be the case, says Towers, if Fv1 competes with TRIM5α for the virus. “They may both act at the same early point of the lifecycle, but end up killing the virus in different ways,” he says. In addition Towers' team has found that the TRIM5α protein from squirrel monkey actually blocks retroviral replication after DNA synthesis, showing the exact point at which TRIM5α acts may not be as fixed as originally thought. 

Deeks then presented preliminary data on drug resistance to the fusion inhibitor T20, which suppresses viral load to undetectable levels in many patients, even those with virus resistant to many other ARVs. His team found that when T20 drug resistance emerges it does so very quickly, in as little as 2 weeks, and viral load rebounds back toward baseline. Even so, T20 treatment still resulted in a higher CD4<sup>+</sup> T cell count that persisted for more than 2 months and data suggested this improvement was driven by a fitness defect in the virus caused by the drug resistance, an idea that may have implications in vaccine design (see Perspective, page 1). Deeks concluded that the study of the interplay between drug-resistant virus and the immune system holds important clues for clinicians, immunologists and vaccinologists. “Attenuating the virus with drugs may be the closest thing we have to an attenuated vaccine for humans,” he says.

### Monkey business

Primate SIV models are also being used to study immunological control of viral replication or to reveal aspects of HIV/SIV pathology. David Watkins of the University of Wisconsin began his talk presenting what may be the Indian rhesus macaque equivalents of LTNP.

When these animals are challenged with the highly pathogenic SIVmac239, the infection typically settles to a baseline viral load of over 1x10<sup>6</sup> vRNA copies/ml and the animals progress to and die from AIDS. But Watkins' team has found some rare spontaneous “controllers” that maintain viral load below 50,000 vRNA copies/ml and “elite controllers” below 1,000 vRNA copies/ml. These controller populations often express the Mamu-B\*17 MHC class I molecule and tend to mount broad, low frequency SIV-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses, but show no sign of neutralizing antibodies in conventional PBMC neutralization assays.

While these controllers may be valuable for elucidating immune correlates of protection, Watkins noted it argued for excluding Mamu-B\*17<sup>+</sup> animals from vaccine studies. He also presented encouraging data from such a study of Mamu A\*01<sup>+</sup> macaques using a DNA prime, adenovirus serotype 5 (Ad5) boost vaccine regimen with both vectors expressing Gag, Tat, Rev and Nef immunogens, but no Env component. After repeated low-dose SIVmac239 challenge at

6 months, the regimen suppressed peak viremia more than 10-fold, suppressed chronic viral load set point by 1.5 logs, and was able to slow the loss of memory T cell subsets that are now thought to play a critical role in establishing the course of HIV disease.

Watkins noted the need to test the vaccine regimen in genetic backgrounds other than Mamu-A\*01, and while his DNA/Ad5 vaccination protocol proved effective, he noted that the experiment did not reflect a realistic course of HIV infection since the vaccine and challenge viruses were exactly matched and the challenge came just 6 month after vaccination. His team plans to address these issues in future experiments.

Jeffrey Lifson of SAIC Frederick, Inc. at the US National Cancer Institute presented preliminary data on correlates of viral control in Indian rhesus macaques, characterizing virus-specific responses of circulating immune cells using polychromatic flow cytometry with intracellular cytokine staining. The animals had controlled replication of pathogenic SIV isolates (SIVmac239 or SIVsmE660) either spontaneously or after short term ARV treatment during primary infection, or after various vaccination regimens.

The results suggest that more than one pattern of immune response may be associated with effective virus control. One macaque that had controlled infection with SIVmac239 for 5 years with minimal viral load (~ 100 vRNA copies/ml) demonstrated robust, broad CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses to SIV antigens. At the other end of the spectrum was an animal that was “vaccinated” by transient early ARV treatment during the first weeks of infection with SIVsmE660. Except for a few occasional blips, this monkey held viral load to below 100 vRNA copies/ml despite high dose intravenous rechallenges with the virulent viruses SIVsmE660 and SIVmac239. Yet this animal's T cell responses in peripheral blood were barely detectable. “This animal is a poster child for the argument that in focusing on higher frequency immune responses measured in peripheral blood, we are almost certainly missing an important part of the story,” says Lifson. He plans to follow up the work by looking at qualitative features of responding cells and antiviral responses in lymphoid tissues.

Ronald Veazey of Tulane University in New Orleans used data from a number of

primate species to argue that pathogenesis of HIV/SIV infection is a function of target cell availability, turnover and destruction of CD4<sup>+</sup> T cells in the gut—what he called a unifying hypothesis. His team has been building on their work suggesting that the infection and subsequent direct viral destruction of gut CD4<sup>+</sup> T cells is a crucial part of SIV pathogenesis. This observation has proven to be also true in humans and has recently been independently supported by other research groups (see Research Briefs, page 30). His new data show that the sudden loss of these cells can't be accounted for by three other mechanisms: CD4<sup>+</sup> T cell redistribution, cytotoxic T lymphocyte (CTL) killing, or lowered CD4<sup>+</sup> T cell production.

Veazey's team looked for redistribution of CD4<sup>+</sup> T cells during SIV infection in rhesus macaques and found no evidence for migration of these cells to other compartments, including blood, spleen, liver or bone marrow. “If they are redistributing, it must be to hair or toenails because we've looked at every other tissue,” he joked. To address whether CD8<sup>+</sup> cells could be targeting infected CD4<sup>+</sup> T cells, the researchers treated animals with anti-CD8 antibodies before infection. CD4<sup>+</sup> T cell destruction was even greater in the CD8<sup>+</sup> cell-depleted animals. Finally, they measured CD4<sup>+</sup> T cell replication by pulsing recently infected animals with BrdU label for 24 hours. They found steady production of CD4<sup>+</sup> T cells, especially in the bone marrow.

He then talked about sooty mangabeys and African green monkeys, natural hosts of SIV that maintain high plasma viral loads but don't develop disease. His work suggests these animals can withstand infection because their gut CD4<sup>+</sup> memory T cell population lacks the CCR5 coreceptor for the virus and, as a result, aren't targets for SIV and aren't destroyed.

Veazey ended his talk with a cautionary tale for vaccine researchers based on a vaccine study using a SHIVsf162p3 vector. His team found this virus, which Chinese macaques easily clear in about 90 days, appeared to give robust protection against the otherwise deadly SIVmac251. Two out of 5 animals had a 100-fold viral load reduction over an unvaccinated control animal, and the remaining trio had no detectable plasma viremia. Nonetheless, when Veazey examined the gut-associated CD4<sup>+</sup> T cells in these 3 “protected” animals, he found the



cells were completely destroyed. “This argues we need to really monitor mucosal immune response in vaccine studies or we might be missing something very important,” he says.

### **Clinical update**

Barney Graham of the National Institutes of Health Vaccine Research Center (VRC) began his presentation with an analogy to the setting of the meeting, saying that we were stuck in the foothills and “we still have to get over the mountain in AIDS vaccine research.” He gave an interim report on three ongoing multi-center safety, immunogenicity and dose-escalation clinical trials of DNA and Ad5 AIDS vaccine candidates, preliminary trials with an eye to future prime/boost trial regimens.

Trial VRC 004 is testing a 4 plasmid DNA vaccination protocol (subtype A, B, C *env* genes plus a subtype B *gag/pol/nef* fusion construct) administered three times at four-week intervals to 40 volunteers at 2, 4, and 8mg doses. Encouragingly almost all vaccinees had CD4<sup>+</sup> T cell responses against Env (and 25% against Gag) detectable by intracellular cytokine staining (ICS) that persisted for a year. After 6 weeks (between the second and third vaccine dose) these were of broad phenotype—either IL-2<sup>+</sup> only, IFN $\gamma$ <sup>+</sup> only, or IL-2<sup>+</sup>/IFN $\gamma$ <sup>+</sup>. CD8<sup>+</sup> T cell responses were detectable by ICS against Env in 40% and Gag in 25% of subjects who received the two higher doses of DNA vaccine, the majority of these cells being IFN $\gamma$ <sup>+</sup> only, with some IL-2<sup>+</sup> only and very few expressing both. Graham said that these cellular responses evolved over time so that a large proportion of cells switched to the IL-2<sup>+</sup> only phenotype by the one year mark. At 6 weeks, 40% and 80% of vaccinees given the 4 and 8mg doses respectively also showed Env-specific antibody detected by immunoprecipitation Western blot.

He then presented data from trial VRC 007 involving 15 volunteers each given 4mg of six DNA plasmids—subtype A, B, C *env* genes and subtype B *gag, pol,* and *nef* genes, each on separate plasmids—three times at four-week intervals. At 6 weeks, CD4<sup>+</sup> T cell responses were again seen in all volunteers against Env, but there were also more vaccinees with responses against Gag than was evident with the *gag/pol/nef* fusion construct used in trial VRC 004. About half the vaccinees had CD8<sup>+</sup> T cell responses against Env

and some also against Gag and Nef. Eight of the volunteers also had detectable Env-specific antibodies.

Trial VRC 006 used a cocktail of four Ad5 recombinants (expressing either subtype A, B, or C Env or a Gag/Pol fusion) to vaccinate 10 volunteers each three times with either 10<sup>9</sup>, 10<sup>10</sup>, or 10<sup>11</sup> particles at four-week intervals. At the highest dose about half the volunteers had mild systemic symptoms (fevers, chills). CD4<sup>+</sup> T cell responses to all of the immunogen proteins were seen in nearly all vaccinees and the majority also had CD8<sup>+</sup> T cell responses, some even in the face of pre-existing anti-Ad5 antibody responses at titers as high as 1:8000. Antibody responses were dose-dependent, with nearly all vaccinees given the 10<sup>11</sup> dose showing a response but only 20-30% of vaccinees given the lower doses.

“The most exciting thing is that the data from this combination of trials has led to a set of protocols that will be submitted to the FDA for approval for use in Phase II DNA prime/adenovirus boost trials” says Graham. These trials will be an “exciting collaboration” between the HIV Vaccine Trials Network (HVTN), the US Military Research Program and IAVI at sites in eastern and southern Africa, Haiti, Puerto Rico, Jamaica, Brazil and the US. A priority trial that is already recruiting is the six plasmid prime combined with the four Ad5 recombinant boost.


John Shiver of Merck Research Laboratories gave an update on various aspects of their AIDS vaccine program. Merck are finding the ELISPOT assay more sensitive than ICS—they can detect more than twice as many CD8<sup>+</sup> T cell responders with ELISPOT, in contrast to researchers at the VRC. Shiver described a study designed to see if higher doses of Ad5-Gag (from subtype B) can overcome the blunting effects of pre-existing anti-vector antibody immunity on induced CD8<sup>+</sup> T cell responses. Eight weeks after vaccination with Ad5-Gag at one of four doses (10<sup>8</sup>, 10<sup>9</sup>, 10<sup>10</sup>, or 10<sup>11</sup> particles) the expected lowered frequency of responders was evident when anti-Ad5 antibody titers had exceeded 1:200 before vaccination. The highest 10<sup>11</sup> dose was not able to improve either the frequency or magnitude of response.

He then described a trial with a trivalent Ad5 recombinant expressing Gag, Pol, and Nef (all subtype B) administered to volun-

teers at the same four doses. After 8 weeks there was a dose-dependent frequency of CD8<sup>+</sup> T cell responders—for instance, 24%, 38%, 76%, and 68% of vaccinees given 10<sup>8</sup>, 10<sup>9</sup>, 10<sup>10</sup>, or 10<sup>11</sup> respectively had responses against more than one protein. However there was little difference between the 10<sup>10</sup> and 10<sup>11</sup> doses, suggesting that the highest dose may not be worth pursuing given the mild systemic symptoms seen in trial VRC 006 at this dose.

Merck is also looking to see how effective their candidates might be against other subtypes of HIV. CD8<sup>+</sup> T cells from vaccinees given the subtype B trivalent Ad5-Gag/Pol/Nef were tested for responses against subtype B, A, and C derived peptides. Gag-specific responses were not drastically lower—61% responded to subtype B Gag peptides, whereas 43% and 45% responded to subtype A and C peptides respectively. But Nef-specific responses were very different—47% to subtype B but only 19% and 7% to subtypes A and C respectively.

Shiver also described a clinical trial of Ad5-Gag prime, ALVAC canarypox vector (expressing an almost identical Gag) boost, a vaccine modality that has previously been shown to induce potentially synergistic immune responses in monkeys. In humans, however, there appeared to be no such effect and the frequency of responders (and magnitude of response) remained the same.

Merck, in collaboration with the HVTN, is currently conducting a proof of concept clinical trial with their trivalent Ad5-Gag/Pol/Nef candidate to assess the efficacy of the vaccine-elicited immune response. 

*Simon Noble contributed to this report.*

# HIV in the aftermath

*Breaking the HIV/AIDS and disaster connection*

By Sheri Fink

In the province of Aceh, Indonesia, in the weeks following last December's tsunami disaster, children began showing up at medical clinics hot with fever and covered with the characteristic red rash that spells measles. The cramped tent camps where the children had taken shelter were a catalyst for the spread of the disease, which takes an alarmingly deadly toll in displaced populations. Our team of health workers ventured out daily with national health authorities to prick arms with vaccination needles and pop vitamin A capsules—which can lessen measles complications—into tiny mouths.

As we traveled from camp to camp, the reproductive health nurse on our team found time, even in the midst of the outbreak, to identify midwives, learn about the situation of women, and distribute kits filled with obstetrical supplies and materials.

She had been hired for this job. While once emergency relief was synonymous with providing food, shelter, clean water, and basic medical services, now supporting good reproductive health has unquestionably joined these as a top priority. Part of the reason is HIV/AIDS. Relief experts now recognize that devastation—such as that caused by the tsunami—can heighten AIDS risk factors, making HIV prevention efforts a vital part of an emergency response.

"Many of the conditions that facilitate the spread of HIV are worsened in a post-disaster context," says Yannick Guegan, who works with the humanitarian affairs department of UNAIDS, the Joint United Nations Programme on HIV/AIDS.

Guegan points to the mass displacement of people, social instability, worsening poverty due to income loss, and the influx of new populations, including reconstruction and relief workers, soldiers and transporters, as factors associated in the past with the transmission of HIV/AIDS. "The experience from other emergency situations, like in South Africa some years ago or in East Timor, has demonstrated an increased vulnerability in emergency situations, and that can change the incidence of sexually-transmitted disease, including HIV/AIDS."

But survivors have many competing needs in the aftermath of disasters, so only simple methods of promoting HIV prevention are feasible. In the mid-1990s, aid agencies developed the Minimum Initial Service Package (MISP), a set of actions to counter HIV risk and sexual violence and attend to other reproductive health requirements in the midst of acute emergencies.

MISP addresses HIV prevention in two key ways: making condoms freely available and ensuring that medical equipment and blood for transfusion are free from infectious agents. "Anything more comprehensive than that wouldn't really be appropriate in the first few weeks," says Sandy Krause, who directs the reproductive health project

of the Women's Commission for Refugee Women and Children.

Krause and a colleague set out for Asia soon after the tsunami hit, visiting emergency responders in Aceh to talk about MISP and assess its implementation. The results were disappointing. Workers providing assistance on the ground often hadn't heard of MISP and were not implementing its precepts.

Over the past several years MISP has received a stamp of approval from many of the "key players" in disaster and emergency response—from the United Nations refugee agency UNHCR to the disaster relief standards organization SPHERE. These top-level backers are urging relief workers to integrate simple approaches to HIV/AIDS prevention into emergency disaster assistance activities.

They are also calling for a transition to more comprehensive HIV/AIDS prevention and education activities in the post-emergency response, taking the opportunity to help communities develop programs that will be sustainable over the long term. "The approach of the UN in the post-tsunami period is trying to develop a 'recovery plus' plan, making things better than they were before," says Guegan. "We see it as an opportunity to accelerate the response to HIV."

## ***Understanding the risk after the tsunami***

The tsunami struck low HIV-prevalence countries such as Sri Lanka and Indonesia, where UNAIDS estimates less than 1:1000 adults aged 15-49 is infected with HIV. The disaster also hit countries such as India and Thailand where adult prevalence runs higher (0.4 - 1.3% in India and about 1.8% in Thailand), and Somalia where HIV prevalence is not

known. Most worryingly, India's tsunami-stricken state of Tamil Nadu has the highest HIV infection rates in the country; 2003 data suggested that 83.8% of injection drug users and 8.8% of female commercial sex workers were HIV infected.

The fear is that these rates could escalate if post-tsunami hardships and stress push survivors into drug use and other high-risk activities. Untold numbers of families have lost boats, businesses and other sources of income. Guegan fears that resulting widespread destitution could pressure women into commercial sex work.

"In big towns like Chennai there are a lot of refugees who have been coming there after the tsunami, living in harsh conditions which may put them at risk of unsafe sexual behavior," he says.

Sex workers will not lack potential customers. Thousands of outsiders have poured into tsunami-devastated areas to lend a hand in the reconstruction efforts, among them soldiers from around the world. Although there is no direct evidence linking military person-



***Many of the conditions that facilitate the spread of HIV are worsened in a post-disaster context***



***Yannick Guegan***

nel to increased HIV transmission in disaster relief settings, in some countries HIV prevalence among military personnel is 2-5 times that of respective civilian populations. The presence of soldiers could logically contribute to the spread of HIV/AIDS, and history has highlighted that risk during wartime; in 1991 researchers found a link between the geographic pattern of AIDS and the placement of the Ugandan National Liberation Army during six years of civil war.

Tsunami-related HIV risks extend beyond drug use and sex work. Displaced persons' camps and barracks, home to hundreds of thousands of tsunami survivors, pose their own threats. In hard-hit Aceh in February and early March, Krause and her team conducted interviews and focus groups. Acehese women reported being uncomfortable living in camps with strangers and "going off to latrines that women and men share," she says. "If there is an incident, are all the NGOs prepared to provide care for rape survivors? Basically we found none were prepared."

Her team heard rumors of rapes. When Krause's colleagues sought out one rape survivor they were told that her family had left the area. "The community was saying she wanted it, she wanted the sex," says Krause. "She was isolated socially and then the family moved away."

Similar stigma extends to HIV/AIDS. "I don't think I've been anywhere where people knew less about HIV. And the stigma was so high, even people who knew [about HIV/AIDS] didn't want to be seen as knowing."

The tsunami survivors' lack of HIV/AIDS awareness and their unwillingness to even discuss the subject underscores an urgent need for HIV prevention activities. Two months after the tsunami, Krause's team found that in some areas condoms were not easily available; rather than being distributed, they sat behind the counter at health clinics. Young men reported that they had to pretend to be married in order to obtain condoms. "It's unethical not to make condoms available," she says.

Krause found it difficult to convince aid workers they could take simple steps like making condoms available without having to establish an extensive reproductive health program. "That's what we can't get people to comprehend. They try to set up more comprehensive services; people don't think you can do something without doing something grand."

Reproductive health training programs, for

example, can wait. The first line of defense, condoms, should be made readily available, even if they cannot be actively distributed in the immediate aftermath of a disaster. "In a new emergency, there's not time for that," says Krause.

### ***Ideas into reality: A slow metamorphosis***

A relatively small portion of the disaster assistance pie is devoted to HIV prevention. As part of a major United Nations funding appeal for tsunami relief, the United Nations Population Fund in January requested US\$6 million to reduce HIV transmission, implement MISP, prevent sexual violence, and ensure safe births. This represented a mere 0.6% of nearly \$1 billion requested by fourteen United Nations and non-governmental organizations. UNFPA's request was the only mention of HIV prevention in the entire 95-page appeal.

Still, \$6 million buys quite a few condoms. Henia Dakkak has been working for UNFPA in Aceh and her group has brought ample supplies of male and female condoms, obstetric equipment, and other reproductive health materials into tsunami-affected nations. UNFPA provides these as pre-packaged kits tailored to the needs of small clinics and larger hospitals. Most recently, post-exposure antiretroviral (ARV) prophylaxis for rape survivors were added to the kits.

Places like Banda Aceh have a particular need for the supplies because the tsunami devastated the city's existing family planning network. "They lost their offices, they lost their warehouses, they lost all their supplies," says Dakkak.

UNFPA has even distributed condoms to militaries, including those that came to Aceh to provide medical assistance. "They were thinking we have trauma, we have emergency, so let us bring the emergency things that were needed," she says. "So when it came to the basics, like having condoms, they were not available."

Some UNFPA kits provide materials for HIV and hepatitis screening of blood to help avoid a tragedy like the one that resulted from Sierra Leone's decade-long civil war, which ended in 2002. "During the war, a lot of people got infected through blood transfusions. People wanted to give blood immediately and there was no way of screening it."

### ***Taking the long view***


Assistance for tsunami survivors will be required for years to come and Guegan says




***When it came to the basics, like having condoms, they were not available***



***Henia Dakkak***



**While disasters like the tsunami may heighten HIV risk factors, they do not have to increase HIV transmission**



that HIV prevention and control programs will become even more important with the passing of time. “There are more risks in the [long-term] post-tsunami period than the [immediate] crisis itself,” he says, such as sexual violence and trafficking in the camps, and the long-term presence of transporters and workers in disaster-affected areas.

With the emergency phase of the tsunami disaster over, Dakkak says UNFPA is now harnessing all forms of media to spread information on preventing HIV infection, including billboards. “People need to protect themselves, people need to understand the risks,” she says. UNFPA has even turned to religious leaders in Aceh to inform the population about prevention methods ranging from abstinence to condoms, pointing out that the use of condoms is not contradictory to Islam. “We are using the mosques and the imams to talk about this,” says Dakkak. “They are open to talking about this and making sure that the community is protected.”


The influx of funding and aid workers also provides a chance to counter the risks by strengthening national AIDS programs in the tsunami-affected areas. Already UNAIDS has called on the Indian government to expand its ARV-treatment program to regions hit by the tsunami that weren’t previously covered. UNAIDS officials have been visiting donors and relief agencies to promote the idea that HIV/AIDS programs be “mainstreamed” into a range of regular assistance activities, to avoid stigmatizing those who access the services and to improve each step of the aid response—from considering women’s safety when designing camps to considering the special needs of people living with HIV/AIDS when designing food distribution programs. This idea was endorsed by the humanitarian coordinating body, the Interagency Standing Committee, which in 2004 laid out guidelines for a cross-cutting, multi-sector approach to HIV/AIDS in all phases of emergency response and recovery.

If the efforts go well, the tsunami response promises to improve HIV/AIDS programs in future disasters, conflicts and other emergencies. In the past, those involved in relief work have not given sufficient consideration to HIV/AIDS and, conversely, those involved in AIDS prevention and treatment have not adequately considered refugees; few programs have involved refugees in HIV surveillance, voluntary counseling and testing, prevention of mother-to-child transmission, and ARV treatment programs. As of 2004, the Global

Fund to Fight AIDS, Tuberculosis and Malaria had funded work in 23 refugee-hosting, sub-Saharan African states, and analysis by Paul Spiegel and Alia Nankoe of UNHCR revealed that only one fifth of these programs included reference to refugees.

There is also a need for research to better understand the relationships between societal disruptions and the spread of HIV. Anecdotal evidence suggests that some conflict zones, such as war-ravaged eastern Democratic Republic of Congo, are associated with an increased HIV prevalence, whilst refugee populations in Kenya, Rwanda and Tanzania, and war-affected populations in southern Sudan, Sierra Leone and Angola appear to have lower HIV rates than surrounding populations. Decreased mobility, isolation, and, arguably, better standards of healthcare and education provided in refugee camps may lie behind these differences. The results of research into HIV and its relationship with war and displacement could help guide policymakers and healthcare providers seeking ways to keep infection rates low when wars end and refugees return home.

Research on HIV prevention has already demonstrated that while disasters like the tsunami may heighten HIV risk factors, they do not have to increase HIV transmission. The knowledge and the tools to prevent HIV infection now exist in all tsunami-affected countries.

Leaving Aceh two months after the tsunami struck, knowing of the tremendous outpouring of generosity from donors around the world, it was disappointing to witness families still withering in broiling tent camps with few latrines and sources of clean drinking water. Some residents were cajoled against their will into cramped wooden barracks with paper-thin walls and rickety, hazardous stairways. Clearly the job of rebuilding real homes and restoring livelihoods remains urgent, but national governments and aid agencies have the resources to accomplish all this and more. Those involved in the recovery effort must seize the opportunity to strengthen public health structures, and ensure that prevention and care programs for HIV are not forgotten. 

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## Anti-HIV neutralizing antibodies delay viremia rebound

What will be most important in an effective AIDS vaccine—robust humoral or cellular immunity? The current consensus among HIV researchers is that most likely both will be required, at least for a vaccine that provides sterilizing immunity.

Passive administration of neutralizing antibodies (NAbs) can protect nonhuman primates from SIV and SHIV infection, at least when they are administered at or soon after the time of exposure to virus. Evidence that the NAb response is important in humans remains largely associative, the strongest evidence being the rapid selection of antibody escape mutants in natural HIV infection.

In a recent paper (*Nat. Med.* **11**, 615, 2005) Trkola and colleagues have tried to better define the potential benefit of NAbs in humans *in vivo* through passive administration of monoclonal NAbs to try to control viremia in HIV-infected patients. Three well-characterized monoclonal NAbs (2G12, 2F5 and 4E10) directed against either gp120 or gp41 were administered to 6 acutely and 8 chronically infected patients who had recently been taken off antiretroviral therapy (scheduled treatment interruption), selected because their autologous viruses were sensitive to those antibodies *in vitro*. Patients received several doses of the 3 NAbs during an 11-week period, the first dose given immediately before ARV suspension to mimic a successful therapeutic vaccination, and then followed immunologically and virologically until week 24.

Two chronically and 4 acutely infected individuals showed a pro-

longed delay (>9 weeks) in viral rebound compared to both their own rebound times during treatment interruptions prior to the current trial and rebound times in a control group of HIV-infected patients who were not treated with NAbs, indicating that, in principle, NAbs can control viremia during HIV infection. Acutely infected patients benefited better from the antibody treatment than patients with chronic infection. Overall, NAb-treated patients took about 8 weeks to rebound to levels above 10 HIV RNA copies/ml, whereas untreated patients rebounded in about 3.75 weeks. In all, 12 of the 14 NAb-treated individuals had rebound virus that was resistant to 2G12, indicating that virus had escaped from this antibody and likely accounted for their viral rebound. There was no detectable resistance to either 4E10 or 2F5, suggesting these two antibodies had negligible effect *in vivo*.

This proof of principle trial demonstrates that NAbs can exert some control on viremia in established HIV infection, despite the fact that the treated and the control groups of patients were neither matched for antibody sensitivity nor randomized, so other factors might have influenced the difference in rebound times between groups. The viremic control was transient and very high doses of NAbs were required, two orders of magnitude above the *in vitro* inhibitory concentration, and there was evidence of antibody escape in the majority of subject's virus, so comparable treatment regimens will not be practical in the near future. However, dosage and perhaps even viral escape concerns might be surmountable in a protective setting in uninfected people, before the virus is able to get a foothold.

## HIV transmission during early-stage infection

Heterosexual vaginal transmission accounts for the majority of HIV infections worldwide, yet knowledge of the biology and epidemiology of transmission has remained inadequate. Previous studies have calculated the frequency of transmission to be about 1 per 1000 coital acts, and transmission risk was thought to be greatest during the early post-seroconversion period and during advanced disease, when blood viral loads are highest. Wawer and colleagues (*J. Infect. Dis.* **191**, 1403, 2005) have now examined the rates of HIV transmission per coital act by stage of infection, shedding new light on this important subject.

The researchers studied a cohort of more than 15,000 people in the Rakai district of Uganda, originally designed to determine if intermittent mass therapy against sexually-transmitted diseases could reduce HIV transmission. Although antiretroviral drugs were not available in Uganda at the time, participants were provided with condoms and voluntary counseling and testing. After the end of the trial they identified 235 discordant couples—where one partner is HIV infected and the other is not—who were monogamous and performed a retrospective transmission investigation based on archival samples and collected data. The monthly frequency of intercourse, as reported by the couples, was used to calculate the number of coital acts through the trial. HIV transmission was corroborated by sequence analysis of donor and recipient viruses.

They found that the overall frequency of HIV transmission in these discordant couples was 1.2 per 1000 coital acts, in good agreement with other studies. However, HIV transmission varied markedly

depending on the stage of disease of the infecting partner. During early infection, in about the first 2.5 months after seroconversion, the frequency of HIV transmission jumped to 8.2 per 1000 coital acts. In the period 6 to 15 months after seroconversion, the rate of transmission was 1.5 per 1000 coital acts. The frequency of transmission observed during established infection was 0.7 per 1000 coital acts, a 12-fold lower risk than during acute infection. Later in infection, within the two years before the infecting partner's death, the frequency increased again to 2.8 per 1000 coital acts. So the rate of HIV transmission follows a U-shaped curve, with the highest values seen in early infection.

Higher viremia has been previously associated with increased rates of HIV transmission per coital act, but this work shows for the first time the variable risk of HIV transmission by stage of infection. Diagnosing this stage of disease requires an understanding of who is at risk, but identifying these individuals allows for counseling to prevent further transmission to others and the potential benefits associated with early therapy. The authors acknowledge the implications for a vaccine that could mitigate the initial infection, when a great number of transmission events take place, and state that an AIDS vaccine that reduces primary viremia may have a greater effect than antiretrovirals in controlling the spread of HIV. They warn, however, that a high early viremia may not be the only factor contributing to the increased risk of transmission seen in early infection. Other factors, such as genital ulcer disease, cannot be disregarded. In fact, they claim that a high rate of genital ulcers was observed in participants during early HIV infection, especially in persons who were HSV-2 seropositive.

## SIV wipes out immune cells in first few weeks of infection

Both HIV and SIV destroy CD4<sup>+</sup> T cells, and the slow and steady decline in the number of these cells correlates with disease progression. But the very early events in acute HIV and SIV infection have been more difficult to study since it is hard to identify people immediately after infection. Loss of CD4<sup>+</sup> T cells in that period had been considered moderate and of short duration but two papers published last year (see Research Briefs, *LAVI Report* September–November 2004) confirmed previous data in SIV-infected monkeys: the acute depletion of activated CCR5<sup>+</sup> CD4<sup>+</sup> memory T cells in the gut mucosa of HIV-infected patients, strongly suggesting that these are HIV-infected cells killed directly by the virus, and debunked once again the idea of early HIV disease being relatively benign.

Two studies of SIV-infected monkeys have now examined more closely the depletion of CD4<sup>+</sup> T cells during acute infection. Both find that SIV immediately wipes out more than half of these cells and provide definitive evidence that they are killed directly by the virus.

In the first study, Roederer and colleagues (*Nature* **434**, 1093, 2005) studied flow cytometry-sorted populations of naïve and memory CD4<sup>+</sup> T cells from various tissues of recently-infected rhesus macaques. They found that the acute depletion of CCR5<sup>+</sup> CD4<sup>+</sup> memory T cells from the gut mucosa can be explained by a severe (30-60%) loss of these cells throughout several body tissues of infected monkeys, first appearing in the blood and lymph nodes and then spreading to the gut. Using a quantitative PCR assay for SIV *gag* DNA sensitive enough to detect single copy molecules in cells, they found that cells had been infected by day 10 post-inoculation and had disappeared four days later. How the CD4<sup>+</sup> memory T cells are killed is not clear but the authors suggest it may be due to direct viral cytopathic effects or CD8<sup>+</sup> T cell killing. They found that naïve, resting CD4<sup>+</sup> T cells are not infected with SIV, and since these cells are the majority of CD4<sup>+</sup> T cells in blood, their numbers mask the massive loss seen in the CD4<sup>+</sup> memory fraction.

In a related paper, Haase and colleagues (*Nature* **434**, 1148, 2005) examined SIV replication in 'resting' CD4<sup>+</sup> memory T cells during acute infection. The consensus opinion has been that resting T cells cannot support SIV (or HIV) replication. CD4<sup>+</sup> memory T cells, their resting phenotype and their infection sta-

tus were identified by combined *in situ* hybridization for the presence of SIV RNA and immunohistochemical staining of cell markers of gut cells. Interestingly, they found that resting CD4<sup>+</sup> memory T cells are capable of supporting virus production. In agreement with the Roederer paper, SIV RNA production peaked at day 10 post-inoculation. In contrast to that study, these authors found that CD4<sup>+</sup> T cells may be depleted by Fas-mediated cell apoptosis.

These papers further evaluate SIV pathogenesis in monkeys and show that the acute phase of infection is not only restricted to gut lymphoid tissue as previously thought. Rather, the two studies support the view that the infection is a systemic and huge infection of CD4<sup>+</sup> memory T cells. Previously studied depletion of CD4<sup>+</sup> cells in the gut reflects the predominance of CD4<sup>+</sup> memory T cells over naïve CD4<sup>+</sup> T cells in this tissue compartment. These findings stress the point that an AIDS vaccine will have to produce an effective immune response able to arrest this early peak in virus replication in the early days of the infection to thwart the enormous destruction of CD4<sup>+</sup> memory T cells that seem to have irreversible consequences.

The significance of this very early massive infection of CD4<sup>+</sup> memory T cells on the chronic phase of the infection and disease progression has not been well understood. Evidence pointing to the importance of early events has been accumulating that supports the initiation of antiretroviral treatment in HIV patients as early as possible, regardless of peripheral blood viral counts and CD4<sup>+</sup> T cell counts. Two papers reinforce the importance of this very early immunological insult. In a study in 205 HIV-infected infants, Moldanado and colleagues (*JAMA* **293**, 2221, 2005) found that initiating very early treatment of infants (by 2 months after birth as opposed to 3-4 months) with one or two antiretrovirals was associated with decreased and delayed progression to AIDS. And Wong and colleagues (*J. Infect. Dis.* **191**, 1410, 2005) have studied the effect of initiating antiretroviral treatment during either primary infection (before or less than 6 months after seroconversion) or chronic infection on cellular reservoirs of HIV, as determined by decay kinetics of HIV DNA and cell-associated infectivity *ex vivo*. The data suggest that early treatment initiation controls HIV cellular reservoirs as cell-associated infectivity was not detectable in most early-treated patients after 1 year of treatment whereas all patients that started treatment during chronic infection still had detectable cell-associated activity after 3-6 years of treatment.

## US reverses restriction on Global Fund grant recipients

The US government recently reversed its decision to force all international recipients of funding from the Global Fund to Fight AIDS, Tuberculosis, and Malaria to publicly condemn commercial sex work. Countries that receive money directly from the US government will still be required to comply with this restriction, which is part of the administration's global AIDS initiative. But Randall Tobias, director of the President's Emergency Plan for AIDS Relief (PEPFAR), rejected extending the plan to the 128 countries that currently receive Global Fund grants.

The announcement from Tobias came just weeks after Brazil made headlines for refusing millions of dollars in grants from the US Agency for International Development (USAID). Brazil rejected the grants because accepting the funding required the country to sign a pledge denouncing commercial sex work. A Brazilian national commission of scientists, cabinet members, and activists made the decision to decline the US grants because the country's AIDS outreach and education programs work closely with marginalized, and often stigmatized, groups like sex workers, injection drug users, and other at-risk groups. The Brazilian commission decided that denouncing commercial sex work, which is legal in the country, would jeopardize this work.

"Many NGOs in Brazil are supporting the Ministry of Health position to refuse money from

USAID. I believe the most important thing is to have a clear understanding about institutional interests, independently of who is the sponsor," says Octavio Valente of Grupo Pela Vidda, a non-governmental organization based in Rio de Janeiro.

Brazil was the first country to refuse grant money based on a restriction imposed by the US. Human Rights Watch recently criticized Uganda, a major beneficiary of PEPFAR grants, for altering its HIV prevention programs to emphasize abstinence due to pressure from the US.

Although grants directly from the US government will still require countries to publicly express disapproval of sex work, more than 33,000 other organizations would have been affected if this policy were broadened to include countries receiving donations from the Global Fund. The US contributed a third of the money currently available through the Global Fund, which has thus far committed US\$3 billion to public service organizations throughout the world. Multilateral organizations like the Global Fund had the right to refuse restrictions from donor countries until the US Justice Department revised its policy last year.

Several grass-roots organizations protested extending the limitation to the Global Fund by arguing that there is no advantage to forcing AIDS service organizations to stigmatize commercial sex workers. Tobias rescinded the requirement as he prepared to travel to Africa to meet with grant recipients.

—KJK

## New arm of HVTN trial opens in Botswana

The HIV Vaccine Trials Network opened another arm of its HVTN 059 trial at a site in Gaborone, Botswana. Trial sponsors in Botswana plan to enroll 24 HIV-uninfected volunteers at low risk for HIV infection into this Phase I trial. All volunteers will receive three injections of the vaccine candidate, AVX101.

The Botswana arm of the trial is being run in cooperation with the Botswana Harvard AIDS Institute Partnership and the Ministry of Health. Several other sites in the US and South Africa are already participating in this trial. The National Institutes of Allergies and Infectious Diseases (NIAID) is conducting the US arm of the study along with AlphaVax, the manufacturer of the vaccine.

The Phase I study in Botswana will primarily evaluate the safety of the vaccine candidate. Immunogenicity will also be monitored as a sec-

ondary outcome of the trial. The vaccine candidate utilizes a replication defective alphavirus replicon vector derived from an attenuated strain of the Venezuelan Equine Encephalitis (VEE) virus, developed by AlphaVax. Removal of the capsid gene and insertion of two mutations into the virus's glycoprotein gene render it replication defective and therefore safe for testing in humans. The vector expresses the *gag* gene from HIV subtype C, which is predominant in sub-Saharan Africa. This trial will evaluate four possible doses of AVX101.

The start of this trial in Botswana was met with optimism. The country has one of the most serious HIV epidemics in the world with an adult prevalence estimated at 37% in 2003. Although Botswana has a nationally-sponsored treatment program for HIV-infected individuals, the uptake has been slow. The US Centers for Disease Control and Prevention estimates that only 10,000 people of the 110,000 in need are receiving treatment.

—KJK

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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.

IAVI ([www.iavi.org](http://www.iavi.org)) is a global not-for-profit organization working to accelerate the development of a vaccine to prevent HIV infection and AIDS. Founded in 1996 and operational in 23 countries, IAVI and its network of collaborators research and develop vaccine candidates. IAVI also works to assure that a vaccine will be accessible to everyone who needs it. IAVI's major financial supporters include the Bill & Melinda Gates Foundation; the Rockefeller, Sloan and Starr foundations; the World Bank; BD (Becton, Dickinson & Co.); the European Union; and the governments of Canada, Denmark, Ireland, the Netherlands, Norway, Sweden, the United Kingdom and the United States.

## San Francisco prepares for AIDS vaccine trial by targeting potential volunteers

On the eve of hosting a large-scale AIDS vaccine trial, the San Francisco Department of Public Health (SFPDH) launched a new educational campaign to address public fears and misconceptions about participation in vaccine trials. The SFPDH created the website *SFisReady.org* to educate San Franciscans on AIDS vaccines and to encourage people to enroll in vaccine trials. This project was conducted in partnership with ISIS Inc, a nonprofit sexual health information agency and was announced on May 18th, World AIDS Vaccine Day.

The website provides concise information on AIDS vaccines, the eligibility requirements for participation in the trial, and the commitment required from potential volunteers. But all volunteers will still undergo a rigorous informed consent process detailing all of the benefits and potential risks of trial participation if they are interested in volunteering.

## Merck presents protection data on rotavirus vaccine

New data on the safety and efficacy of a live reassortant human-bovine rotavirus vaccine developed by Merck and Co., known as ROTATEQ, was recently presented at the annual meeting of the European Society for Paediatric Infectious Diseases in Valencia, Spain, on May 18-20. The company submitted a Biologics License Application for this vaccine to the US Food and Drug Administration (FDA) in April. Rotavirus is the most common cause of severe diarrhea among children and kills, due to severe dehydration, 1 in every 300 infants in developing countries, resulting in the death of more than 500,000 children annually worldwide.

In 1998 the FDA approved for use in children a live reassortant rhesus macaque-human rotavirus vaccine called Rotashield, developed by Wyeth. Subsequently the Advisory Committee on Immunization Practices (ACIP) decided that the vaccine should no longer be recommended for infants in the US because of a few rare cases of bowel obstruction (intussusception) among some infants during the first 1-2 weeks after vaccination. The risk of intussusception following Rotashield immunization was estimated to be 1:10,000 to 1:32,000 vaccinees.

The most recent study with the pentavalent ROTATEQ vaccine candidate (against serotypes G1, G2, G3, G4, and P1A) was tested

One common misconception about vaccine trials is that they put volunteers at risk for HIV infection. "Our goal is simple," said Tom Kennedy, Director of Health Communications at ISIS in a statement. "We want to end the myth once and for all. You can't get HIV from doorknobs and you can't get it from vaccine trials."

San Francisco is one of many US cities participating in a large-scale Phase IIb vaccine trial with the Merck vaccine candidate, an adenovirus serotype 5 (Ad5) vector containing the HIV genes *gag*, *pol*, and *nef*. The trial will enroll 1,500 people in 28 cities worldwide. This trial is a 'proof-of-concept' study and will evaluate the candidate's ability to prevent HIV infection or slow disease progression in those who become HIV infected. A Phase III trial involving several thousand volunteers will be necessary if this study generates promising results and more ambitious public education campaigns may be required at this stage. For more information on this vaccine candidate or the trial, see the IAVI Database of AIDS Vaccines in Human Trials at <http://www.iavireport.org/trialsdb/>.

—RFL

for the incidence of intussusception in a group of 70,000 healthy 6- to 12-week old vaccinated infants. They found no statistical difference in the incidence of intussusception in vaccinated infants in the post-vaccination period or through three years of follow-up compared with the placebo group.

The vaccine was evaluated for efficacy against rotavirus dehydrating acute gastroenteritis in a double blind, placebo-controlled study conducted from 2001 to 2004 in 5,700 healthy 6- to 12-week old infants in the US (including the Navajo and White Mountain Apache Nations) and Finland. Infants were randomized to receive either 3 doses of the oral vaccine or placebo at 4- to 10-week intervals. Efficacy of the vaccine against rotavirus acute gastroenteritis of any severity caused by all vaccine serotypes was 74%, but the efficacy against severe rotavirus dehydrating acute gastroenteritis was 98%, the type associated with death by rapid dehydration.

Merck's vaccine is not the only rotavirus vaccine that could soon come to market. GlaxoSmithKline (GSK) has an attenuated human rotavirus candidate vaccine called Rotarix that is also in advanced safety trials.

In February the Global Alliance for Vaccines and Immunizations awarded the Program for Appropriate Technology in Health a US\$30 million grant over 3 years to promote the use of rotavirus vaccines once they are licensed.

—RFL

## World AIDS Vaccine Day commemorated

The annual World AIDS Vaccine Day took place on May 18th, eight years after US President Bill Clinton delivered an historic speech calling for new commitment worldwide toward the development of an AIDS vaccine. Clinton said, "Only a truly effective, preventive HIV vaccine can limit and eventually eliminate the threat of AIDS."

This year was marked by several international community events. Five different regions of Kenya sponsored activities including advocacy walks and rallies featuring speeches by many organizations active in the search for an AIDS vaccine. The community activities

emphasized the importance of partnerships between the potential trial volunteers, the scientific and research community, and the political leaders. Many AIDS organizations also used the day to emphasize the urgent need for an effective vaccine. The International AIDS Vaccine Initiative (IAVI) issued a statement detailing some of the challenges and promises of vaccine research. The AIDS Vaccine Advocacy Coalition also released their updated handbook on AIDS vaccines on the eve of World AIDS Vaccine Day. To view the IAVI statement or for more information on the AVAC handbook, visit [www.iavi.org](http://www.iavi.org) or [www.avac.org](http://www.avac.org).

—KJK