



IAVI Report

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AIDS vaccine researchers STEP up to the challenge

Questions linger, but so does determination, as researchers gather at the AIDS Vaccine 2008 Conference

by **Kristen Jill Kresge**

This year's AIDS Vaccine Conference, which was held in Cape Town, South Africa, from October 13-16, was momentous on both political and scientific fronts. It was the first time the annual conference was held in an African country and Lynn Morris, conference chair and head of the AIDS unit at the National Institute for Communicable Diseases in Johannesburg, kicked off the conference by commenting on the particular significance of it being held in South Africa. "Nowhere else is the need for a vaccine greater than it is here," she said, adding that this conference sent an important signal that "while we've suffered a setback, we're not giving up."

Even more politically significant were the remarks made by the newly appointed South African Minister of Health, Barbara Hogan. After just two weeks on the job, Hogan made one of her first public addresses to the nearly 1,000 conference delegates. "We know that HIV causes AIDS," she said, immediately making her positions clear. "The science of HIV and AIDS is one of the most researched subjects in the medical field." Hogan also praised the conference organizers for holding the meeting in South Africa. "The timing of this conference coincides with a renewed interest in HIV prevention in this country. To the South African government and its people, there can't be any more important meeting to be held at this time." She called for evidence-based public health education as well as the development of evidence-based HIV prevention tools, which she said were critical to changing the course of the epidemic, and confirmed South Africa's commitment to conducting clinical trials of vaccines. Hogan's comments stood in stark contrast to those of her predecessor and were lauded by subsequent speakers.

On the scientific front, this year's meeting was momentous because it was the first to be held following the unexpected failure of Merck's adenovirus serotype 5 (Ad5) vector-based vaccine candidate (MRKAd5) in the STEP trial last fall, just after the 2007 conference. Since then the landscape of the AIDS vaccine field has changed dramatically. "The whole meeting has been

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Mighty mice

Scientists are still improving the humanized mouse model but are optimistic about its future role in evaluating AIDS vaccine candidates


by **Andreas von Bubnoff**

The flying cartoon rodent Mighty Mouse would be proud of his brethren. New mice, which are being developed by researchers, may not don capes or fight villains but they do possess other super powers, brought on by the fact that they have human immune systems. These so-called

"humanized" mice represent a new frontier in the preclinical testing of experimental drugs, and possibly even vaccine candidates.

After about two decades of experimentation in transplanting human tissues into mice, the latest round of rodents can successfully harbor human immune cells and

can be infected with human viruses that mice are usually not susceptible to. This is particularly significant for HIV. Although the mouse model is one of the most fundamental in all of biomedical research, its use as a model system for HIV has been severely hampered by the fact that the virus is a

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held in the fallout of the STEP trial,” said Edward Rybicki, a professor of microbiology at the University of Cape Town who served as a rapporteur on the topic of vaccine concepts and design. The conference provided an opportunity for researchers, clinical trial investigators, and advocates to get the latest data from the STEP and Phambili trials. “We watch with bated breath every new piece of data that comes out of that study,” said David Weiner, chair of the Gene Therapy and Vaccines program at the University of Pennsylvania School of Medicine. Researchers also discussed some of the lingering questions about the potential for cell-mediated immunity candidates and the value of non-human primate (NHP) models for predicting vaccine efficacy. Other areas of focus at the meeting included the role of innate immunity in HIV infection and the exploration of novel viral vectors.

Stanley Plotkin, executive advisor to the CEO of Sanofi Pasteur and veteran vaccinologist, was the final speaker at the opening session and his comments were echoed over the following days by many presenters (see *An Interview with Stanley Plotkin*, page 12). Plotkin summed up his views on the AIDS vaccine field with the expression ‘sang-froid,’ which means to avoid panic when things look bad. “While the situation is serious,” Plotkin said, “it’s not desperate.”

Emerging data

One of the key points of interest at the conference was, of course, the data emerging from the STEP trial. Since the results were first made public last September, they have practically become household news, at least in some circles. Anthony Fauci, director of the National Institute of Allergy and Infectious Diseases (NIAID) at the US National Institutes of Health (NIH), said during his keynote lecture, “even the gardeners at the NIH know the three [key] bullets of the STEP study.”

Julie McElrath, director of the Vaccine and Infectious Disease Institute at the Fred Hutchinson Cancer Research Center in Seattle, outlined progress in analyzing the data from the STEP trial in a plenary talk. She first noted that only 31% of vaccinees in the STEP trial mounted both CD4⁺ and CD8⁺ HIV-specific T-cell responses following three vaccinations with MRKAd5. In his earlier comments, Plotkin said only 0.5-1% of total CD8⁺ T cells in vaccinees were specific to HIV, suggesting to him that the candidate’s failure could be due to the paucity of immune responses it induced. “The responses were inadequate,” said Plotkin. In fact, when McElrath and colleagues compared the magnitude and breadth of the T-cell responses induced by MRKAd5 to those observed in a group of long-term nonprogressors (LTNPs)—individuals infected with HIV who are able to control viral replication or disease progression for an extended period of time without the aid of anti-retroviral therapy—they found the median percentage of vaccine-induced CD8⁺ T-cell responses in STEP volunteers were 43% lower than the average in HIV-infected LTNPs. “If we’re trying to mimic the responses in these individuals, we’re not there,” said McElrath.

However, Bruce Walker, director of the Partners AIDS Research


Center at Massachusetts General Hospital, continues to find that individuals in his cohort of elite controllers—a subset of LTNPs who maintain viral loads less than 50 copies of HIV/ml of plasma—have weaker CD8⁺ T-cell responses than individuals with typical HIV disease progression. One key difference he’s detected so far among elite controllers is that their CD8⁺ T-cell responses are directed primarily toward Gag. Among STEP trial volunteers, McElrath noted that vaccinees without pre-existing Ad5 immunity were “more likely to make a Gag response,” and in individuals with higher levels of Gag-specific T-cell responses, there was a trend toward lower viral load. According to McElrath, there was also an inverse correlation in vaccine recipients, without pre-existing Ad5 immunity, who subsequently became HIV infected between the level of interferon (IFN)- γ -secreting T cells directed toward HIV Gag and

viral load. She referred to these findings as “some potential ray of hope” but also acknowledged that there are “a lot of caveats associated with this,” including the very small number of volunteers that these analyses are based on.


Still, McElrath said these preliminary findings suggest that a CD8⁺ T-cell response capable of reducing viremia may be an attainable goal. However when McElrath and colleagues analyzed the function of the CD8⁺ T cells induced by a similar Ad5 vaccine candidate—in this case the Ad5-vector based vaccine candidate developed by researchers at the Vaccine Research Center (VRC) at NIAID—using a viral inhibition assay, the results were not encouraging. Peripheral blood mononuclear cells (PBMCs) collected from two volunteers in a Phase I trial who received two doses of the VRC’s Ad5 candidate were evaluated. In this assay, CD4⁺ and CD8⁺ T cells were isolated from PBMCs. The CD4⁺ T cells were infected with HIV *in vitro*, and the CD8⁺ T cells were then added to see if they had any effect on HIV replication. The CD8⁺ T cells from these individuals had no effect on suppression of HIV replication in this assay. McElrath said the caveat with this data is that the cell samples tested were collected at 52 weeks, a time when they were “less likely to be effector cells.”

Analysis of granzyme B and perforin expression, two proteins that induce apoptosis of virus-infected cells, are also being conducted with *ex vivo* CD8⁺ T cells from STEP trial volunteers and McElrath says this information may help “tease out what would be a more effective immune response against HIV.” Susan Buchbinder, principal investigator of the STEP trial and a professor of medicine at the University of California in San Francisco, said in another plenary talk that there was also a “tantalizing hint” that protective human leukocyte antigen (HLA) types were associated with a reduction in viral load among vaccinated volunteers, but this is a very preliminary finding based on only a small number of participants.

In the meantime, researchers are still looking for any effect MRKAd5 may have had on HIV progression in vaccinated volunteers. Holly Janes, an assistant member of the biostatistics program at Fred Hutchinson Cancer Research Center (FHRC), presented data in the



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Barbara Hogan

late-breaker session on a small sub-group of HIV-infected male volunteers from the STEP trial—33 who received placebo and 40 who received MRKAd5. Of these volunteers, 25 have already initiated anti-retroviral therapy. Janes reported that there was no significant difference between the median viral load in vaccine and placebo recipients prior to starting therapy, nor was there a difference in the amount of time prior to their initiation of treatment. The pre-treatment CD4⁺ T-cell counts and set-point viral loads were also similar between vaccine and placebo recipients. Janes said the data did not provide any evidence to suggest that the vaccine had exacerbated HIV disease progression.

In another study, researchers investigated a possible correlation between levels of pre-vaccination Ad5 neutralizing antibodies and the induction of Ad5-specific T-cell responses in STEP trial volunteers. High Ad5 antibody levels was one factor associated with an increased risk of acquisition of HIV among vaccine recipients in the STEP trial and one possible explanation for this was that individuals with higher Ad5 antibody levels would also have higher levels of Ad5-specific memory CD4⁺ T cells that would become activated upon vaccination, creating more target cells for HIV. However, according to research presented by Nicole Frahm, a faculty member in infectious diseases at FHCRC, individuals in the STEP trial with high Ad5 neutralizing antibody titers actually had lower amounts of Ad5-specific CD4⁺ and CD8⁺ T cells. “Ad5 [antibody] titers don’t really tell you if someone will make Ad5-specific cellular responses or not,” said Frahm.

She and her colleagues incubated PBMCs from 139 volunteers in the STEP trial who received three doses of MRKAd5 with 10,000 empty Ad5 vector particles per cell and then analyzed the samples for Ad5-specific T cells using intracellular cytokine staining. This study was conducted with the empty Ad5 vector developed at the VRC, but researchers first verified that it produced the same results in their assays as Merck’s Ad5 vector.

Frahm reported that 80% or more of vaccinees mounted Ad5-specific CD4⁺ T-cell responses and 65% or more had Ad5-specific CD8⁺ T-cell responses. The response rates varied based on Ad5 antibody titer, but both CD4⁺ and CD8⁺ T-cell response rates to the Ad5 vector were consistently higher in the Ad5 seronegative group. And when researchers compared Ad5-specific T-cell response rates from individuals who subsequently became HIV infected with those who did not, they found a significantly higher Ad5-specific CD4⁺ T-cell response rate in individuals who did not eventually acquire HIV, indicating that Ad5-specific T cells, at least in peripheral blood, were likely not responsible for the increased risk of HIV infection among vaccinees. “This was not what we expected at all,” said Frahm, who cautioned that researchers still need to look at the level of Ad5-specific T cells at mucosal sites. “We could just be looking at the wrong spot,” she warned.

Dan Barouch, associate professor of medicine at Beth Israel Deaconess Medical Center in Boston, reached a similar conclusion after analyzing samples from 116 individuals who participated in a Phase I trial of an earlier version of MRKAd5 encoding only Gag. He found that

individuals with high levels of pre-existing Ad5 antibodies had higher Ad5-specific antibody responses following vaccination, but not higher levels of Ad5-specific T-cell responses. He is planning NHP studies to see if Ad5-specific T cells are concentrated at the mucosa.

While the reasons for MRKAd5’s failure are still unknown, and may never be completely clear, McElrath said investigators affiliated with the STEP trial have “made further progress in defining threshold responses for T-cell based vaccines.”

Phambili data

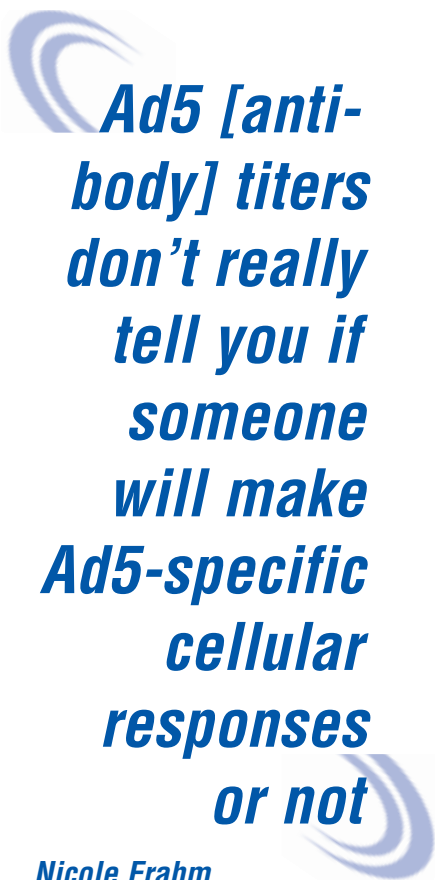
Glenda Gray, executive director of the Perinatal HIV Research Unit in Soweto, South Africa, presented data collected so far from the Phambili study, the second Phase IIb study of MRKAd5, which is being conducted in South Africa. When immunizations in the Phambili trial were stopped last September, 801 volunteers had been enrolled, and 50% were women. While the STEP trial volunteers were primarily men who have sex with men, the intention in the Phambili trial was to evaluate the efficacy of the candidate against primarily heterosexual HIV transmission.

Of the 400 volunteers in the vaccine group when immunizations were ceased, 66% had received two vaccinations and 7% had received all three. Gray reported that so far there have been 29 HIV infections among the 801 volunteers, 17 within vaccine recipients and 12 in the placebo group. Most of these infections were in volunteers with pre-existing immunity to the Ad5 vector, as was the case in the STEP trial, with 16 of 17 infections in the vaccine group and 9 of 12 infections in the placebo group having occurred in individuals with measurable Ad5 antibody titers. Of the seven infections that have occurred among male volunteers in the Phambili trial, six were in uncircumcised men—four in the vaccine group and two in the placebo arm—another risk factor associated with HIV acquisition in the STEP trial.

Gray noted that the unblinding of volunteers in the Phambili trial has had a significant impact on HIV acquisition rates in the study—since the volunteers were told a year ago whether they received vaccine or placebo, no new HIV infections have occurred among vaccinated volunteers. “Unblinding was a major confounder,” Gray said. Because of this, she declined to make any comparisons between the Phambili data and the results of the STEP trial, which suggested that the vaccine candidate may have increased the risk of acquisition of HIV infection in certain subsets of volunteers, primarily uncircumcised men with high levels of pre-existing Ad5 immunity.

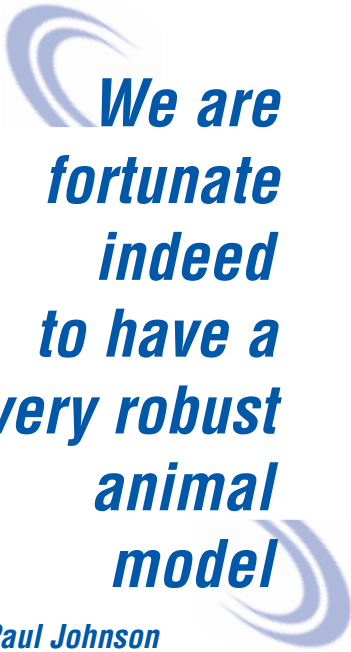
Debating the way forward

At this year’s conference there were two organized debate sessions at which pairs of researchers faced off over central questions currently dominating discussion in the AIDS vaccine field. These debates were peppered with references to the US presidential race



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and often took amusing side-turns as researchers built their arguments by gently provoking their opponents. It was an opportunity for researchers to display their creativity outside the lab, and video clips as well as doctored photos were used to great effect.

The first debate was about whether the NHP model should be used as a gatekeeper to clinical trials of vaccine candidates, or more specifically, whether a candidate vaccine should be required to show efficacy in NHPs to be advanced into Phase I clinical trials. And although there is general agreement that studies in NHPs play an important role in vaccine research, there is some disagreement about just how heavily the field should rely on them.

The utility of the NHP model in preclinical evaluation of vaccine candidates was one theme that emerged from the NIAID-sponsored HIV Vaccine Summit, held earlier this year to discuss future research priorities in light of the STEP trial (see *Balancing AIDS vaccine research*, *LAVI Report*, March-April 2008). In studies with rhesus macaques, MRKAd5 modulated viral load against challenge with a hybrid SIV/HIV or SHIV but did not provide any protection against a more stringent simian immunodeficiency virus (SIV) challenge (see *Getting it right early*, *LAVI Report*, Sep.-Dec. 2007). This was enough to convince some researchers that protection against SHIV was not sufficient to predict efficacy in humans and as Jeff Lifson, head of the retroviral pathogenesis section at the National Cancer Institute said, "SHIVs are [now] out of fashion." But Lifson was quick to point out that "there is not *a* primate model, there are *many* primate models," and he said it was important to understand the different models and use them thoughtfully so they can best inform research.

Paul Johnson, associate professor at Harvard Medical School, said he wanted to dismiss at the outset the idea that monkeys lie, an oft-repeated line in the field. "As long as we ask them the right questions they tell the truth," Johnson said. "We are fortunate indeed to have a very robust animal model." He and Lifson argued that to gain entry into Phase I/II trials vaccine candidates should have to show significant immunogenicity, which they defined as greater than a 1.0 log copies/ml difference in viral load in vaccinated NHPs as compared to controls, following homologous SIV challenge. To warrant testing in a Phase IIb, screening-test-of-concept (STOC), or Phase III trial, they suggested a candidate should have to provide improved protection against homologous challenge when compared to MRKAd5 or provide protection

against heterologous SIV challenge. Johnson and Lifson said criteria other than protection data in NHPs could argue for testing some candidates, including those that elicit neutralizing antibodies. In the case of candidates based on viral vectors with limited replicative capacity in macaques, they said the onus would be on the developers to decide when it was appropriate to advance a candidate into humans.

Johnson said the finite financial, manufacturing, and human resources, along with the need for extensive iterative cycles of testing AIDS vaccine candidates, argued for such guidelines, citing several examples of vaccines that were immunogenic in monkey models and then in humans. He said the positive predictive value of the NHP model could only be proven when a vaccine also showed efficacy in humans, but results from both the STEP trial and the Phase III trial previously conducted by VaxGen have paralleled the results seen in NHP studies.

Weiner and Jerald Sadoff, chief executive officer of the Aeras Global TB Vaccine Foundation, agreed that any vaccine platform should be studied in relevant macaque challenge models, but they argued that protection in NHP studies should not be required prior to advancing a candidate into Phase I trials. "It doesn't matter what works in monkeys," said Sadoff, "it only matters what works in humans." He cited several vaccines, including those against cholera and rotavirus, which were developed without the use of relevant animal models as well as others like the malaria vaccine that is currently in efficacy trials, which were thrown off track by the data collected in NHP studies. "In malaria we were completely misled by the monkey model," he said.

He also argued that although SIV is analogous to HIV, it's not the same. "We have a different physiology in the animal and a different pathogen," added Sadoff. He and Weiner concluded that while monkey models should be used as an immunogenicity marker for Phase I trials, they should not serve as a gatekeeper. Rather, Sadoff suggested that NHP studies should be done in parallel with Phase I trials so that clinical evaluation isn't delayed.

Alan Bernstein, executive director of the Global HIV Vaccine Enterprise, said linking NHP researchers with clinical trial researchers was one project the Enterprise will be spearheading in the coming months. "We need to regard clinical trials as science," he said, adding that the separation of clinical and discovery research "is a false dichotomy." The Division of AIDS at NIAID will hold an NHP

workshop November 12-13, 2008, at which experts on NHP research will offer guidance on how NIAID should invest in NHP research to advance the discovery of an AIDS vaccine.

Size matters

The second debate session was focused on whether additional cell-mediated immunity (CMI) candidates should be advanced into efficacy trials, given the failure of MRKAd5. In this session Gary Nabel, director of the VRC at NIAID, and David Watkins, a professor at the University of Wisconsin-Madison, squared off against Dennis Burton, a professor of immunology at The Scripps Research Institute in California and scientific director of the recently established HIV Neutralizing Antibody Center (see *Vaccine Briefs*, this issue). Robert Gallo, director of the Institute of Human Virology in Maryland, was scheduled to be Burton's debating partner but he did not attend the meeting.

This debate was originally scheduled when Fauci was still considering whether NIAID would fund a Phase IIb trial to test the VRC's DNA/Ad5 candidates, according to John Moore, professor of microbiology and immunology at Weill Cornell Medical College, who served as moderator. That trial was initially postponed after the STEP trial results were released, and in July Fauci rejected the proposed Phase IIb trial design, known as PAVE 100A (see *PAVEing the way to a smaller trial*, *LAVI Report*, July-Aug. 2008). NIAID is still considering conducting a smaller trial to look only at the ability of the prime-boost DNA/Ad5 regimen to lower viral load in vaccinated individuals that become HIV infected, and the protocol for such a trial is still under development.

Nabel argued that efficacy trials of T-cell vaccines should continue and that these trials should be "sufficiently large to be able to address questions related to immune correlates, viral load, and prevention of infection." But Burton said there are "too many uncertainties at this time" to justify large-scale trials. He said large efficacy trials would only distract researchers from the best-case scenario for an effective AIDS vaccine—one that stimulates both arms of the immune system. However, Watkins said testing CMI vaccines was essential since "we don't have any candidate antibody-based vaccines yet."

Nabel cited the increasing evidence of the efficacy of T-cell candidates in relevant nonhuman primate challenge studies as another reason to move forward with large-scale trials. Some of

this data stems from studies by Watkins and colleagues and in a late-breaker talk, Nancy Wilson, an associate scientist in Watkins's lab, presented data from a study in which rhesus macaques were vaccinated with a DNA/Ad5 regimen encoding all of the SIVmac239 genes except *env*. While vaccinees in the STEP trial developed immune responses to three to five HIV epitopes on average, the vaccinated macaques in this study developed immune responses to an average of 20 SIV epitopes. Following five low-dose, mucosal challenges with the heterologous swarm virus SIVsmE660, five of the eight vaccinated macaques were SIV infected. The vaccinated macaques had markedly lower viral loads—the average peak plasma viral load was 12,600 copies/ml, compared to four million copies/ml in unvaccinated control animals. And at eight weeks post-infection, the average viral load in vaccinated animals was undetectable, while the average in unvaccinated controls was 200,000 copies/ml.

On this point Burton didn't disagree. "I have no problem with screening-test-of-concept trials with T-cell based vaccines," said Burton, "particularly ones that show robust responses in the macaque model." He voiced support for smaller studies, like STOC trials, that would involve fewer volunteers but could provide preliminary information about the ability of such candidates to lower viral load. It seemed that the division between the two sides in this debate revolved mainly around the use of the words 'large scale.' "For those of you who are looking for a fight," Burton said, "I'm afraid you're not going to get it."

Better vectors?

The pool of CMI candidates that may be up for testing in future clinical trials will likely be based on novel vectors. One of these is the cytomegalovirus (CMV), which is under investigation by Louis Picker, associate director of the vaccine program at the Vaccine and Gene Therapy Institute at the Oregon Health and Science University. Picker said vaccines that generate typical T-cell memory responses, which are primarily central memory T cells (T_{CM}), may not be able to overcome what he called the "kinetic mismatch" between the explosive replication capacity of HIV and the ability of T_{CM} to expand, differentiate, and migrate to the sites of virus replication. Picker said most prime-boost vaccine regimens currently being tested induce mostly T_{CM} , whereas live-attenuated SIV vaccines, which provide the best protection seen so far in NHP models,



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Dennis Burton



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George Pavlakis

induce mostly effector memory T cells (T_{em}). He therefore proposes that a vaccine candidate that could induce more T_{em} than T_{cm} may increase the potential for protection.

To study this hypothesis, Picker chose to evaluate a CMV-vector based vaccine, which he called the “quintessential inducer of effector memory dominant T-cell responses.” Picker vaccinated 12 macaques with a rhesus CMV vaccine encoding SIV *gag*, *rev*, *nef*, *tat*, and *env* and then repeatedly challenged them with low-dose SIVmac239. The vaccinated animals required a median of eight doses of challenge virus to develop a progressive infection, compared to only two doses in the 16 unvaccinated control monkeys.

Remarkably, 4 of the 12 vaccinated animals resisted progressive SIV infection altogether, though they were demonstrably infected by the challenge virus because they developed *de novo* immune responses to SIV antigens *pol* and *vif*, which were not included in the vaccine. Two of these four animals had transient, very low plasma virus levels, while the other two had no detectable virus in their plasma at all. There was still no sign of viral replication even after depleting the $CD8^+$ T cells of these monkeys 133 days post challenge. Picker said this suggests that early T_{em} recognition of viral antigens may lead to an effector response that is capable of controlling viral replication early, likely at the sites of viral entry. Vaccine-elicited effector T-cell responses may need to be induced at sites of viral entry to control replication of the founder population of virus before a systemic infection is established, according to Picker. He said this also emphasizes the importance of conducting such low-dose mucosal challenge studies with vaccine candidates.

Another vector under development is the measles virus. Hussein Naim, director of vaccine research at the biotechnology company Crucell Berna Biotech in Switzerland, presented data on a replication-competent measles virus vector his company is developing based on a commercially-available live-attenuated measles vaccine. The measles vaccine, which is administered to children as part of a combination vaccine that also protects against mumps and rubella, provides long-lasting protection against the disease and has reduced morbidity associated with the virus by between 95% and 100%.

One advantage of using the paramyxovirus that causes measles as a vector is its ability to infect numerous types of cells, including macrophages, dendritic cells, lymphocytes, and monocytes. It also has a favorable safety profile,

since it has been administered to millions of people, and is inexpensive to manufacture. The potential disadvantage to using measles as a vector is pre-existing immunity—measles vaccination is nearly universal among infants in developed countries. In studies with mice, passive administration of measles antibodies did somewhat inhibit induction of cellular and humoral immune responses following immunization with a measles vector encoding HIV antigens, according to Naim, but he said the vaccine candidate was still able to induce HIV-specific immune responses. Naim and his colleagues are now considering evaluating intranasal administration of an aerosol measles vector, in collaboration with researchers at NIAID, to further evaluate its ability to induce immune responses against HIV. Researchers at the Institut Pasteur in Paris are also collaborating with GlaxoSmithKline Biological to develop a measles vector.

Meanwhile Barouch and colleagues have initiated a Phase I trial with an adenovirus serotype 26 (Ad26) vector, which in a prime-boost combination with Ad5 provided better results than a heterologous Ad35/Ad5 combination in NHP studies. The Ad26/Ad5 prime-boost combination resulted in a 1.4 log copies/ml reduction of peak viral load and a 2.4 log copies/ml reduction in viral load set point in rhesus macaques, which persisted for more than 500 days following SIVmac251 challenge. Barouch observed that the correlate of viral control with this regimen was anamnestic SIV Gag-specific responses, which he said “looks similar to Bruce Walker’s data from elite controllers.”

DNA 2.0

Other researchers are continuing to explore different formulations and administration techniques to enhance the immune response profiles induced by DNA vaccine candidates. “DNA vaccines are improving rapidly,” said George Pavlakis, head of the Human Retrovirus Section at the National Cancer Institute. His group found that using different HIV antigens affects the immune responses induced by their DNA construct. Pavlakis and colleagues also found additional benefit in terms of immunogenicity using *in vivo* electroporation, a vaccine administration technique that uses electric pulses to disturb cellular membranes and thereby allows molecules like DNA to cross into cells more easily. Vaccinating rhesus macaques by electroporation with DNA vectors encoding the majority of SIVmac239 proteins, together with interleukin (IL)-12 DNA as an adjuvant, increased

the level of antigen expression, improved cellular (central memory CD4⁺ T cells and effector CD8⁺ T cells) and humoral immune responses, and resulted in an improved level of protection following SIV challenge. The SIV-specific T-cell responses were able to secrete multiple cytokines, including IFN- γ , IL-2, and TNF- α , in response to SIV peptides and lymphocytes recovered from bronchioalveolar lavage showed that there was migration of SIV-specific memory T cells to this peripheral mucosal site. These immune responses were long-lived in the animals—they could still be detected in both blood and mucosa 10 months after vaccination.

When vaccinated animals were challenged mucosally with SIVmac251, they had greater than 10-fold lower viral loads in comparison to unvaccinated control animals. Pavlakis said IL-12 continues to be a good adjuvant for DNA vaccines, even with electroporation, and that this data shows that DNA vaccines can control viremia following highly pathogenic SIV challenge. He said this DNA construct is “as good, or as bad, depending on your perspective, as any other vaccine modality save for live-attenuated [SIV] vaccines.”


Innate immunity in vertical transmission

As researchers continue to develop vaccine approaches that could elicit antibody and cellular immunity, a growing level of interest is also being paid to the role of innate immunity in HIV infection. “Innate immune responses are clearly valuable,” said Plotkin. Both he and Fauci mentioned the importance of exploring the role of innate immunity, and of natural killer (NK) cells—a major component of the innate immune system, which also play a role in adaptive immunity—in particular.

In a late-breaker talk, Caroline Tiemessen, a

professor of virology at the University of the Witwatersrand in Johannesburg, presented intriguing data on the role of NK cells in preventing vertical transmission from HIV-infected mothers to their infants. Tiemessen and colleagues looked at CD4⁺ and CD8⁺ T cells, as well as several subsets of CD3⁺ cells, in 79 HIV-infected mothers and their 76 infants and found that CD3⁺ NK cells that respond specifically to HIV peptides were associated with protection against vertical transmission.

According to Tiemessen, 43% of mothers and 16% of infants had CD3⁺ responses specific to Env, and 22% of mothers and 5% of infants had CD3⁺ responses specific to Reg [Tat, Rev, Vif, Vpu, and Vpr combined]. Most of the infants with HIV-specific CD3⁺ NK-cell responses were born to mothers who also had high levels of these cells, and in mothers they were associated with lower viral loads and higher CD4⁺ T-cell counts, two factors that reduce the likelihood of vertical HIV transmission. Tiemessen also observed that HIV-specific NK cells were also dependent on a soluble plasma factor, as well as interactions with CD4⁺ T cells and HLA Type I molecules.

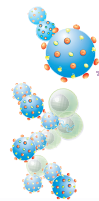
NK cells, which were assumed to non-specifically control virus, may “see” HIV in a specific manner and also play a role in ADCC, according to Galit Alter, an instructor at the Partners AIDS Research Center in Boston, who served as conference rapporteur on the topics of T-cell immunology and innate immunity. Unraveling the precise role of NK cells may lead to new avenues in vaccine research; then researchers will have to figure out how to harness the innate immune system, according to Fauci. “This is a scientific problem and there will be a scientific solution,” he said, “I believe we are on our way toward that goal.” 


***Innate
immune
responses
are clearly
valuable***

Stanley Plotkin

**Based on the results of our reader survey,
we are proud to introduce the new...**

IAVI Report



Be sure to check out the November-December 2008 issue for the debut of the new design.

Featuring full-color graphics throughout, additional coverage of conferences and meetings, interviews with key figures in the field, in-depth feature articles tracking trends in the AIDS vaccine field, and more detailed scientific images to accompany various articles.

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uniquely human pathogen. Studies in non-human primates are conducted with simian immunodeficiency virus, which is the closest approximation of HIV infection researchers suspect, but is still a different virus.

Humanized mice are already being used preclinically to evaluate new antiretrovirals, but most researchers say these mighty mice will require some further optimization before they can be used as a preclinical screen for AIDS vaccine candidates. Still, many are optimistic that the humanized mouse model will someday soon provide valuable preclinical information on human immune responses to candidate vaccines.

Early findings

The first humanized mice were generated around 20 years ago by transplanting human tissue into strain CB17 severe combined immunodeficiency (SCID) mice. These SCID mice have a mutation in a DNA repair enzyme that leads to impairment in the genomic DNA rearrangements, which are responsible for creating the cornucopia of different B and T cells. As a result, SCID mice can't make B or T cells, and therefore don't reject grafts of human tissue.

At that time, several groups transplanted human tissues into SCID mice. In 1988, a group led by Joseph McCune, then at Stanford University School of Medicine and now a professor of medicine at the University of California in San Francisco (UCSF), transplanted human fetal liver and thymus tissue under the kidney capsule, a well vascularized organ where transplants survive especially well (*Science* **241**, 1632, 1988). The resulting mice, known as SCID-hu mice, developed a thymus-like structure containing human T cells that could be infected with HIV (*Science* **242**, 1684, 1988). Another group led by Donald Mosier, then at the Medical Biology Institute and now a professor at the Scripps Research Institute in California, injected human peripheral blood leukocytes (PBL) into the peritoneal cavity of SCID mice, generating Hu-PBL-SCID mice (*Nature* **335**, 256, 1988).



But these early SCID models had their drawbacks, says Markus Manz, who studies humanized mice as a group leader and vice director at the Institute for Research in Biomedicine in Switzerland. Manz says the recipient mice only produced human immune cells for a limited time, maybe for a few weeks or months. "All these [models] did not

really sustain the human hematopoiesis," Manz says. This is partly because CB17-SCID mice still maintain some innate immune responses such as natural killer (NK) cells and therefore eventually develop some mouse T and B cells, which causes them to reject some of the human cells. "The CB17-SCID mouse has quite active NK cells, and when the mice get older, T and B lymphocytes develop," says Leonard Shultz, who studies humanized

autoimmune diabetes, but NOD-SCID mice don't because they lack the ability to generate immune responses and, as it turned out, they were also much more accommodating for transplanted human tissue. These NOD-SCID mice supported better engraftment with human CD34⁺ hematopoietic stem cells (HSCs), the cells from which most other immune cells develop, for up to six months following transplantation, which is much longer than HSCs last *in vitro*, Manz says.

It was known that lower NK cell activity contributed to the increased ability of NOD-SCID mice to support human HSC engraftment, according to Shultz. But "the reason why this worked so much better was never clear," says Manz. Then, a study published last year showed that the transplanted human cells engraft better in these mice than in SCID mice because of an additional impairment in their innate immune system. Macrophages in NOD-SCID mice have a version of a receptor that can recognize the human cell surface protein CD47 in the transplanted human cells and as a result, the NOD-SCID mouse macrophages don't reject the transplanted human cells (*Nat. Immunol.* **8**, 1313, 2007). "Now it's become clear [that not only] is it the adaptive immune system that is impaired, but also the innate immune system [that] needs to be impaired to have the graft surviving," says Manz (*Nat. Immunol.* **8**, 1287, 2007). Still, even in NOD-SCID mice, the transplanted human HSCs did not develop into a functional immune system, Manz says, precluding any meaningful human-like immune responses. But a few years ago, several groups developed strains of mice with further impairments in their innate immune responses that allowed these mice, for the first time, to develop functional human B and T cells from the transplanted human HSCs. Some groups achieved this by crossing mice lacking the common gamma chain cytokine receptor—a common element of several different cytokine receptors—with NOD-SCID mice.

Manz transplanted human tissues into mice that lacked part of the recombining activating gene (RAG2) and the common gamma chain cytokine receptor and as a result, these mice lacked B and T cells, as well as NK cells. His group also injected human CD34⁺ HSCs from cord blood into the liver of newborn mice instead of adult mice, which Manz says


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mice as a professor at the Jackson Laboratory, a leading genetics research lab in Maine.

Another drawback was that the HIV particles in infected SCID-hu mice remained mostly confined to the transplanted thymus tissue where the infection occurred, and as a result, biopsies were required to analyze the infected cells.

The next advance came in 1995 when a team led by Shultz crossed non-obese diabetic mice (NOD) with SCID mice (*J. Infect. Dis.* **172**, 974, 1995). NOD mice develop

allowed the transplanted cells to be in a better growth environment (*Science* **304**, 104, 2004). This is similar to the natural situation in humans where blood cells are formed in the liver during intrauterine development and immediately following birth. In the recipient mice, just like in humans, the transplanted human CD34⁺ cells then migrate from the liver into the bone marrow.

Still, even the new RAG2/common gamma chain mice can't maintain transplanted human HSCs forever. Six months after transplanting 100,000 human HSCs in a mouse, most of them are gone, Manz says. By comparison, when the transplanted HSCs come from another mouse, they survive much better and expand 10- to 100-fold in just a few months. However, Ramesh Akkina, a professor of microbiology, immunology, and pathology at Colorado State University, says he has been able to maintain human cell engraftment in these mice for up to a year and a half by using CD34⁺ cells derived from fetal human liver (see Figure 1).

The new RAG2/common gamma chain humanized mouse model allows researchers to maintain HIV infection in the mice longer and with higher viral titers than before (*Proc. Natl. Acad. Sci.* **103**, 15951, 2006; *Retrovirology* **3**, 76, 2006). In addition, HIV can also be found in the blood of these mice, with intraperitoneal HIV injection resulting in an HIV titer of about 10,000-100,000 copies/ml of blood. The infected mice also show chronic viremia, because the transplanted human HSCs keep making T cells that serve as HIV targets, as well as CD4⁺ T helper cell depletion.

"For the first time we seemed to have a real model of the adaptive immune system," Manz says. "There was disappointment but now with the new models there is great hype."

BLT: More than a sandwich

Meanwhile, J. Victor Garcia-Martinez, a professor of internal medicine at the University of Texas Southwestern Medical Center, developed another humanized mouse model called BLT (bone marrow-liver-thymus). In an extension of the 1988 McCune SCID-hu model, Garcia added intravenously injected fetal liver CD34⁺ cells that repopulate the bone marrow, which is where the reconstituted human immune cells come from, he says, adding that the bone marrow is a niche for the stem cells to

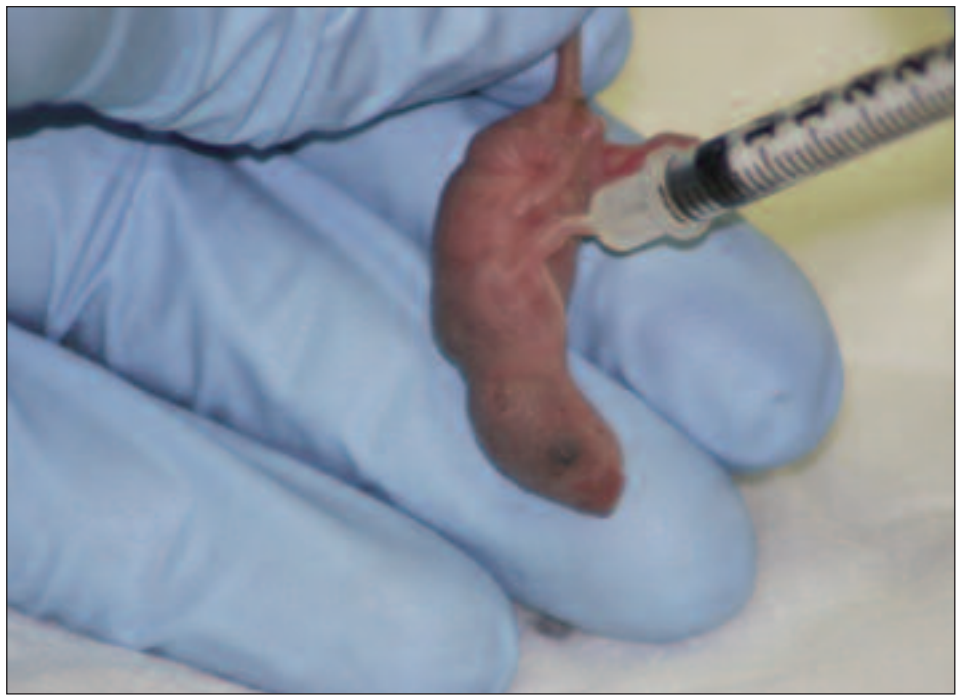


Figure 1. One approach for generating humanized mice. Injection of human CD34⁺ hematopoietic stem cells derived from human fetal liver into the liver of a newborn RAG2/common gamma chain mouse. Ramesh Akkina, a professor of microbiology, immunology, and pathology at Colorado State University, says human cell engraftment in humanized mice generated using this method can be maintained for up to a year and a half, generally the life of the engrafted mouse. Image provided by Akkina.

grow and proliferate. In addition, he uses adult NOD-SCID mice as the recipients of the human cells.

Like RAG2/common gamma chain mice, the BLT mice also developed a human adaptive immune system, including human B and T cells, dendritic cells, and macrophages in blood, as well as in the spleen, lung, liver, and even the gut (*Nat. Med.* **12**, 1316, 2006). "The entire gastrointestinal tract was reconstituted with human lymphoid cells, and that had never been seen before," Garcia says. His group then showed that BLT mice could be infected with HIV by both rectal and vaginal transmission, since these sites were reconstituted with human cells (*J. Exp. Med.* **204**, 705, 2007; *PLoS Med.* **5**, e16, 2008). "Everything that we know is important for [HIV] transmission, pretty much was there," says Garcia. Akkina's group also showed successful mucosal transmission of HIV via vaginal and rectal routes in RAG2/common gamma chain mice (*Virology* **373**, 342, 2008).

Putting them to use

Since these current humanized mice can be infected with HIV, they can already be used to test the efficacy of antiretrovirals

(ARVs), according to Harris Goldstein, a professor of pediatrics, microbiology, and immunology at Albert Einstein College of Medicine. "We have tested ARVs that have been shown to work in humans and they all work in the mouse model," she says. "I would suspect that that indicates that it's predictive [of the human situation]. HIV is HIV, and it's replicating in a human cell, so [if a] drug blocks replication it's going to block it in the model."

But researchers have observed that different doses of ARVs are required for humanized mice because they have a larger renal filtration surface area in proportion to their weight and therefore excrete drugs faster than humans. A study last year with the four licensed classes of ARVs showed that SCID-hu mice need to be dosed with about 12 times more of a given drug per kg body weight per day than humans to reduce viral load in the mice to a similar degree (*PLoS One* **2**, e655, 2007).

That study's lead author Cheryl Stoddart, an assistant professor in the division of experimental medicine at UCSF, has also been using humanized mice to pre-clinically test drug candidates as part of a program



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four legs**



Fatah Kashanchi

funded by the Division of AIDS at the National Institute of Allergy and Infectious Diseases, which enables small companies to have drug candidates screened free of charge. As part of that program, Stoddart has tested dozens of potential drugs, including the candidate drug Bevirimat, an HIV maturation inhibitor developed by Panacos Pharmaceuticals. This drug was able to reduce viral load in humanized mice and protect them from T cell depletion at plasma concentrations that are also achievable in humans after oral dosing (*PLoS One* **2**, e1251, 2007). This drug candidate is now in Phase IIb clinical trials, according to a company spokesperson.

Fatah Kashanchi, a professor of microbiology and tropical medicine at the George Washington University Medical Center, is using RAG2/common gamma chain and NOD-SCID mice to test drug candidates such as an inhibitor of the kinase CDK9, a host cell factor that is needed for HIV transcription. He says using humanized mice for such experiments is far better than using nonhuman primates. “[Using nonhuman primates] would have taken me probably twice the amount of time [and] at least 100 times more money,” Kashanchi says. “These animals are almost like test tubes with four legs.”

The fact that both the BLT and the RAG2/common gamma chain deficient humanized mice can be infected rectally and vaginally with HIV may also enable researchers to test preventive approaches such as pre-exposure prophylaxis (PrEP) and microbicides. For example, Garcia recently showed that BLT mice are protected from vaginal HIV challenge after PrEP with truvada, a combination of the antiretrovirals emtricitabine and tenofovir (*PLoS Med.* **5**, e16, 2008). “If you give the humanized mice that drug on a daily basis for seven days and in the middle of that expose them to HIV intravaginally,” Garcia says, “they are completely protected.” Garcia and Akkina have both started to use the humanized mice to test microbicides. “It offers additional relevant information regarding their potential to actually work when they are eventually tested in humans,” says Garcia, referring to previous microbicide candidates that have failed to protect against HIV infection in clinical trials. “There was very little, if any, evidence that these products would actually work.”

Others are using humanized mice to test novel approaches to treating HIV infection. For example, Pin Wang, an assistant professor

of chemical engineering at the University of Southern California, in collaboration with David Baltimore, a professor of biology at the California Institute of Technology, is using the RAG2/common gamma chain deficient humanized mouse model to test if a lentiviral gene therapy vector is able to bind human target cells and introduce genetic material into them (see *Engineering immunity, LAVI Report*, July-Aug. 2008). And recently Shultz was involved in a study that used NOD-SCID/common gamma chain deficient humanized mice to show that anti-HIV small interfering RNA (siRNA) delivery to human T cells could suppress viral load in these humanized mice (*Cell* **134**, 577, 2008). “Even though these humanized mice might need some further development [to test] primary immune function from HIV vaccines,” Shultz says, “I think they are ready right now to test other approaches such as siRNA.”

Limited response

Before these humanized mouse models will be viable for screening AIDS vaccine candidates, further optimization is required since the immune responses produced in response to infection are somewhat limited. For example, when Manz and his colleagues infected the humanized RAG2/common gamma chain mice with Epstein Barr Virus (EBV), which only infects human B cells, some of the humanized mice eventually developed B cell lymphomas (*Science* **304**, 104, 2004). In EBV-infected humans, this only occurs in immune-suppressed individuals, suggesting the human immune cells in the mice could not control EBV infection in the same way as humans with a fully functional immune system. “This is proof that this [human immune system in the mice] is not the real thing yet,” Manz says.

In a later study, only one of 25 HIV-infected humanized mice showed HIV-specific antibodies and none showed obvious T-cell responses to the virus (*Proc. Natl. Acad. Sci.* **103**, 15951, 2006). None of the other studies with the same mouse model showed strongly convincing HIV-specific immune responses, Manz adds.

In response to Dengue virus, however, Akkina’s lab has observed neutralizing antibody responses in RAG2/common gamma chain mice (*Virology* **369**, 143, 2007). Still, in general the immune responses are sometimes limited, in part because the human T cells mature in the thymus of the mouse and are therefore “educated” on a mouse major histo-

compatibility complex (MHC) instead of a human MHC. This should be less of an issue in BLT mice because they contain human thymic tissue, and in fact Garcia has already observed immune responses to EBV infection. “They mount a classical MHC class restricted human T-cell response,” he says, adding that these were the first HLA-restricted cellular immune responses seen in a humanized mouse model (*Nat. Med.* **12**, 1316, 2006). “[These] are probably the most comprehensive and most clear-cut human immune responses to a specific pathogen in a way that you measure it for humans,” Garcia says. He also demonstrated the production of human antibodies to HIV proteins in BLT mice (*J. Exp. Med.* **204**, 705, 2007). Still, he points out that measuring immune responses in humanized mice will require developing new, more sensitive assays. “You need to miniaturize everything because the volumes and the number of cells are small,” says Garcia.

A mightier mouse?

To ready the humanized mouse model for evaluation of preclinical AIDS vaccine candidates, researchers are now trying to improve upon the immune responses seen in the current models. Manz is collaborating with Richard Flavell, a professor at Yale University School of Medicine, and the Tarrytown, New York-based company Regeneron Pharmaceuticals, on such a project with support from a grant through the Grand Challenges in Global Health Initiative. To further improve the immune responses, researchers generate RAG2/common gamma chain immunodeficient mice that express certain human cytokines important for maintaining human HSCs and supporting myelopoiesis. These mice will also express human MHC class I and II so that the T cells that develop from the transplanted human HSC are “educated” properly. The strategy is to replace the respective mouse genes with their human counterparts in mouse embryonic stem (ES) cells and then make transgenic mice from these ES cells.

The project is now in its third year and Manz says his group and his colleagues at Yale are now testing the first mice. So far Manz says he has some “promising” data. “We think it’s going in the right direction,” he adds. Eventually Manz says this model will be made available to researchers who want to test candidate vaccines.

Another project—also funded by a grant through the Grand Challenges in Global Health Initiative—led by Rudi Balling, scien-


tific director of the Helmholtz Centre for infection research in Braunschweig, Germany, is trying to achieve similar goals by crossing existing mouse strains, according to Manz.

Still, even without the full and appropriate immune responses, there is a way the current humanized mouse models could already be used for HIV vaccine research, says Shultz. “You could take PBL from people who got experimental HIV vaccines and put the PBL in the mice and then challenge with HIV,” he says. “People should be doing that.”


Got stem cells?

Even if researchers succeeded in developing a humanized mouse model with a perfect immune response, researchers will still face another challenge—a limited supply of human CD34⁺ HSCs. “From one cord blood [sample] you can maybe transplant four to eight mice,” Manz says. As a result, experiments with humanized mice are hard to compare with each other because of the low numbers of animals and/or genetic variation between different human HSC sources. The situation is better with SCID-hu mice, in which thymus and liver tissue from one fetal donor is sufficient for 50-60 mice, according to Stoddart. “It remains to be seen yet whether those kind of numbers can be made of any of these other models,” she adds. Still, fetal tissue that can serve as a source for CD34⁺ HSCs is hard to come by—it requires fetal tissue from pregnancies aborted after three months.

To find a source that’s independent from donated tissues, researchers are trying to generate an unlimited number of CD34⁺ HSCs *in vitro*. Hongkui Deng, a professor of cell biology at the University of Peking, is working on another project funded by a grant through the Grand Challenges in Global Health Initiative in which he treats human embryonic stem cells with small molecules that induce differentiation (induction factors) to make them develop into CD34⁺ HSCs. One problem, he says, is that there is no functional HSC marker yet; CD34⁺ expression alone is not enough to be sure a cell is an HSC. Still, he is able to get CD34⁺ cells that, after transplantation into immunodeficient mice, lead to up to 6% engraftment. This means that up to 6% of the blood cells in the recipient mice are human. That’s much less than the up to 80% engraftment one gets after transplanting human cord blood cells, he says, but “that’s already very promising.”



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Leonard Shultz



The state of the field

Stanley Plotkin, MD, is widely regarded as a leading authority on vaccines. He invented the rubella vaccine now used exclusively throughout the world and worked extensively on vaccines for polio, rabies, cytomegalovirus, and rotavirus. As the medical and scientific director for Pasteur-Mérieux-Connaught Vaccines in Paris from 1991-97 and now an executive advisor to the CEO of Sanofi-Pasteur, he has also been at the forefront of AIDS vaccine development. In recent months he has been one of the leading voices in the field as researchers struggle to find new directions in AIDS vaccine research after the halting of immunizations in Merck's STEP trial last year.

Plotkin, a soft-spoken man who cites "Henry V" as easily as he does scientific abstracts, credits two widely-acclaimed books—"Arrowsmith" by Sinclair Lewis and "The Microbe Hunters" by Paul de Kruif—with inspiring him to pursue a career in medicine and research. The New York native earned his medical degree from State University of New York College of Medicine in Brooklyn and trained at hospitals both in the US and abroad. Prior to joining Pasteur-Merieux-Connaught, he was a professor of pediatrics and microbiology at the University of Pennsylvania and a professor of virology at the Wistar Institute, a major player in vaccine discovery.

More than 600 scientific publications are to his credit, and Plotkin is also editor of "Vaccines," now the standard textbook in the field. He has received numerous awards, including the Sabin Foundation Gold Medal and the French Legion of Honor, and has served on several boards related to infectious diseases and vaccinology. He has been chairman of the Infectious Diseases Committee, the AIDS Task Force of the American Academy of Pediatrics, and the Microbiology and Infectious Diseases Research Committee of the US National Institutes of Health, and has been a liaison member of the Advisory Committee on Immunization Practices.

During the early years of the AIDS epidemic, Plotkin advocated strongly for the development of live-attenuated HIV vaccines but US funding agencies thought the strategy was too risky and refused to back his research proposal. He also believes partially effective vaccines that slow disease progression could offer public health benefits in mass vaccination campaigns.

Plotkin recently spoke with IAVI Report Science Writer Regina McEnery about the state of AIDS vaccine research and development.

You have a long history of working in vaccinology but how did you become involved in AIDS vaccine research specifically?

I got involved in AIDS vaccines after I had moved to Paris in 1991 to join what was then called Pasteur-Mérieux-Connaught, now Sanofi Pasteur, and that was because HIV obviously was a significant issue. At that time, Sanofi had just begun to develop an avian pox virus as a vector and so applying that vector to HIV was an obvious thing to do. Parenthetically, that vaccine is now being tested in an ongoing Phase III trial in Thailand, the results of which will be reported in 2009.

So at that point I began to be involved in the problems of developing an HIV vaccine. There was optimism then that a solution was possible. Of course, by that time it was also known that it was not going to be easy and that it wouldn't be a classical vaccine, but there was not a sense that all avenues had been exhausted.

You were one of the few people in the early years of AIDS vaccine development who advocated for developing live-attenuated vaccines. What made you so fervent about this particular strategy?

Well, it's sort of obvious. Live vaccines have the virtue of stimulating all arms of the immune system so they could stimulate production of neutralizing antibodies as the result of the vaccine, and they typically provide the best protection. And actually, one can see that in the SIV system the only thing that is really effective is the live-attenuated virus. I guess you could also say that my experience was largely in the development of live vaccines and certainly, as a class, they are usually effective, but I would also acknowledge that they often bring safety issues with them.

Now the criticism of the approach, which I acknowledge, is that attenuation would not prevent incorporation of the viral genome into the cellular genome. It's a

legitimate objection. Even today, few people are willing to try to continue to develop such a vaccine. Ron Desrosiers [director of the New England Primate Research Center] developed an attenuated virus and has been an advocate of attenuated vaccines, but he also acknowledges the time may not be right for that kind of approach.

What type of attenuation were you proposing when you suggested exploring live-attenuated HIV vaccines?

Well, the basic idea, which was naive, was to deprive the candidate virus of its functional reverse transcriptase to try to prevent or reduce incorporation. I think that early assumption was basically wrong, but we hoped to develop a virus that would have limited replication but no latency.

Did you think then it was feasible to pursue a high-risk idea like live-attenuated HIV vaccines, and is it more or less feasible now?

It certainly was and is a high-risk idea. You could still say that a live-attenuated vaccine candidate is foolish, but one could also say, in light of what's happened since, that it's an idea still worth pursuing if we're ever going to have an effective vaccine.

In the absence of a successful vaccine all ideas should be explored, and I think that the exploration of attenuated SIV vaccines may shed some light on what could be a valuable approach to an attenuated HIV vaccine. Based on that knowledge one might be able to attenuate HIV with more understanding.

However, I think one has to admit that having zero risk associated with any live vaccine is probably a dream, and not something that we should anticipate. But everything in life has a risk/benefit analysis and if we had a vaccine that was highly effective and which rarely caused a problem, one could argue that the advantages and the benefits of a vaccine would outweigh the risks.

The history of live vaccines in general goes along with that. Perhaps the best analogy would be to the oral polio vaccine. It's very clear that there is a risk of paralysis from the vaccine, which is variously estimated but probably is around 1 in 700,000 first doses. That level of risk has been accepted because the oral polio vaccine has been able to eliminate wild-type virus completely in many countries. But in the US, safety frequently takes precedence over everything else.

Which of the other AIDS vaccine strategies that are currently under investigation do you think hold the most promise?

I think at this stage the first thing we need to do is confirm or disconfirm the results of the STEP trial. Leaving aside for the moment the safety issues that arose during the trial, we need to know whether a non-replicating vector will give any efficacy or not and unfortunately we didn't learn that from the STEP trial. When I say efficacy, I mean control of viral replication using adenovirus serotype 5 (Ad5) in people without pre-existing immunity. If some control of viral replication was to be shown, then we need to deal with the potential safety issues involved with using Ad5 vectors by choosing some other non-replicating vector.


I'm not saying anything that's new here. If the result in a future trial is negative, meaning no effect of cellular immunity on viral replication is observed, then we have to acknowledge that inserts in non-replicating vectors will not give sufficient stimulation of the immune system and we have to move toward replicating vectors. In addition, no vaccine is going to prevent infection if it doesn't involve stimulation of antibody responses. So the issue of finding a way to produce antibodies that neutralize primary HIV isolates is still the major issue on the table today.

What do you think is the best hope for vaccines that would not be able to prevent infection?

A vector-based vaccine may allow us to prolong people's lives in a reasonable state of health, especially if used in combination with antiretrovirals, and also might inhibit transmission by lowering the viral load, but I don't see it providing substantial protection against infection unless there's an antibody component.


HIV could be similar to other diseases, like pneumococcal disease, in that reducing the levels of the virus in semen or in the vagina will result in lower transmission rates. With HIV the rate of transmission is already very low, so if you were able to reduce viral load significantly you're certainly going to diminish the risk of transmission and therefore obtain herd immunity. Mathematical modeling shows us that even a partially-effective vaccine, in the sense of reducing viral load, would reduce HIV transmission to the point where the so-called reproductive number would be less than one and, therefore, the prevalence of infection would gradually decrease.

What do you think might explain the observation in the STEP trial that uncircumcised vaccinees



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Stanley Plotkin



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with pre-existing Ad5 immunity were at higher risk of acquiring HIV?

It caught the best minds by surprise, and many are puzzled about it. There's work from Rafi Ahmed [an immunologist at Emory University] suggesting that adenoviruses may be peculiar in their long-term stimulation of T cells, which may increase susceptibility to infection. But I think at this stage one can only guess.

I remain deeply puzzled by the fact that the so-called enhancement is the result of a lower infection rate in the placebo recipients who had high titers against adenovirus. John Moore [professor of immunology and microbiology at Weill Cornell Medical College] has tried to explain this but today we really only have hypotheses. I still think it's possible that the result was due to confounding demographic factors and circumcision distribution. That being said, it's going to be extremely difficult, if not impossible, to go back to Ad5 vectors in a population that has pre-existing Ad5 immunity.

Do you think that a heterologous prime-boost regimen is more likely to provide some degree of effect on viral load?

I think the idea that a vector that is strong in terms of presentation of HIV antigens, such as Merck's Ad5, was worth trying. It's easy to criticize in retrospect, but I think it was a logical thing to do. What could be criticized, I suppose, is the choice of Ad5 alone versus a prime-boost schedule. The criticism could be lodged, and has been lodged, that Merck's results with this regimen in the SIV model were not good enough, but again one has to appreciate the lead time that precedes going into a clinical trial. It's not that you wake up in the morning and say, 'Let's try this vaccine in an efficacy trial.' It requires years of work and so changing the trial because you have a new idea is not something that is likely to happen. The apparent effect of adenovirus immunity on the result was not something that anyone had predicted.

What do you think of the decision not to go forward with the proposed PAVE 100A trial of the DNA/Ad5 prime-boost regimen developed by researchers at the Vaccine Research Center?

The PAVE trial would have had the virtue of telling us whether or not there was any efficacy from non-replicating vectors, or whether non-replicating vectors are simply not strong enough in antigen presentation and therefore are not going to work. I can accept the eventual compromise of reducing the trial size, on the

condition that the new trial is sufficiently powered to tell us whether the vectors employed can reduce viral load. The beauty of science is that you can confirm or disconfirm an idea. It's not like philosophy where all ideas are equal.


What impact has the STEP trial had on industry's involvement in research and development of AIDS vaccines?

It's fair to say that Sanofi Pasteur, like other manufacturers, has been dismayed by the result of the STEP trial. Although let me say that I am a consultant and do not speak for the company, I see no inclination to leave the HIV vaccine field. Sanofi is still developing and testing pox virus vectors, but it remains to be seen whether any non-replicating vector is going to work. If not, for Sanofi and for other companies, it's back to the drawing board.

The fact is that there are many vaccines for other diseases on the table. Vaccine development is expensive and unless there are new basic discoveries I think the companies will probably not exert themselves strongly in the HIV field. I have suggested to Alan Bernstein [executive director of the Global HIV Vaccine Enterprise] that he really should visit the chief executive officers of the major companies to try to influence them to stay in HIV vaccine development. Companies by and large are not there to do basic research. They are there to develop something that has been discovered in an academic laboratory or at a biotechnology company and to take it to a licensed vaccine, which is a major and expensive effort. Now you cannot reasonably expect a company to spend millions of dollars unless there are promising approaches, realistic approaches, to a vaccine. I think every company is looking for a brilliant idea.

What do you think has been the biggest obstacle to AIDS vaccine development?

Unquestionably, it is technical feasibility. The problem with vaccine development has not been a lack of effort. A lot of money and a lot of scientific effort have been put into it, but it has just been an intractable problem. However, by no means would I give up because vaccine development has never been easy.

You know, people say the easy vaccines have been developed, but it's always easy in retrospect. The basic work on the Merck rotavirus vaccine was done in my lab in the mid-1980s but the vaccine was not licensed until 2006. So to say that vaccine development takes a long time is a cliché, but it's true. 

New center focuses on neutralizing antibodies

A new research center, dedicated to developing AIDS vaccine candidates that can elicit broadly neutralizing antibodies against HIV, was established recently by The Scripps Research Institute and IAVI. The new HIV Neutralizing Antibody Center will be housed at Scripps in La Jolla, California, and was established with an investment of US\$30 million from IAVI, extending the existing collaboration between the two institutions. The center will bring together researchers from diverse fields to work on solving what is arguably the single biggest biological obstacle blocking the discovery of an AIDS vaccine—identifying which HIV immunogens are capable of inducing neutralizing antibodies against the virus.

None of the AIDS vaccine candidates or approaches tested so far in clinical trials has induced broadly neutralizing antibodies against HIV. Yet such immune responses play a critical role in many, if not all, of the currently licensed vaccines against other viruses and bacteria, and are believed to be critical to the development of an AIDS vaccine that could effectively block transmission of the virus.

Dennis Burton, an immunology professor at The Scripps Research Institute, says researchers at the new center will be venturing into “uncharted waters” that hopefully will yield a greater level of understanding about the mechanisms that enable vaccines to shield people from infection. “Current vaccines simply mimic natural infections,” says Burton, who directs the HIV Neutralizing Antibody Center. “But it turns out for HIV, simple mimicry has been shown not to be effective.”

Scientists affiliated with the Neutralizing Antibody Consortium (NAC), an international consortium of researchers established by IAVI in 2002, will now collaborate with researchers at the HIV Neutralizing Antibody Center, as well as with scien-

tists in IAVI’s own research and development program. This expanded network will focus on immunogen design and identification of neutralizing antibodies against HIV.

Seth Berkley, president and CEO of IAVI, says the creation of the center will ensure that the best minds and institutions are dedicated to solving one of the biggest challenges facing AIDS vaccine researchers today. “We are excited and hopeful that this collaboration will help to bring us closer to developing a vaccine that will end the AIDS pandemic,” adds Berkley. Burton says the new center will also make it easier to recruit top young scientists to the field. Three new scientists will soon be joining a team of researchers who were already working with Burton. David Montefiori, director of the Laboratory for AIDS Vaccine Research and Development in the Department of Surgery at Duke University Medical Center, says housing this many scientists devoted to the HIV neutralizing antibody question under one roof is “quite an extraordinary thing,” and he is hopeful that it will stimulate a more rapid pace of exploration.

“We have a number of groups working together on various aspects of this problem,” says Montefiori, whose own research involves neutralizing antibodies. “But rarely do you have members of that group in close proximity who are sharing ideas and data in real time. It’s something that is needed.”

Barton Haynes, director of the Duke Human Vaccine Institute and the Center for HIV/AIDS Vaccine Immunology at Duke University, says that the Scripps team has been a leader in structural analysis of neutralizing antibody epitopes and has made enormous strides in understanding how HIV evades an antibody response. “Having the HIV Neutralizing Antibody Center will be a terrific help to the field,” says Haynes. “We shouldn’t give up on this problem and the funding of this center is a signal of renewed commitment.” —*Regina McEnerg*

products, and eventually the development of anti-retrovirals, according to the Nobel assembly which appoints the winners of the US\$1.4 million prize.

German researcher Harald zur Hausen shared the prize for the discovery of human papilloma virus (HPV) types that are linked to the development of cervical cancer, the second most common cancer among women. This research eventually led to the development of vaccines against HPV which provide 95% protection against these two high-risk types. —*Andreas von Bubnoff*

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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 Foundation for the National Institutes of Health, The John D. Evans Foundation, The New York Community Trust, the James B. Pendleton Charitable Trust, The Rockefeller Foundation, The Starr Foundation, The William and Flora Hewlett Foundation; the Governments of Canada, Denmark, India, Ireland, The Netherlands, Norway, Spain, Sweden, the United Kingdom, and the United States, the Basque Autonomous Government, the European Union as well as The City of New York, Economic Development Corporation; multilateral organizations such as The World Bank; corporate donors including BD (Becton, Dickinson & Co.), Bristol-Myers Squibb, Continental Airlines, Google Inc., Henry Schein, Inc., Merck & Co., Inc., Pfizer Inc, and Thermo Fisher Scientific 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.

Nobel awarded for discovery of HIV

This year’s Nobel Prize in Physiology or Medicine was shared by French researchers Françoise Barré-Sinoussi and Luc Montagnier for the discovery of HIV. Barré-Sinoussi and Montagnier discovered the retrovirus now known as HIV in 1983, just two years after the first reports of cases described what is now known as AIDS. The finding allowed cloning of the HIV genome, paving the way for the development of methods to diagnose HIV infection, the screening of blood

Apobec3 may restrict retroviral infection by controlling antibody response

Researchers have found evidence indicating that a cellular antiviral factor called Apobec3, which is associated with anti-HIV activity, can restrict infection with another retrovirus called Friend virus in mice (*Science* **321**, 1343, 2008; *J. Virol.* doi:10.1128/JVI.01311-08). The results of the *Science* study also suggest that Apobec3 may restrict infection with Friend virus by improving the production of virus-specific neutralizing antibodies, leading the authors of this study to suggest that in humans, Apobec3 might fight HIV infection at least in part by improving neutralizing antibody responses against the virus.

Human Apobec3G and 3F are among a growing number of cellular factors that are thought to naturally inhibit HIV infection. Apobec3G and 3F interfere with HIV's reverse transcription process in several ways. They encode an enzyme thought to introduce mutations in the reverse transcribed cDNA made from the retroviral RNA, which inhibits retroviral replication by destroying the cDNA's ability to encode viral proteins. However, HIV has evolved a factor called Vif (viral infectivity factor) that inhibits Apobec3G and 3F, in part by targeting it for accelerated degradation in the proteasome. The role of human Apobec3G as an anti-HIV factor and its inhibition by Vif were first described in 2002 (*Nature* **418**, 646, 2002). Researchers are trying to find molecules that could inhibit Vif and therefore allow Apobec to perform its natural function.

The *Science* study suggests that in the absence of Vif, Apobec may also keep HIV at bay by improving neutralizing antibody responses against the virus. In a series of genetic experiments in mice, Warner Greene, director and senior investigator at the Gladstone Institute of Virology and Immunology in San Francisco who led the study in collaboration with researchers at the National Institute of Allergy and Infectious Diseases (NIAID), and colleagues showed evidence that *Rfv3* (recovery from Friend virus 3), a genetic trait that has been known for 30 years to be important for the recovery of mice from Friend virus by promoting the formation of neutralizing antibodies, is encoded by the murine *Apobec3* gene. "When you remove *Apobec* in the *Rfv3* resistant strain, [the mice] become susceptible [to Friend virus]," Greene says.

People have been wondering about which gene encodes Rfv3 for 30 years, says B. Matija Peterlin, a professor of medicine, microbiology, and immunology at the University of California in San Francisco, who was not connected to the studies. Friend virus is only the second type of retrovirus that has been shown in *in vivo* animal experiments to be repressed by Apobec3, according to Peterlin, who showed last year that the absence of *Apobec3* in mice leads to an increased susceptibility to infection with mouse mammary tumor virus (*Nature* **445**, 927, 2007).

However, Peterlin says the new studies do not completely exclude the contribution of a gene other than *Apobec3* in restrict-

ing Friend virus infection. Mario Santiago, a postdoctoral fellow at the Gladstone Institute and the first author of the *Science* study, however, says that "if those genes exist, their contribution would be negligible."

Additionally, when Greene and colleagues infected *Apobec3*-deficient mice with Friend virus, they observed that these mice also produced fewer Friend virus-specific neutralizing antibodies, resulting in a higher titer of Friend virus. This is consistent with the previous observation that Rfv3 mediated recovery from Friend virus correlates with Friend virus-specific neutralizing antibody responses.

"For the first time we link [Apobec3] to the adaptive immune response and more specifically to [the production of virus-specific] neutralizing antibodies," says Greene. But the authors of the other study showing that Apobec3 plays a role in inhibiting infection with Friend virus are more cautious. "We think there is no direct connection between the known Apobec3 functions and the control of antibody production," says Masaaki Miyazawa, lead author of the *Journal of Virology* study and a professor and chairman at the department of immunology at Kinki University School of Medicine in Japan.

Greene says the next experiments will try to elucidate the mechanism of the Apobec3/neutralizing antibody connection. "Now the question is how Apobec is eliciting these neutralizing antibodies," says Greene.

One possible mechanism is that simply by lowering virus levels, Apobec might make the immune system less overwhelmed, thereby allowing it to mount a better antibody response. "It's a little counterintuitive," Peterlin says. "If you have less virus, you make more antibodies and [a better] cytotoxic T lymphocyte response." Another possibility is that Apobec may be limiting viral replication in immune cells important for the production of neutralizing antibodies like T cells or dendritic cells, Greene says. It's also possible that Apobec3, which according to Greene is expressed in B cells, might have a direct role in shaping the antibody repertoire by increasing the mutation rate in the DNA encoding antibody immunoglobulins.

For HIV, a possible involvement of Apobec3 in the neutralizing antibody response could mean that Vif antagonists, should they be identified, would have the added benefit of eliciting a strong humoral immune response, according to Greene. And higher Apobec levels may explain why some individuals known as exposed seronegatives (ESNs) are able to resist HIV infection despite sometimes repeat exposure. A 2005 study of ESNs mapped their resistance to HIV and production of antibodies to the same chromosomal region as the Apobec locus (*AIDS* **19**, 1015, 2005). Perhaps, Peterlin says, increased Apobec3 levels could protect ESNs from HIV infection because the virus might not be able to make enough Vif protein to counteract Apobec3 levels in these people. "It's a very attractive notion," he adds. —*Andreas von Bubnoff*