



IAVI Report

Contents

1 And the winner is... HIV treatment and prevention

1 Molecular snapshots from CROI 2007

10 An interview with Dennis Burton

14 Advisory panel considers complexities of HIV prevention trials

17 In memoriam: Prof. Job Bwayo

18 In memoriam: Dr. Kenneth Anthony Kalanyi Kebba

19 Vaccine Briefs

And the winner is... HIV treatment and prevention

The red carpet was rolled out at CROI, where talk centered on exciting new treatments and prevention strategies and the need to continue progress on both fronts

By Kristen Jill Kresge

The 79th Annual Academy Awards were the big news in Los Angeles on the evening of Sunday, February 25. Headlines around the world focused on this year's Oscars ceremony—who won, who lost, and what everyone was wearing. American film director Martin Scorsese finally won an Oscar and former US Vice President Al Gore also took home a gold statuette for his documentary film about global warming, *An Inconvenient Truth*. But on that same night a red carpet was also rolled out at another ceremony across town.

This affair, though decidedly less glitzy and glamorous, also grabbed its share of headlines in the following days, including a front-page article in the *New York Times*. The occasion was the 14th annual Conference on Retroviruses and Opportunistic Infections (CROI), and instead of attracting movie stars from Hollywood it drew nearly 4000 HIV researchers and clinicians from around the world.

Although CROI may not attract the same level of celebrity as the Academy Awards, it is still a highlight on the conference calendar of researchers working in the HIV/AIDS field—an opportunity to showcase the latest advancements in prevention and treatment. This year's conferences satisfied all of those interests, ushering in two new classes of antiretrovirals (ARVs) to the treatment armamentarium and focusing on several HIV prevention strategies of the past, present, and future. Prevention of mother-to-child transmission (PMTCT), male circumcision, suppression of herpes simplex-2 virus (HSV-2), and vaccines topped the agenda while many other presentations at the meeting covered the nitty-gritty of HIV transmission and pathogenesis. Researchers shared their gains in the fundamental understanding of HIV biology and possible ways to exploit the dynamics of infection in order to control the virus (see *Molecular snapshots from CROI 2007*, this page). And although the Oscars probably inspired better afterparties, the research presented at CROI could have much more profound and enduring effects.

continued on page 2 

Molecular snapshots from CROI 2007


Highlights in virology and immunology, from HIV variation and endogenous retroviruses to T-cell memory and exhaustion

By Richard Jefferys

Historically, the annual CROI meeting has concentrated heavily on virology, while immunology and vaccines commanded the attention that you might expect for, say, the Oscar for best animated short film. But the first day of this year's CROI offered two parallel morning

sessions on virology and immunology and, surprisingly, it was the latter session that was standing room only. In a similar shift, vaccines commanded a special symposium that lasted the entirety of Tuesday afternoon. The increased balance of the program is perhaps a welcome sign that

the relative success of antiretroviral therapies has sharpened the focus on the complex immunological questions that confront researchers in the disparate fields of AIDS pathogenesis, preventive vaccine research, and immune-based therapy development.

continued on page 6 

'A convenient truth'

The Bernard Fields Lecture on the opening night of CROI was delivered by Edward Berger of the US National Institute of Allergy and Infectious Diseases (NIAID), who told the story of the seminal discovery of the cellular co-receptors on the surface of CD4⁺ T cells that HIV uses during entry into the cell. Early work in this area was motivated by the study of some rare individuals that were immune to HIV infection, and this immunity was eventually attributed to a genetic deletion, known as a $\Delta 32$, in their CCR5 gene. The lack of CD4⁺ T cells expressing CCR5 at the surface is still the only molecular explanation for resistance to HIV infection, despite repeated exposure to the virus.

Subsequent work has illustrated the precise interaction between gp120 and CD4⁺ T cells, which triggers fusion of the virus and therefore subsequent infection. This original discovery held great promise for both prevention and therapy and recently significant advances occurred in both areas.

Just a few weeks ago AIDS vaccine researchers came a step closer to understanding how this interaction between CD4⁺ T cells and a highly conserved region of the viral surface protein gp120 can be disrupted by a broadly neutralizing antibody known as b12 (*Nature* 445, 732, 2007). Dennis Burton of Scripps Research Center in La Jolla, California who participated in this research with Peter Kwong and colleagues at the Vaccine Research Center (VRC) at NIAID said, "This provided us for the first time with very detailed molecular information," which can now be used to design improved immunogens. "That's still an enormous structural challenge," said Burton, "but we have some of the best structural scientists in the world working on this." (See *Interview with Dennis Burton*, page 10.)

Also at CROI, the field of HIV treatment was reenergized by news of the first drugs in two novel classes of ARVs. The first is a small molecule designed to obstruct the CCR5 receptor-binding pocket and effectively block attachment. This CCR5 inhibitor, known as maraviroc, dramatically reduced the level of viral replication in HIV-infected individuals (Abstract 104aLB and 104bLB, www.retroconference.org/2007). The data presented at CROI is now also being considered by the US Food and Drug Administration as the basis for licensure.

Another novel ARV is also a step closer to becoming a reality. For more than a decade researchers have been trying to interfere with HIV's integrase to prevent the virus from inserting its genetic material into chromosomal DNA, but identifying safe and effective compounds has proven an arduous task. Now, based on Phase III trial results presented by US pharmaceutical company Merck, a new drug called raltegravir is highly effective at lowering viral replication, as measured by a significant drop in viral load in individuals given the drug in addition to their highly-active antiretroviral therapy (HAART) regimen (Abstract 105aLB and 105bLB).

News of these novel ARVs caused quite a stir at CROI and many insisted that there hasn't been this much enthusiasm since the first studies showed that HAART was an effective strategy for controlling HIV infection.

And the nominees for HIV prevention are ...

In the prevention sessions the enthusiasm was more tempered. "This has been quite a remarkable year in the study of HIV interventions," said Judy Wasserheit of the University of Washington in Seattle. She mentioned both the highs—the very encouraging results of the circumcision trials—and the lows, including the recent results from the cellulose sulfate trial that show the candidate microbicide may actually enhance risk of HIV acquisition (see *Advisory panel considers complexities of HIV prevention trials*, page 14).

...Circumcision

Ronald Gray of Johns Hopkins University in Baltimore presented results from the US National Institutes of Health-sponsored trial in Rakai, Uganda that enrolled 5000 men who were randomized to be circumcised either immediately or after two years (Abstract 155aLB). This trial was stopped prematurely by the data safety monitoring board in December 2006 because of indications that the intervention could lower the risk of HIV acquisition by greater than 50%, supporting earlier results from another randomized, controlled clinical trial of circumcision in South Africa (see *Cutting HIV transmission, LVI Report* 9, 3, 2005).

At the time the trial was stopped, 44% of the men had completed the full two-year follow-up. Only 22 of the circumcised men acquired HIV during this time, compared to 45 men in the control group—a cumulative HIV incidence rate of 0.7% in circumcised men compared to 1.3% in uncircumcised men over the two-year study.



This has been quite a remarkable year in the study of HIV interventions

Judy Wasserheit

All circumcised men were counseled to avoid sexual contact for the first 30 days following surgery while the wound was still healing and 89% said they followed these instructions. Despite this reported curtailing of sexual activity, researchers observed that HIV incidence actually decreased more among circumcised men during the second year of the trial. Gray hypothesized that this may be due to complete keratinization of the wound, but researchers are unsure how long this process actually takes. There is some concern, based on data shared with the World Health Organization (WHO) after CROI's completion, that if men do engage in sexual activity before the wound heals, they may be more likely to transmit HIV to their female partner(s).

Investigators in the Rakai study also collected data from the trial volunteers on sexual risk behaviors, including number of sexual partners and condom use. This data indicated that behavioral disinhibition was not a dominant influence in this trial.

The investigators also found that circumcised men who reported multiple sexual partners or partners outside of marriage had even lower HIV incidence rates than monogamous circumcised men. Circumcised men who reported having symptoms of genital ulcer diseases—including herpes, syphilis, or chancroid—were also significantly less likely to contract HIV (Abstract 155bLB); researchers observed a 0.6% HIV incidence among circumcised men who reported a genital ulcer disease and 1.8% in those who didn't, compared to 1.1% and 6.3% among uncircumcised men with or without genital ulcer diseases respectively. Overall, circumcision reduced the rate of symptomatic genital ulcer diseases by 47%. Although circumcision is protective irrespective of co-infection with these STIs or number of sexual partners, this data indicates it could have the most profound impact in men who are at the highest risk of HIV infection.

...HSV suppression

The role that other STIs play in HIV transmission has long been speculated—especially for HSV-2, the cause of genital herpes. There are currently several ongoing studies to test whether treating HSV-2 with acyclovir or valacyclovir can reduce the levels, or frequency, of HIV shedding in the genital tract or reduce transmission of the

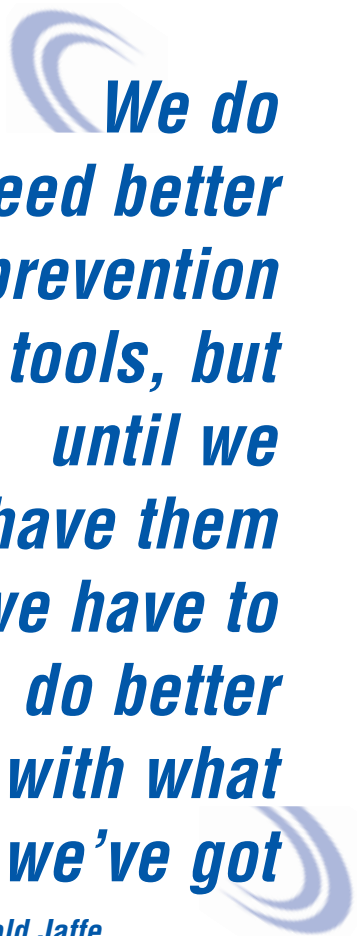
virus by reducing the symptomatic ulcerations (see *HIV prevention in a pill?*, *LAVI Report* 9, 4, 2005).

Two studies presented at CROI looked specifically at the genital shedding of HIV in women given acyclovir to treat their HSV-2 co-infection. The first study of 67 women in Chang Rai, Thailand, conducted by the US Centers for Disease Control and Prevention (CDC), found that women taking acyclovir had a modest reduction in HIV shedding (-0.4 log copies of virus) as determined by cervical vaginal lavage (Abstract 30). Eileen Dunne of the CDC suggested this could predict acyclovir's protective effect in preventing HIV transmission in women co-infected with both viruses, particularly in women experiencing symptomatic HSV-2 infection or in those who are more severely immunocompromised and therefore have higher viral loads.

The second study, a Phase IIb trial involving 299 HIV/HSV-2 co-infected women presented by Sinead Delany of the Reproductive Health and HIV Research Unit in Johannesburg, South Africa, indicated that there was no statistically significant difference in the levels of HIV in the genital tract of women given acyclovir over a four-month period (Abstract 154LB). Acyclovir therapy did however seem to reduce the periodic frequency of HIV shedding in the genital tract. Delany said further study is required to determine whether acyclovir therapy can limit the HSV-2 ulcerations and thereby lower HIV transmission rates. Results from these ongoing trials won't be available until 2008.

...PMTCT

If interventions like HSV suppression or pre-exposure prophylaxis (see *Treatment as prevention*, *LAVI Report* 10, 3, 2006) are found to work, the big challenge will be delivering them. That's where the provision of ARVs for PMTCT can provide a sobering lesson (see *New strides in protecting infants from HIV*, *LAVI Report* 9, 2, 2005). The first trial showing that this simple intervention could protect infants from contracting HIV was completed 13 years ago but currently only 9% of pregnant women globally have access. The number of pediatric AIDS cases in the US reached an all-time low of 58 in 2005, but "each of these cases represents a failure of prevention," said Harold Jaffe from Oxford University in the UK. "We do need better prevention tools," he said, "but until we have them we have to do better with what we've got."



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Harold Jaffe

We really are entering a new era of vaccine development. We have vaccines now that are immunogenic, at least in early phases of development

Scott Hammer

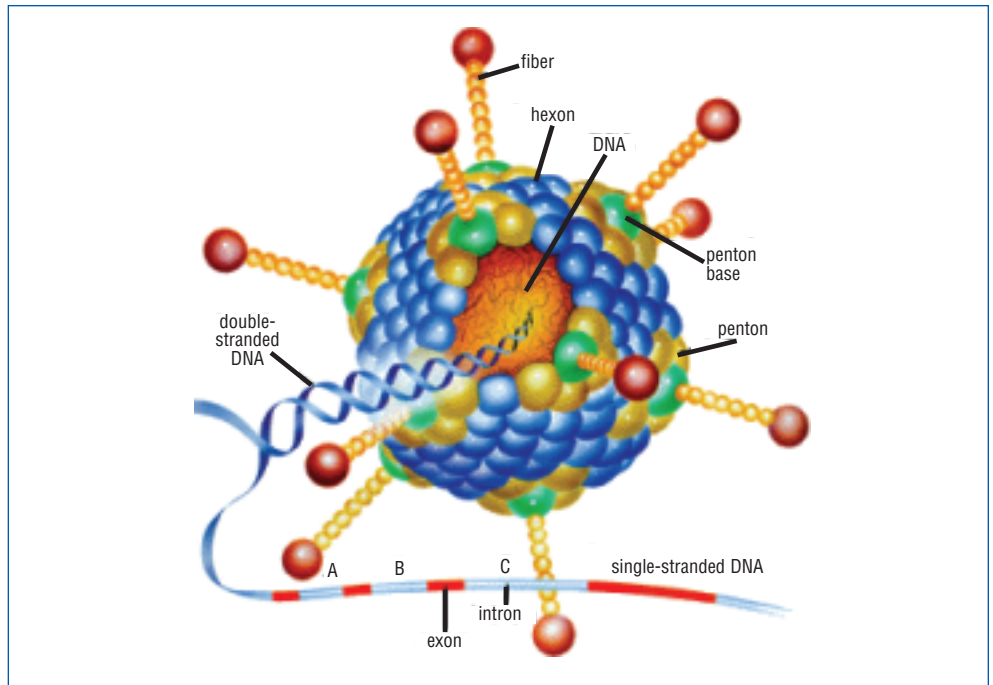


Figure 1. Adenovirus particle. Adenoviruses are non-enveloped, double-stranded DNA viruses with icosahedral symmetry. The viral capsid is composed of 240 hexon proteins (shown in blue) and 12 penton base proteins (shown in green). The fiber spike associated with each penton base allows the virus to attach to human cells using the coxsackie-adenovirus receptor. There are 51 serotypes of human adenoviruses identified so far, three of which are currently in or soon to enter clinical trials as AIDS vaccine vectors (Ad5, Ad35, and Ad26). To circumvent pre-existing immunity to these vectors, Dan Barouch and colleagues have constructed a chimeric Ad5/Ad48 virus where the hexon proteins, the primary target of antibodies, are substituted with the corresponding proteins from Ad48.

Measure twice, cut once

Researchers are now facing this challenge as they begin to consider recommending and implementing safe circumcision programs. WHO officials are currently compiling guidelines on how circumcision should be utilized as an HIV prevention tool. Their primary concern now is safety, not acceptability. Surveys done during the Rakai study showed that 60% of men said they were willing to be circumcised and some of the volunteers randomized to the control arm actually tried to re-enter the trial under false names in the hope of getting circumcised, said Gray. The biggest concern with broad implementation of circumcision is that the procedure must be performed in a sterile setting to avoid the risk of HIV infection. During the Rakai trial, almost 4% of the surgeries led to a modest or severe adverse event, although Gray believes this to be an overestimate. At a meeting in March the WHO discussed surveillance plans for monitoring safety outcomes once circumcision becomes more widespread outside the controlled setting of a clinical trial.

The HIV envelope please

Interest in the somewhat beleaguered research into AIDS vaccine candidates that

induce potent humoral immune responses was boosted by recent structural work by Peter Kwong of the VRC and colleagues and some of this excitement spilled over into CROI. Burton summarized the importance of this study in a plenary talk entitled “Toward broadly neutralizing antibodies against HIV,” but also made it clear that it’s a long way until this work is translated into the design of better vaccine candidates (Abstract 99). “Studying natural infection doesn’t give the answer.” Instead, he said, AIDS vaccine researchers are left to “make up new rules.”

Meanwhile some of the leading cellular immunity-focused vaccine candidates are now, or will soon be, in preliminary efficacy trials. A summary of these candidates—including Merck’s adenovirus serotype 5 (Ad5) vaccine and the DNA and Ad 5 candidates developed at the VRC—was provided by Merlin Robb of US Military HIV Research Program (USMHRP) and was greeted with optimism (Abstract 103). “We really are entering a new era of vaccine development,” said Scott Hammer of Columbia University in New York City. “We have vaccines now that are immunogenic, at least in early phases of development.”

But David Ho of the Aaron Diamond AIDS Research Center in New York City cast some

doubt on this immunogenicity. In his overview presentation on the evidence to support possible clinical efficacy of T-cell based vaccines, he reminded the mixed audience of vaccine researchers and HIV clinicians that there is still no way of knowing if the number of spot-forming cells (SFCs) determined by the ELISPOT assay actually corresponds to T cells that can limit the virus's spread. "Reason tells us that they might, but formally this has not been documented." He was much more careful in his assessment of current research efforts. "Incremental progress is happening in the field, but it is happening at a rather slow pace," he said. "We should've appreciated earlier how difficult the task at hand is."

Even if the leading Ad-5 based candidates are immunogenic their efficacy in humans may be compromised by pre-existing immunity to the viral vector, which has motivated researchers to start looking at other serotypes or novel chimeric adenovirus vectors that can be used in heterologous prime-boost regimens with Ad5 (see Figure 1). Dan Barouch, of Beth Israel Deaconess Medical Center in Boston, and his colleagues determined first the seroprevalence of 51 known serotypes of human adenovirus in different countries in Africa and then the immunogenicity of four of these serotypes (Ad26, 48, 49, and 35) in rhesus macaques (Abstract 98) and found that Ad26 was the most immunogenic. They also constructed a chimeric Ad5 vector with all of the hypervariable regions of the hexon capsid protein—the viral protein to which antibodies are directed—replaced by the same regions of a much lower seroprevalent adenovirus, Ad48. They then tested the immunogenicity of both the Ad5/Ad48 chimera and the Ad26 vectors with HIV inserts in rhesus macaques who had already received two injections of an unadulterated Ad5 vaccine candidate. After boosting, the animals had T-cell responses that ranged from 1000 to 15,000 SFCs per million peripheral blood mononuclear cells (PBMCs), indicating that pre-existing immunity could be overcome with these non-Ad5 vectors.

Barouch also conducted a series of experiments with different heterologous prime-boost regimens, including Ad26/Ad5, Ad48/Ad5, and Ad49/Ad5. Data he presented at CROI showed that the Ad48 primed better than Ad49, but to a lesser extent than Ad26. The Ad26/Ad5 combination also "boosted remarkably well," Barouch said, with responses in the range of 2400 SFCs. This het-

erologous prime-boost was 5-fold more immunogenic than a homologous prime-boost with Ad5 in rhesus macaques.

Both the Ad26 and the Ad5/Ad48 chimeric vaccine candidates encoding clade A HIV *env* genes will go into Phase I clinical trials to assess their safety and immunogenicity in humans. Pending regulatory approval, Barouch expects these trials to start before the end of the year.

Protocols are also in development for other new vaccine trials that will test Merck's Ad5 vaccine candidate and the VRC's DNA/Ad5 candidates in different populations. The VRC is currently planning a Phase IIb test-of-concept trial with this prime-boost regimen, in partnership with IAVI, USMHRP, and the HIV Vaccine Trials Network (HVTN). This trial, known as PAVE 100, may start before the end of the year and the VRC is now also considering testing these candidates in a cohort of adolescent volunteers.

Merck's Ad5 vaccine candidate is already in two Phase IIb test-of-concept trials in the Americas, Caribbean, and Australia, as well as in South Africa (see *Vaccine Briefs*, *IAVI Report* 10, 6, 2006), and now the company is also preparing a protocol to evaluate the candidate in a trial involving infants born to HIV-infected mothers to see if the vaccine could protect babies from HIV infection through breastfeeding. This is particularly important in light of new research at CROI that showed that replacing breastfeeding with formula feeding in developing countries, where women have limited access to clean water, can be equally problematic. For several years researchers have promoted formula feeding as a safer alternative to breastfeeding for infants of HIV-infected women; however, a study in Botswana found that use of infant formula increased a baby's chance of dying from a diarrheal disease by 50 times (Abstract 9).

Early weaning is another alternative strategy researchers tried to control the transmission of HIV through breastfeeding—instead of breastfeeding for more than a year women were asked to stop after four months. A study measuring the efficacy of this approach in Zambia found that early weaning had absolutely no effect on the number of HIV infections or mortalities by age two (Abstract 74LB). A trial with Sanofi Pasteur's canarypox vaccine candidate, vCP1521 is already being tested in infants born to HIV-infected mothers in Uganda. ■



Incremental progress is happening in the field... We should've appreciated earlier how difficult the task at hand is



David Ho

HIV variation

During the CROI symposium on AIDS vaccines, Francine McCutchan from the US Military HIV Research Program (USMHRP) delivered one of her signature talks on global HIV diversity and inter-subtype recombination. McCutchan reviewed some of the ways HIV variation impacts vaccine research, including how it affects choices regarding vaccine antigens, clinical trial site selection, and also evaluation of breakthrough infections in vaccinees to assess whether they resulted from a mismatch between vaccine antigens and the infecting virus.

In terms of the current global pandemic, McCutchan listed the nine known HIV subtypes (A through K, except E which had its subtype status rescinded when it was found to be a recombinant) and reported that there are now 35 circulating recombinant forms (CRFs) in the Los Alamos HIV sequence database, as well as many unique recombinant forms (URFs) that have been reported from single individuals that are a potential reservoir of new CRFs.

Despite this extensive variation, McCutchan estimates that—at least currently—six globally prevalent subtypes (A, B, C, D, CRF01 and CRF02) account for more than 95% of HIV infections worldwide. Vaccine candidates in advanced stages of testing are based on one or more of these subtypes but many trial sites have a complex mix of subtypes and recombinant forms co-circulating. In preparation for vaccine trials in East Africa, McCutchan's team sequenced 120 full-length genomes from Kenya, Uganda, and Tanzania. While the majority of sequences were subtypes A, D, and C respectively, all three subtypes were present in each country and, unexpectedly, a third or more of the sequences represented URFs.

McCutchan explained how such recombinant forms arise: HIV is a diploid virus—each virion contains two RNA templates—and during each replication cycle the viral reverse transcriptase switches back and forth between the templates to generate its proviral DNA. Usually the RNA templates are virtually identical, but in individuals infected dually (or multiply) with different subtypes the RNA of two different viruses can become co-packaged into the same viral particle. Subsequently, when replication occurs, a provirus is generated that is a chimera of the two subtypes; the RNA tem-

plate switching causes the new virus genome to contain alternating sections from each subtype.

McCutchan's logical inference is that circulating virus/vaccine antigen mismatch are more likely to occur in high-risk populations where dual infections and recombination are more common, and most vaccine trials are, by necessity, carried out in these populations. She has assessed the extent to which there is an epidemiological link between high-risk groups, dual infections, and recombinant forms, using a novel assay that can distinguish between these various viral forms using probes specific to the subtypes of interest. The technique was employed to study viruses from 1420 HIV-infected individuals in East Africa and 806 individuals in Thailand whose risk for HIV infection varies from low to high.


McCutchan showed that, as predicted, the proportion of individuals infected either dually or with inter-subtype recombinant forms increased based on their degree of risk; more than half the individuals from an urban high-risk cohort in East Africa were infected dually or with recombinant forms, and in Thailand these forms were more often seen in injection drug users. She also found that, over time, dually-infected individuals typically generate 5-10 different URFs that replicate to high enough levels to become detectable.

To look at how recombinant forms spread among high risk social networks, McCutchan and her colleagues developed a novel technique that analyzes the breakpoints in the recombinant HIV genome where the different subtypes have been joined together, and these breakpoints can potentially track inter-subtype recombinants through different social networks and populations. They conducted a painstaking analysis of breakpoints in 125 complete HIV sequences from Uganda, Kenya, Tanzania, Thailand, Myanmar, and China, and after protracted analysis created a database of each breakpoint. A grand total of 896 breakpoints were eventually identified, 521 in viruses from East Africa and 375 in those from Asia.

Using the data to look at the connections between different risk groups and countries, they found that in Thailand the recombinant viruses among IDUs shared more breakpoints and yet also contained more novel breakpoints compared to those found in lower risk heterosexuals. However, the analysis also



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showed that the heterosexual and IDU networks were interconnected and had been since the beginning of the Thai epidemic. Compared to the East African countries studied, there were fewer connections across borders and the breakpoints also showed that HIV strains found in Myanmar bridged the epidemics in Thailand and China. In East Africa, recombinants with shared breakpoints were also commonly identified in different countries, suggesting long chains of transmission of viruses from specific recombinant lineages.

McCutchan's groundbreaking work promises to add another dimension to the study of the molecular epidemiology of HIV infection. Among her conclusions from these initial studies is that dual infection—the proximal source of recombinant forms—is occurring at a higher rate than had previously been surmised. She also believes that CRFs may represent recombinant viruses that have entered lower-risk social networks and therefore have fewer opportunities to recombine due to the lower incidence of dual infections, a phenomenon evolutionary biologists call reproductive isolation. In terms of vaccine trials, McCutchan suggested the optimal approach will be to test candidates in many different types of social networks with varying degrees of risk, so that efficacy in multiple settings can be evaluated.

Electrifying DNA vaccines


Initial excitement regarding the immunogenicity and efficacy of DNA vaccines in small animal models largely dissipated when results in larger animals and humans proved far less impressive. At CROI, Michael Egan from Wyeth Vaccines explained why DNA vaccines may be about to undergo a renaissance. He first cited recently published data from the NIH's Vaccine Research Center showing that an optimized DNA vaccine consisting of multiple plasmids could induce detectable, albeit low-level and limited, T-cell responses in the majority of recipients in a Phase I trial, a first for any DNA vaccine (*J. Infect. Dis.* **194**, 12, 1650). Egan then outlined the strategies now being pursued to try to build on this important milestone. Wyeth has long been interested in 'molecular adjuvants,' such as cytokine genes that are delivered in tandem with DNA-encoding vaccine antigens, but Egan showed data indicating that T-cell responses to an HIV DNA vaccine construct were only

marginally enhanced by the inclusion of an interleukin (IL-) 12 gene. As a result of this and similar data from other groups, Egan explained that the focus has now shifted to new delivery methods. He listed the variety of ways DNA vaccines can be administered, including particle and jet delivery devices (intramuscularly or intradermally), topical applications, and—the focus of Wyeth Vaccines and the remainder of his talk—electroporation.


Electroporation involves delivering brief electrical pulses to the muscle after the injection of the DNA vaccine using a special 'wand.' The pulses induce transient pore formation in cell membranes, facilitating entry of the DNA and then the production of vaccine-encoded antigens. Electroporation also attracts inflammatory cells, including antigen-presenting cells, to the site of immunization. Egan showed data from an experiment in macaques demonstrating a massive enhancement of DNA vaccine immunogenicity using this approach. Two weeks post-immunization, animals that received a multi-gene HIV DNA vaccine plus IL-12 showed HIV-specific T-cell responses that averaged 816 spot forming cells (SFCs). The same vaccine, delivered with electroporation, led to responses that averaged 8141 SFC. Over longer term follow-up the differences between the two groups of animals increased. At 22 weeks post-immunization, T-cell responses averaged 3394 SFCs in electroporation recipients versus 74 SFCs, only marginally above background, in animals that received injection only. Egan pointed out that this represented a roughly 225-fold increase in DNA vaccine immunogenicity. However, Egan stressed that data from non-human primates has often failed to be duplicated in humans so confirmation of the electroporation buzz awaits results from Wyeth's ongoing Phase I clinical trials.


HERVing into view

Two novel presentations at CROI described interactions between HIV and human endogenous retroviruses (HERVs), which may have intriguing implications for vaccine research. HERVs are essentially fossil remnants of retroviruses that humans encountered many millennia ago. These retroviruses can no longer replicate but are incorporated into our chromosomes and now make up a surprising 8% of the human genome. The envelope protein of one




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Around one-fifth of all the central memory CD4⁺ T cells... were lost during acute SIV infection ... followed by a more gradual attrition and abrogation of proliferative capacity among the remaining central memory population



HERV has been co-opted by humans and plays an indispensable role during pregnancy, illustrating the ancient and ongoing relationship between humans and retroviruses. Certain rare HERV sequences, for example HERV-K, exist as full length proviruses, but fragments known as human endogenous retrotransposable elements (HERE) are more common. Human cells have restriction factors like the recently discovered APOBEC proteins which suppress retroviral activity (see *Guardian of the genome, LAVI Report 9, 2, 2005*) and these factors are believed to be involved in keeping endogenous retroviral sequences dormant. Only a few instances of HERE transcription have so far been described in breast and testicular cancer tissue. However, HIV has mechanisms, such as the viral protein Vif, which disable host restriction factors. This led researcher Brad Jones from the University of Toronto to ask whether HIV infection of CD4⁺ T cells would awaken endogenous retroviral sequences and transcriptionally activate them.

When Jones looked at HIV-infected primary CD4⁺ T-cell lines *in vitro*, this is exactly what he found. These cells accumulated increasing numbers of genomic copies of the HEREs that Jones tested for (AluSX, LINE-1, and segments of HERV-K), but uninfected CD4⁺ T cells did not. To take a preliminary look at whether such events occur *in vivo*, Jones searched databases of HIV sequences and found a primary HIV isolate into which a HERE (LINE-1) had become inserted, strongly suggesting that HIV and LINE-1 can be active in the same cell.

Keith Garrison from UCSF, one of Jones's collaborators, also presented a poster at CROI (Abstract 457). Garrison studied 16 people with HIV and found they had higher levels of HERV-K genetic material in their blood compared to four uninfected controls. Garrison also looked for evidence of T-cell responses to HERV-K derived epitopes and found that they could be detected in individuals with primary HIV infection but not in uninfected controls. This finding held true even for HERV-K epitopes with little similarity—less than three amino acids in common—to HIV epitopes. There was even a weak but statistically significant inverse correlation between HERV-K-specific T-cell responses and HIV viral load.

The authors of these studies note that induction of CD8⁺ T cell responses to HERV epitopes may offer a novel way of selectively

targeting HIV-infected cells, with the advantage that HERV epitopes cannot mutate to escape immune surveillance. Further work will be needed to demonstrate whether this interesting idea can be translated into a safe vaccine strategy.

Memory center

Louis Picker From the Vaccine & Gene Therapy Institute at Oregon & Health Science University delivered a plenary talk on pathogenesis that focused on lessons learned from the SIV/macaque model of HIV infection. Picker is focused on understanding the host factors that distinguish progressive from non-progressive disease using macaques infected with either the highly pathogenic clone SIVmac239 or its attenuated derivative SIVmac239 nef. Picker stressed a basic concept that underpins his work: T cells (both CD4⁺ and CD8⁺) can be grossly subdivided into long-lived resting T cells (either inexperienced naïve T cells or antigen-experienced central memory T cells) and short-lived CCR5-expressing effector memory T cells. Resting T cells primarily circulate through the lymphoid system while effector memory T cells migrate to the tissues. Importantly, central memory T cells represent a reservoir of effector memory T cells because they generate these cells upon activation. Picker has previously shown that loss of effector memory CD4⁺ T cells from tissue (e.g. the bronchioalveolar lavage and lamina propria) is a critical determinant of disease progression in SIV-infected macaques (*J. Exp. Med.* **200**, 1299, 2004). At CROI, he described his research team's efforts to understand how this loss occurs.

Picker homed in on SIV's effects on central memory CD4⁺ T cells as they represented the likely source of the vanishing effector memory cells. Around one-fifth of all the central memory CD4⁺ T cells detectable in lymph nodes were lost during acute SIV infection, and this was followed by a more gradual attrition and abrogation of proliferative capacity among the remaining central memory population. SIV DNA was always detectable in a small proportion of the central memory CD4⁺ T cell pool, even though effector memory CD4⁺ T cells were more extensively infected. In addition to direct infection, Picker also found that immune activation drove the accelerated conversion of central memory CD4⁺ T cells into effector memory cells and he suggested that this may

lead to replicative senescence or other functional disturbances.


Picker concluded by proposing a “two tier” schema of HIV infection wherein there is an early assault on effector memory CD4⁺ T cells, such as those in the gut, that the immune system can withstand due to replenishment from the central memory CD4⁺ T cell pool. The second tier is the less efficient and more covert infection of central memory CD4⁺ T cells that occurs during chronic infection, ultimately leading to disease progression and AIDS. Picker’s argument appears to dovetail neatly with many recent papers and a presentation at CROI by Mario Roederer from the VRC suggesting that vaccine-mediated protection of the central memory CD4⁺ T cell pool is a—perhaps *the*—crucially important correlate of protection against disease in SIV-infected macaques.

Memory cells defend themselves

A presentation that shed some light on factors protecting memory CD4⁺ T cells from HIV infection was given by Joseph Casazza from Rick Koup’s group at the VRC. Casazza compared CD4⁺ T-cell responses specific for the pp65 protein from CMV, from both HIV-infected and uninfected individuals, to those specific for HIV’s Gag protein. He found that Gag-specific CD4⁺ T-cell responses were less poly-functional (and therefore unable to produce multiple cytokines and chemokines) than pp65-specific responses. In particular, far fewer Gag-specific CD4⁺ T cells produced the chemokine MIP-1 β . Notably, however, the functional profile of the pp65-specific CD4⁺ T-cell responses from HIV-infected versus uninfected individuals was similar. To ascertain whether production of MIP-1 β was exerting a protective effect, Casazza sorted pp65-specific CD4⁺ T cells from HIV-infected study participants based on their ability to make the chemokine and then assessed their infection history by looking for the presence of HIV Gag DNA. The results showed that MIP-1 β -producing CD4⁺ T cells consistently had a lower content of Gag DNA than non-producing cells. Casazza suggested that this finding may account for the relative conservation of the CMV-specific CD4⁺ T cell response in people with HIV. He also noted that MIP-1 β production may be a desirable property for vaccine-induced HIV-specific memory CD4⁺ T cells.

Measuring T-cell exhaustion

Early last year a study from Rafi Ahmed’s group at Emory University identified the molecule PD-1 (programmed death 1) as a marker of CD8⁺ T cell exhaustion (*Nature* 439, 682, 2006). Since then a plethora of papers have shown that PD-1 is strongly upregulated on the CD4⁺ and CD8⁺ T cells of HIV-infected individuals, particularly on their HIV-specific T cells (*Nature* 443, 350, 2006; *Nature Med.* 12, 1198, 2006). These papers also reported that PD-1 expression correlates with viral load and inversely with CD4⁺ T cell counts. At CROI, Brent Palmer from the University of Colorado Health Sciences Center reported on the relationship between viral load and PD-1 expression on HIV-specific CD4⁺ T cells. Palmer’s study cohort included 14 untreated individuals and 17 on ART with viral loads less than 20 RNA copies/ml of blood. PD-1 expression was significantly higher on HIV-specific CD4⁺ T cells compared to those specific for CMV and was also elevated in untreated individuals versus those on ART. Statistical analyses revealed a strong correlation between PD-1 expression on HIV-specific CD4⁺ T cells and viral load; however, there was no correlation between viral load and PD-1 expression measured on total CD4⁺ T cells. Looking at HIV-specific CD4⁺ T cells based on cytokine production, Palmer found that cells producing only interferon- γ expressed the highest levels of PD-1 whereas cells producing IL-2 alone showed low levels of PD-1 expression.

In a subsequent talk on immunopathogenesis, Bruce Walker from Partners AIDS Research Center at Massachusetts General Hospital also touched on the role of PD-1 in HIV infection, adding the information that another molecule associated with T-cell dysfunction, CTLA-4, is also upregulated on HIV-specific CD4⁺ T cells in untreated HIV infection. Walker pointed out that engagement of PD-1 and/or CTLA-4 *in vitro* can lead to an apparent restoration of HIV-specific CD4⁺ T cell function, but it remains uncertain whether these mechanisms can safely be exploited for the purposes of immunotherapy. For the AIDS vaccine field, it remains to be seen whether vaccine-induced HIV-specific CD4⁺ T cell responses will be less prone to exhaustion than those that differentiate in infected people in the absence of prior vaccination. 

Richard Jefferys coordinates the Michael Palm Basic Science, Vaccines & Prevention Project at the Treatment Action Group, a New York-based community organization advocating for HIV research.



PD-1 is strongly upregulated on the CD4⁺ and CD8⁺ T cells of HIV-infected individuals, particularly on their HIV-specific T cells





Structure-function in HIV research

Dennis Burton, PhD, is one of the world's leading experts on antibodies. After gaining his PhD from the University of Lund, Sweden, in physical biochemistry, Burton returned to the UK and held faculty positions in biochemistry and molecular biology at the University of Sheffield. He then took a position at The Scripps Research Institute as Professor of Immunology and Molecular Biology, where he has been for the last 16 years.

Burton holds a number of scientific advisory positions, including at the University of California, San Diego, the HIV Vaccine Trials Network, the Foundation for AIDS Research (amfAR), and the Conference on Retroviruses and Opportunistic Infections, and has had a hand in organizing many prestigious scientific conferences, including this year's Keystone Symposium on HIV vaccines. He also sits on a number of grant review committees, including at the National Institutes of Health, amfAR, and the Wellcome Trust in the UK. Since 2001 he has been the Scientific Director of IAVI's Neutralizing Antibody Consortium (NAC).

At the end of the 1980's, Burton was a key member of the team that first demonstrated the expression of antibodies from a combinatorial phage display library, a technique that greatly facilitated the study of antibody. Soon after he turned his attention to human antibody responses against HIV and how they might be manipulated in a vaccine setting. Burton has called his approach retrovaccinology, the idea being that by fine mapping broadly-neutralizing antibodies one can work back from that point to design an immunogen that will effectively elicit such antibodies. His work in nonhuman primates has shown that neutralizing antibody can completely protect from infection with immunodeficiency virus, studies that contribute to the hope that an effective vaccine will one day be developed.

Burton is widely acknowledged by his peers as a front-runner in AIDS vaccine research, but still manages to be well known for his affable nature and for inspiring teamwork among colleagues. The success of the NAC is due in no small part to Burton's personal qualities that enable him to foster a spirit of cooperation and collaboration. He recently spoke with IAVI Report Editor Simon Noble about structures—from HIV's proteins to research consortia—and the functions they bring about.

How successful do you think IAVI's Neutralizing Antibody Consortium has been to date?

As far as I know it was the first research consortium of this type in AIDS vaccine research, and I think it's been amazingly successful, it has driven the field in lots of aspects and has been very, very influential. I think you'd expect it to be, because the individual scientists in the NAC are just outstanding. I've never worked with such an extremely talented group of individuals. Collectively they have defined what is, I think, the only sensible approach to coming up with an antibody component for an AIDS vaccine—a rational, molecular approach. The members of the consortium are right at the frontier of understanding at the molecular level the interaction between neutralizing antibodies and the HIV envelope, and that's where a vaccine is going to be developed.

How do you think the NAC could be improved?

The NAC started out as a group of investigators studying a relatively narrow area covering the structure and function of HIV envelope and antibodies. So it could always be improved in terms of the breadth of talent and, as we progress, then we'll need new expertise in many areas, for example, in B cell biology, in understanding immunogenicity, and in understanding better how to elicit antibody responses generally. So there's always great scope for improving the quality of the whole outfit.

A key organizational feature of the NAC has been 'enabling projects' that accelerate research by pooling certain resources—for example, we have common repositories of antibodies and envelope protein reagents—which is straightforward but has enormous impact because individual members can get hold of

these standard reagents very quickly and easily.

The other organizational aspect that helps greatly is standardization of assays. We've put into place standard neutralization assays and immunization protocols, and run these as much as possible on a consortium-wide basis, freeing up the individual investigators to be innovative. That's really what we want the members to be free to do, to innovate, to take those tedious and labor-intensive tasks out of the research equation.

What about the intellectual openness that the NAC has fostered?

That's been very important in two aspects. One is in the sharing of information, ideas, reagents, and so on, and that has been quite exemplary, and you can clearly see that in the number of publications that contain multiple authors from the NAC. The second aspect has been in terms of intellectual property, where the NAC has pioneered the sharing of intellectual property between multiple laboratories so that discoveries made by investigators will favor them proportionately more, but would also benefit anyone within the consortium. That's a healthy and stimulating environment to do research in. The agreements also give IAVI the opportunity to develop vaccines so they'll be available where they're most needed, in developing countries.

What are your impressions of the other consortia in AIDS vaccine research?

The first organization that I would point out is the Vaccine Research Center (VRC), which I think has been a huge success. I think Gary Nabel's recruiting to the VRC has been inspirational. I usually visit the VRC several times a year and it's just a real pleasure to go because there are so many really, really great people there and the work they're doing is so good. I think that is a great institution for HIV vaccine research and its success certainly underlines what can be done when you bring people together working on a common problem.

The other consortia, the NIH in the form of the CHAVI [Center for HIV/AIDS Vaccine Immunology] and the Gates Foundation, in the form of CAVD [Collaboration for AIDS Vaccine Discovery], I think that undoubtedly they took note of the success of the NAC. Right now it's too early to say how successful they're going to be.

I think the CHAVI, obviously, has some outstanding individuals involved, the likes of Joe Sodroski at Harvard and Andrew McMichael at Oxford, they are clearly world class AIDS researchers. It'll be interesting to see how it goes, they are trying to cover a lot of ground in the CHAVI and of course, given the amount of money involved, there are huge expectations. If you take one research area, primary acute infection, there's no doubt that prominent researchers outside the CHAVI have found it very difficult after the formation of the CHAVI. These guys are far too valuable to the overall research effort to lose.


The CAVD has been formed only recently so it's very difficult to make a judgment. Some components of the CAVD are following a closely parallel track to that of the NAC, which is flattering. I'm very impressed with Tachi Yamada, having met him he seems like a really terrific choice for that job.

The VRC is a real bricks-and-mortar, vaccine-devoted institution, existing under one roof. Do you think that's significant to its success?


I think that the neutralizing antibody problem, for example, could definitely benefit from being focused into one, two, or three centers, rather than as it is now, dispersed over many centers. It could also benefit from a re-thinking about mission-driven research. To take one example: grants. If you look at the RO1 [NIH funding] structure, the classical way to keep your RO1 grants flowing is to move quickly, seize on opportunities, write another grant, something else comes up, seize on that, raise another grant, and so on.


What we're trying to do in the HIV vaccine design field is somewhat different from that—it's more mission-oriented. We don't necessarily want people running to the next opportunity, to some degree you want to keep people on mission and that is not particularly well-suited to the RO1-style of funding.

I'm not criticizing RO1s or peer-reviewed research at all, I want to really emphasize that, I think that the NIH peer-reviewed process has been massively successful and has produced a huge amount of science that's been of great benefit for mankind. So I don't think that should be replaced at all. What I'm talking about here is an alternative, given a specific set of problems—HIV vac-




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cine design. In this instance, one needs different structures.

What's required is not quite the funding that happens in biotechnology either, because this is much riskier than classical biotech. It's something in between classical biotech and classical RO1 investigator-initiated funding. So we need some sort of funding mechanism that recognizes the unique nature of this mission and seeks to deal with it accordingly.

I think an R&D vaccine institute, or institutes, could be very useful for this type of problem. This would also affect the career structures of not just the PIs, but of the individuals actually doing the bench research. Some of this research is not necessarily going to produce the same amount of publications that one typically has to accrue to make an academic career, so one needs to think about alternate career structures that can hold the best researchers into this mission-driven project.

To bring young people in to HIV vaccine research, they'll probably have to be guaranteed a longer than usual tenure to do certain research projects and their accomplishments recognized as being somewhat different from traditional academic research. This can be done, people have found ways to do this, for example, on the Human Genome Project. So it just takes some imagination and the political will to take a crack at it.

Obviously all of this requires a lot of money, and it would also involve bringing in some of the tools of biotechnology and project management and so on. I think that would be helpful.

But the Human Genome Project gave rise to a whole new career ladder in bioinformatics, which didn't really exist before that.

Right, and if HIV vaccine design can be cracked, then I think that will open up the whole field of vaccinology and we'll then have rational vaccine design for a whole slew of vaccines. There is no health measure that's done more for humankind than vaccines, and if we could actually get control of vaccines so that we could rationally design them, rather than be at the mercy of a slightly altered pathogen, then we'd be in control of a whole new area of human health.

You were involved in the very recent structural definition of an HIV neutralizing antibody, b12, binding to the CD4 binding site of gp120 (Nature

445, 732, 2007), a paper that has generated a fair bit of enthusiasm, even in the non-scientific press. How big a step forward do you think this represents?

I think it's a major step. We have known about this site of vulnerability since we discovered the antibody in the early '90s, but this report from Peter Kwong's lab provides the molecular level details of this HIV:antibody interaction. That's key because vaccine design now, I believe, has to be done at the molecular level. This new molecular understanding, hopefully, will transfer into molecular design of vaccine candidates.

So in light of that paper, what are the prospects of now designing an immunogen that will induce similar neutralizing antibodies?

We absolutely should not underestimate the gargantuan size of that task. We have now defined the shape of the HIV surface that the antibody recognizes, the epitope that is part of the CD4 binding site. Now we have to recreate that molecular shape with some high degree of precision in some other protein environment where we can use it as a vaccine candidate. That, in essence, is the approach that people are going to try. This is a great starting point on the molecular scale. It will be tough, there's undoubtedly lots and lots of work to be done and it's really pushing at our understanding of structure and of immune responses, but I think it can be done.

Supposing this immunogen design problem is surmounted, what then would be necessary for an effective AIDS vaccine?

Even if we could elicit antibodies like this one, b12, very effectively, that wouldn't be sufficient for an AIDS vaccine. First, we will need to elicit multiple other broadly-neutralizing antibody specificities. Second, I think that an effective HIV vaccine is going to require two components; a component eliciting multiple broadly-neutralizing antibodies, and another eliciting a robust, rapid, potent cellular immune response. Research into both of those areas needs to be maximized, because I think only when those two components are brought together are we going to have real success.

Will the antibody response need to be mucosal?

I think that's a very, very open question. I'm not sure that HIV really is a mucosal pathogen—it goes in via the mucosae and it

does replicate early on in the gut, but there are pathogens that just do all their damage in the mucosae, and you can show mucosal immunity. I think most important is going to be a systemic response to control the virus. So I think there is still very, very many question marks about the requirement of mucosal immunity. And also it's much more difficult to elicit long-lasting mucosal immunity than systemic immunity.

So you don't agree that there is this really short window of opportunity to snuff out the virus immediately after an infective event?

I'm not even sure about that, because in the experiments we've done, we can protect monkeys with systemic antibody; we can also protect them with mucosal antibody but we needed a very high concentration of mucosal antibody. It may be that virus infects only when the mucosae are damaged, and it's not completely clear to me that any level of mucosal immunity would necessarily protect you if the mucosae are broken, for example. If you look at the initial integration events, they may well be systemic; activated T cells can be wherever. I think there are a lot of question marks, but in the first instance, I'd rather have systemic immunity than anything else.

What do you think is the best we could hope for in the near term of a partially effective vaccine?

Probably the best that we can hope for in the medium term is that we get some indication of control from the T-cell vaccines, that the vaccinated patients do better than those who receive the placebo. We'll find out in the next two years or so how effective they are. I'll be absolutely delighted if T-cell responses alone can provide some clearly measurable benefit, but I'm not convinced that that's what we're going to see.

There's currently an extremely limited repertoire of well-characterized neutralizing antibodies. Do we need more novel neutralizing antibodies, and a better understanding of their original biology in their host?

Absolutely, and there are a lot of efforts going on there, including in the NAC. John


Mascola and Rich Wyatt at the VRC have some very nice data showing that at least one donor has broadly-neutralizing antibodies directed to the CD4 binding site, a bit like b12. I'm sure a lot more will be identified—the NAC is also targeting such donors in Africa—and we'll understand more how they're neutralizing. We'll then have many more monoclonal antibodies that will be useful for molecular vaccine design.


What's the next important HIV structure that the field really needs?

The trimer—that's what everybody wants to see, the native infectious form of the trimer. It's going to be very difficult because it's such an unstable, transient structure, and that's part of the problem with making a vaccine. We have to chemically engineer the proteins to stabilize them and still retain native conformation, and as the technologies of structural genomics and robotics are applied, hopefully sooner or later a crystallizing, stable trimer will appear.


What other areas of HIV research, particularly vaccine research, do you find particularly interesting or promising?

I think the restriction factors are very interesting and are indicative of how, possibly, drugs could be developed. They're almost like natural drugs, in a sense. I also think the whole biochemistry of viral entry is very interesting, and there'll be more drug targets there.

The other area that I find quite interesting, which is vaccine-related, is trying to elicit better immune responses through understanding the interplay of innate and adaptive immunity. Everyone has jumped on TLRs [Toll-like receptors], but I'm in the same institute as Bruce Beutler and even though he is one of the discoverers of TLR function, he also believes there's a lot more to it than TLRs. I think that's going to be a huge developing area. I can foresee a future where cellular immunity is elicited not by viral vectors, as it is now, but by designed immunogens that elicit strong, directed cellular immune responses. That could be terrific for vaccinology, I think. 



I'll be delighted if T-cell responses alone can provide some clearly measurable benefit, but I'm not convinced that that's what we're going to see



Advisory panel considers complexities of HIV prevention trials

The Bill & Melinda Gates Foundation seeks advice on conducting HIV prevention trials from an advisory panel convened by the Institute of Medicine

By Kristen Jill Kresge

HIV prevention trials have been grabbing many headlines in recent months. Some heralded results from trials that showed adult male circumcision was around 60% effective at lowering a man's chance of acquiring HIV. These trials were terminated early because the evidence overwhelmingly showed circumcision's protective effect and the board that monitors the safety and ethics of the trial decided it would be unethical not to also offer the procedure to the men in the placebo arm (see *And the winner is... HIV treatment and prevention*, page 1).

Unfortunately some other HIV prevention trials have been stopped in recent months for other, potentially graver, reasons. In January researchers from a US public health organization called CONRAD—a collaboration between the US Agency for International Development (USAID) and Eastern Virginia Medical School—announced that it was halting its Phase III efficacy trial of a microbicide gel in women volunteers from India, Benin, Uganda, and South Africa because the safety committee monitoring the trials had found a higher number of HIV infections in women receiving the microbicide than in those receiving the placebo. Data from this trial is still being analyzed and for now the reasons for the discrepancy remain unclear, but news of the trial sent ripples through the HIV community.

Other trials of new prevention technologies, including microbicides and pre-exposure prophylaxis (PrEP; see *Treatment as prevention, LAVI Report 10*, 3, 2006), were stopped prematurely in the past for a variety of reasons, including a lower than expected HIV incidence in the community that rendered the trial futile, pressure from community activists over trial design, and the controversy of providing life-long access to antiretrovirals (ARVs) for volunteers who became incidentally infected during the study.


These events have provoked the Bill & Melinda Gates

Foundation, which funds many trials evaluating the efficacy of new prevention technologies, to investigate some of the challenges of conducting this research. Recently they requested that the prestigious Institute of Medicine (IOM), part of the well-respected National Academies in the US, convene an independent committee on methodological challenges in HIV prevention trials. This advisory panel of outside experts held its first public

meeting focusing on microbicide and PrEP trials from February 6-7 in Washington, DC, and the 15-person panel heard from many of the organizations that are involved in designing and conducting these trials. A second meeting will be held on April 19 in London and the final guidance from the committee on the methodology, design, and conduct of HIV prevention trials—with an emphasis on microbicide and PrEP studies in particular—will be issued in a report to be released in the fall of this year.

Renee Ridzon, a program officer at the Gates Foundation who oversees non-vaccine HIV prevention trials, opened the first meeting by addressing the committee and highlighting some of the main areas where they are seeking guidance. These included the impact of prevention trials on HIV incidence, the need to enroll and keep women in trials, and the future role of male circumcision in other prevention trials. Another important consideration for any funding agency is, of course, the price of running these complex tri-

als. Ridzon said that many prevention trials actually cost more than double what was originally calculated. The Gates Foundation is hopeful that the committee's suggestions will increase the likelihood that HIV prevention trials will be successful and enable donors to invest their resources wisely. "Trials to look at different interventions are crucial," said Ridzon.



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“And it’s obvious that one round of trials isn’t going to provide all the answers that are needed.”

Predicting incidence

A common thread discussed for many of the microbicide and PrEP trials was the difficulty in estimating HIV incidence in a population where a trial will be conducted. Accurate incidence estimates are necessary to determine the number of volunteers required to demonstrate efficacy of the intervention. But this has become a growing concern for organizations conducting or funding prevention trials since several trials have recently been stopped or scaled back because of lower-than-anticipated HIV incidence rates.

In the absence of cohort studies to determine incidence, researchers rely on other methods, including using a p24 antigen assay to estimate the number of new infections, the BED assay that estimates HIV incidence based on a measurement of the level of HIV-specific antibodies in a serum sample as a marker of recent infection, or data collected from past studies done in these communities. But according to Salim Abdool Karim, a principal investigator of both microbicide and PrEP trials in South Africa, all of these methods tend to overestimate HIV incidence.

Leigh Peterson of Family Health International (FHI), a public health organization based in North Carolina, reported on a Phase III efficacy trial with the candidate microbicide gel Savvy that was stopped prematurely at sites in Ghana due to lower than expected incidence. The Savvy microbicide trial in Ghana was designed with an HIV incidence estimate of 5% in the placebo group, the actual observed incidence turned out to be just under 2%. Trials with the same product at sites in Nigeria were also closed early, but this time because the data safety monitoring board (DSMB) concluded during an interim analysis that the product was unlikely to be effective.

Another Phase III microbicide trial conducted by CONRAD was also stopped because the actual HIV incidence in the study was 2%, less than half that anticipated. This was a companion trial to the one being run in India, Benin, Uganda, and South Africa where the DSMB found a higher number of infections among women receiving the cellulose sulfate gel than those receiving placebo, but this effect was

not observed in Nigeria. Researchers initially planned to expand the number of women in the trial to compensate for the lower HIV incidence but, partly because of additional complications with working in the war-torn delta region of the country, the trial was closed instead.


Other trial sponsors have also had to choose between drastically increasing the number of volunteers—which substantially increases the logistic complexity and cost of these trials—or scaling planned Phase III trials back to smaller, Phase IIb test-of-concept trials, which are most likely not sufficient to support licensure.

Even if researchers have accurate incidence estimates in a community at the start of a trial, the HIV incidence observed in clinical trial participants is often lower since trial volunteers receive regular risk-reduction counseling and have steady access to other prevention methods, like condoms. But Doug Taylor of FHI warned the IOM committee against concluding that behavior change is responsible for the lower-than-expected incidence in the cellulose sulfate trial. Other factors like the increasing number of HIV-infected people who are on ARV therapy may also be contributing to lower HIV incidence rates, especially in Nigeria and other countries where the US President’s Emergency Plan for AIDS Relief (PEPFAR) is operating. While the proliferation of ARV programs in developing countries is a great success, it may also make it more difficult for organizations to conduct HIV prevention research in these same communities.


Time out

Another confounding factor raised is the difficulty of enrolling and retaining women in HIV prevention trials. Almost unanimously, presenters reported surprisingly high pregnancy rates in these trials. In the cellulose sulfate microbicide trial in Nigeria, 7% of women were found to be pregnant in the trial screening process and so could not participate. During the trial, 30% of the 2160 women became pregnant. This was particularly surprising since the candidate microbicide showed efficacy as a contraceptive in previous trials, and 60% of women claimed they were regularly using condoms.


In a PrEP trial run by FHI in Cameroon, Nigeria, and Ghana the pooled pregnancy rate for all sites was an astounding 56%.




Trials to look at different interventions are crucial. And it’s obvious that one round of trials isn’t going to provide all the answers that are needed



Renee Ridzon



Several trials have recently been stopped or scaled back because of lower-than-anticipated HIV incidence rates



Peterson said such high pregnancy rates are predictable when women are having more than three sexual acts a week, as was the case in this trial. Due to potential safety concerns women have to suspend use of the microbicide while they are pregnant. In the Savvy trial in Ghana, women spent 10% of their overall time in the trial not using the microbicide, making it difficult to interpret the data.

Based on these results many presenters urged the IOM panel to consider recommending that women in these trials be allowed to continue using the microbicide candidate during pregnancy. Sharon Hillier, a professor of obstetrics, gynecology, and reproductive sciences at the University of Pittsburgh, said that the places where microbicides are needed most also have some of the highest fertility rates, and since women are unlikely to suspend use of a microbicide—if it is eventually approved and available—during pregnancy, researchers should keep these women actively involved in the study. She also reminded the committee members of the evidence that pregnant women are at an enhanced risk of contracting HIV.


During all of these HIV prevention trials women are offered free hormonal contraception, but many choose not to use it. The US Centers for Disease Control and Prevention is now making the use of hormonal contraception a requirement for enrollment in a PrEP study they are conducting in Botswana; however even this does not guarantee that women will actually take the contraception during the course of the trial.

Lessons learned

Some of the other Gates-funded trials that garnered widespread attention when they were closed early were the PrEP studies in Cameroon, Nigeria, and Ghana (see *Treatment as Prevention, LAVI Report 10, 3, 2006*), and Peterson shared some of the sobering lessons from these trials with the IOM committee.

Before being shut down in 2006, the PrEP trial in Cameroon was suspended for several months while FHI satisfied study design questions at the behest of the government. During this time researchers continued to follow the women in the study, although they couldn't give them pills (either the ARV tenofovir or placebo). Despite the fact that women were receiving absolutely no intervention, researchers still retained 80% of the women through this seven-month period. Peterson said this was one of the most troubling aspects of all since it demonstrated the intense motivation these women had to participate in the trial.

Eventually questions over this trial became a “big scandal” in Cameroon, said Peterson. Cartoons in the local newspaper inaccurately depicted FHI staff members purposely injecting volunteers with HIV. Several of the IOM panelists peppered Peterson with questions on the activities FHI engaged in to prepare the community in advance of the trial. She said FHI thought there was a sufficient level of transparency about the design of the study, but obviously learned some valuable lessons. In Nigeria, Peterson reported that the trial was stopped because of inaccuracies in the laboratory data from this site, and not because of community or government pressure.

If all this makes it sound difficult to conduct HIV prevention research, it is only likely to get harder. Now that three studies have confirmed the protective effect of male circumcision, organizations sponsoring and funding trials are facing the possibility that this procedure will have to be offered to volunteers in other HIV prevention studies, making them even more complicated and expensive. This will be one of the topics the IOM advisory panel will discuss at the April meeting. Sten Vermund, director of the Institute for Global Health at Vanderbilt University, told the committee that researchers need to start thinking about testing prevention interventions in combination, much like drugs are used to treat HIV infection. 

Professor Job Bwayo

The International AIDS Vaccine Initiative (IAVI) mourns the tragic death of our friend and colleague, Professor Job Joab Bwayo, co-founder and director of the Kenya AIDS Vaccine Initiative (KAVI) in Nairobi, Kenya. Prof. Bwayo, a passionate AIDS advocate, dedicated much of his distinguished career to developing an HIV vaccine for communities throughout the African continent. Killed in a senseless act of violence on Sunday evening, February 4, he was unwavering in his belief that Kenya had a significant contribution to make to the global effort to end the AIDS pandemic, and was instrumental in building a world class clinical research facility in the country.

“This is a devastating loss for the entire AIDS vaccine field,” said Seth F. Berkley, President and CEO of IAVI. “Prof. Bwayo, a warm and engaging man, was a talented scientist, working diligently to find a final solution to the AIDS crisis. Under his stewardship, KAVI has played a leading role in driving research for AIDS vaccines globally.”

Born in the Bungoma District, Prof. Bwayo was a frequent lecturer at the University of Nairobi and former chairman of the Department of Medical Microbiology, College of Health Sciences. He also served as co-director of the Regional AIDS Training Network for STD/AIDS and as a senior member of the World Health Organization Collaborative Centre for STD/HIV Research Training. Prof. Bwayo’s research interests included the epidemiology of sexually-transmitted diseases and HIV in men and women in Kenya, intervention studies to control and prevent STD/HIV infection, and immunobiology. He is the author of dozens of publications on public health issues and HIV/AIDS.

“Kenya’s well-earned prominence in the global HIV vaccine research arena is a great testimony to Prof. Bwayo’s scientific leadership and determination, and to his broad vision that getting a vaccine required understanding and commitment across all of Kenyan

society, as well as strong research partnerships to bring African and international capabilities together,” said Geoffrey Lamb, Chairman of the Board, IAVI. “What a great achievement, and what a bitter loss.”

“Prof. Bwayo’s contributions to fighting the AIDS epidemic and more recently to the HIV vaccine field will be remembered,” said Pontiano Kaleebu, Chairman of the African AIDS Vaccine Programme. “He was very active in our AAVP activities, especially in the development of national strategic frameworks. His critical work in shaping HIV vaccine policy in East Africa will be one of his many lasting legacies.”



A Kenya-based research organization developed under the University of Nairobi and in collaboration with IAVI and Oxford University, KAVI is one of the premier AIDS vaccine development organizations in East Africa. Led by Prof. Bwayo, it strongly endorsed links among Kenya scientists, community organizations and the greater global AIDS vaccine research and development field. Its successes included helping to conduct the first five AIDS vaccine trials in Kenya, beginning in 2001; building a world-class, accredited laboratory facility and sci-

entific infrastructure to prepare for larger-scale trials; establishing rigorous quality control programs; and contributing to Kenya’s National AIDS Vaccine Plan.

“Prof. Bwayo was a renowned leader in the AIDS community, in the AIDS vaccine development field and in his own country, understanding that Kenya was at the forefront of AIDS research and discovery,” concluded Berkley. “He often commented, ‘HIV vaccine development is a marathon, not a sprint and—as we all know—Kenyans are very good at marathons.’ IAVI and KAVI will continue to work together to find an effective AIDS vaccine, our best hope of ending the AIDS epidemic—a goal Prof. Bwayo, above all else, cherished.”

Dr. Kenneth Anthony Kalanyi Kebba

The International AIDS Vaccine Initiative (IAVI) mourns the loss of Dr. Kenneth Anthony Kalanyi Kebba, a partner and colleague with the Medical Research Council (MRC) and the Uganda Virus Research Institute (UVRI) in Uganda, and a brilliant young scientist who played a large role as a clinical investigator for the first HIV vaccine trial undertaken in Africa. Dr. Kebba died tragically on February 15 as a result of a brain aneurism. He is survived by a wife and two small children.

“Anthony was known as one of the most committed and diligent leaders in the battle to defeat HIV/AIDS in the East Africa region,” said Seth F. Berkley, President and CEO of IAVI.

“I have personally lost a colleague, but Africa has also lost a young scientist in an area where there is very limited human capacity,” said Dr. Pontiano Kaleebu, Principal Investigator of the UVRI/IAVI HIV Vaccine Program. “It is my hope that Anthony’s life as a scientist will be an inspiration to the younger generation—to emulate his dynamism, interest in science and above all build on and sustain the work that he had initiated.”

Dr. Kebba worked passionately in the field of AIDS vaccines over the past decade. As a young doctor, he worked at Kabarole Hospital in Fort Portal, Uganda and then served as coordinator of the Sleeping Sickness Control Programme for the Southeast region of Uganda, based in Jinja. In 1997, Anthony decided to join the Joint Clinical Research Centre in Kampala, serving as a clinical investigator in the ALVAC HIV vaccine trial.

Subsequently, Dr. Kebba obtained a Rogers Research Fellowship and began conducting intensive research on HIV infection at the Uganda Research Unit on AIDS of the UK MRC, which is based at the UVRI in Entebbe. Anthony received a PhD training fellowship from the Wellcome Trust, and completed his

work in 2004. By that time, seven of his publications had appeared in world-class journals; he was the lead author of six of them.


“Anthony was one of the most dedicated scientists I’ve ever met—very committed to Uganda’s struggle against HIV,” said Leslie Nielsen, IAVI’s Country Director in Uganda. “He was a remarkable team player and was enormously liked and respected by everyone who knew him.”



Only 36 years old, Dr. Kebba most recently served as a principal investigator with the MRC and UVRI. His research interests included the pathology of HIV infection in developing countries and the biology of exposed seronegative cohorts—which Anthony dubbed as the “Rubicon Study” after Cesar’s crossing of the River Rubicon two thousand years ago to change the course of the history of the Roman Empire.

Dr. Kebba worked closely with many organizations, including colleagues at Entebbe Hospital, The AIDS Support Organisation (TASO), and the AIDS Information Centre in Kampala. He also

received funding from donor agencies including IAVI and the Centre for HIV-AIDS Vaccine Immunology (CHAVI), and was appointed as an honorary lecturer in the department of Immunology within the Division of Investigative Science in the Faculty of Medicine at Imperial College in London.

“Anthony was someone I respected as a scientist. He was an extremely hardworking, determined individual with great humility and good humor,” said Jill Gilmour, Research & Development Senior Director at IAVI. “Anthony was a scientist who would always work until a job was done. He is greatly missed by his young research team,” added Andrea von Lieven, IAVI Clinical Program Manager. 

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The International AIDS Vaccine Initiative (IAVI) is a global not-for-profit organization whose mission is to ensure the development of safe, effective, accessible, preventive HIV vaccines for use throughout the world. Founded in 1996 and operational in 24 countries, IAVI and its network of collaborators research and develop vaccine candidates. IAVI's financial and in-kind supporters include the Alfred P. Sloan Foundation, the Bill & Melinda Gates Foundation, The John D. Evans Foundation, The New York Community Trust, The Rockefeller Foundation, The Starr Foundation, The William and Flora Hewlett Foundation; the Governments of Canada, Denmark, Ireland, The Netherlands, Norway, Sweden, the United Kingdom, and the United States, the Basque Autonomous Government as well as the European Union; multilateral organizations such as The World Bank; corporate donors including BD (Becton, Dickinson & Co.), Continental Airlines, Google Inc., Merck & Co., Inc. and Pfizer Inc; leading AIDS charities such as Broadway Cares/Equity Fights AIDS and Until There's A Cure Foundation; other private donors such as The Haas Trusts; and many generous individuals from around the world. For more information, see www.iavi.org.

US Congress to consider vaccine-focused legislation

Senator Richard Lugar and Congressman Pete Visclosky of Indiana, introduced companion bills in both houses of the US Congress calling for the enactment of a comprehensive strategy to accelerate the development, evaluation, and distribution of vaccines and other prevention technologies against diseases, including HIV/AIDS, tuberculosis, and malaria. This legislation is part of the "Vaccines for the Future Act of 2007" and calls for the US government to introduce new incentives that could increase the investment of the private sector in developing vaccines against diseases that primarily affect developing countries.

One recommendation specifically mentioned in these bills is the provision of US funding to support a pilot Advanced Market Commitment (AMC; see *If you build it, they will pay*, *LAVI Report* 9, 3, 2005) for a vaccine against *Streptococcus pneumoniae*, the bacterium (also known as pneumococcus) that causes meningitis and pneumonia and is responsible for more than a million childhood deaths

each year, according to the WHO. This AMC project was already endorsed by Italy, Canada, Norway, and the UK earlier this year. These countries pledged US\$1.5 billion as part of a larger program, called the Advance Market Commitments for Vaccines Against Neglected Diseases, to promote industry involvement in developing vaccines that are most common in resource-poor nations. Currently two US-based pharmaceutical companies, Wyeth and Merck, have pneumococcal vaccines but it is unknown whether these current products would be appropriate for use in developing countries. If this model AMC proves successful at prodding industry into developing vaccines intended for developing countries, it could serve as the basis for similar agreements in the future related to HIV/AIDS, tuberculosis, or malaria.

The legislation also highlights, more generally, other areas where the government could help spur the development of effective vaccines through industry involvement, including endorsing improved regulatory procedures and providing support for intellectual property issues involved in the development of these life-saving interventions.

Canada launches new HIV vaccine development program

The Canadian government, with additional funding from the Bill & Melinda Gates Foundation, is establishing a research institute dedicated to the development of an effective AIDS vaccine. In February Prime Minister Stephen Harper announced his government's pledge of just over US\$95 million to fund the new program, which is called the Canadian HIV Vaccine Initiative, and the Gates Foundation is also committing up to US\$24 million to the project. The Foundation's contribution is another component of the Global HIV Vaccine Enterprise, which was established in 2003 as a way to further accelerate AIDS vaccine research and development.

The primary goals of the Canadian HIV Vaccine Initiative are to support Canadian scientists who are working on the scientific challenges of developing promising AIDS vaccine candidates, construct a new facility capable of manufacturing vaccine candidates for testing in clinical trials, and foster collaboration between researchers, both in Canada and internationally. Canada was one of the first countries to create a national AIDS vaccine plan and the government recently awarded IAVI a CAD\$20 million to continue its work on the development of a safe and effective AIDS vaccine. "Canada has been a long-time supporter of AIDS vaccine research and has demonstrated laudable leadership in tackling the AIDS epidemic comprehensively, with a dual focus on both treatment and prevention," said Seth Berkley, CEO of IAVI.

Therapeutic vaccine trial shows no benefit

At the 14th annual Conference on Retroviruses and Opportunistic Infections, Brigitte Autran of the Hospital Pitié-Salpêtrière in Paris presented results showing that therapeutic vaccination with the recombinant canarypox vaccine candidate vCP1452, developed by Sanofi Pasteur, offered no benefit to individuals interrupting treatment (CROI; www.retroconference.org/2007). Volunteers in this trial received either three or four injections (three primes and one boost) of the vaccine candidate encoding several HIV genes, including gp120, *gag*, *pro*, *Nef*, and RT, or placebo and were given the option to suspend their current highly-active antiretroviral therapy (HAART) regimen after the first dose of vaccine. Researchers then monitored these individuals closely and placed them back on therapy if their CD4⁺ T cells declined below 250 cells/ml of blood.

According to Autran the vaccine candidate showed significant immunogenicity in HIV-infected volunteers and provided modest clinical benefit, lowering the viral set point after treatment interruption, in previous studies. But at CROI she reported that in this latest study, MANON-02, all volunteers who received vCP1452 actually had to resume HAART sooner than those who received placebo (Abstract 126LB). Those who received four immunizations of the canarypox vaccine candidate actually had a five-fold increased risk of needing to

resume treatment; 10 of 20 volunteers who received three immunizations had to resume therapy compared to 14 of 19 in the four injection arm. Meanwhile only 3 of the 15 volunteers who received placebo had a decline in CD4⁺ T cell count that warranted resuming treatment.

Autran called these results “very disappointing” but said that she didn’t think this trial should stop further study of this therapeutic vaccination approach. Researchers have long hoped that therapeutic vaccination would boost HIV-specific immune responses in HIV-infected individuals and therefore allow them to prolong the duration of treatment interruptions, without future therapeutic consequences. Treatment interruptions were first explored as a way to help individuals avoid some of the toxic and unpleasant side-effects of taking antiretrovirals (ARVs) and many different strategies have since been evaluated in clinical trials with mixed results.

The Sanofi-Pasteur vaccine candidate is also currently being tested in a preventive AIDS vaccine clinical trial, alone or in combination with a LIPO-5 vaccine, at HIV Vaccine Trials Network (HVTN) sites in the US. This trial is sponsored by the US National Institute of Allergy and Infectious Diseases and the Agence Nationale de Recherches sur le SIDA (ANRS). Several other trials with canarypox-based vaccine candidates are also ongoing. For more information about these or other preventive AIDS vaccine trials, visit the *LAVI Report* clinical trials database (www.iavireport.org/trialsdb).

LAVI Report awardees to attend HIV vaccine symposium

This year IAVI is sponsoring *LAVI Report* Travel Award scholarships for four researchers from developing countries severely affected by HIV/AIDS to attend the upcoming Keystone Symposium: HIV vaccines from basic research to clinical trials, which is taking place in Whistler, Canada from March 25 to 30. This preeminent meeting on AIDS vaccine research is part of Keystone’s Global Health Series and is supported with funding from the Bill & Melinda Gates Foundation. Keystone is trying to expand the involvement of developing-country researchers in this annual meeting, as well as other global health-focused symposia, and this year marks the first time that *LAVI Report* is providing travel awards. The goal of these awards is to provide scientists, physicians, post-graduate, or graduate students in resource-poor nations with the opportunity to attend important scientific meetings on AIDS vaccine research and development

to broaden their understanding of the topic.

This year’s awardees were selected by Keystone’s scientific organizing committee and include Rugare Abigail Kangwende, who is an investigator at Africa University in Mutare, Zimbabwe. Kangwende is preparing to lead a team of investigators in a Phase I clinical trial, the first in the country. Gaudensia Nzembi Mutua, another travel-award recipient, is from the University of Nairobi in Kenya and is currently working on feasibility studies at a trial site that is now preparing for a Phase IIb test-of-concept AIDS vaccine trial that will be starting later this year. Ajay Wanchu is an HIV clinician at the largest clinic in the region, an immunologist, and a faculty member at the Postgraduate Institute of Medical Education and Research in Chanigarh, India. The final award-recipient, Louis Marie Yindom, is a PhD student at the Medical Research Council in Fajara, Gambia, who is studying the role of human leukocyte antigen (HLA) and killer immunoglobulin-like receptor in HIV-2 infection.