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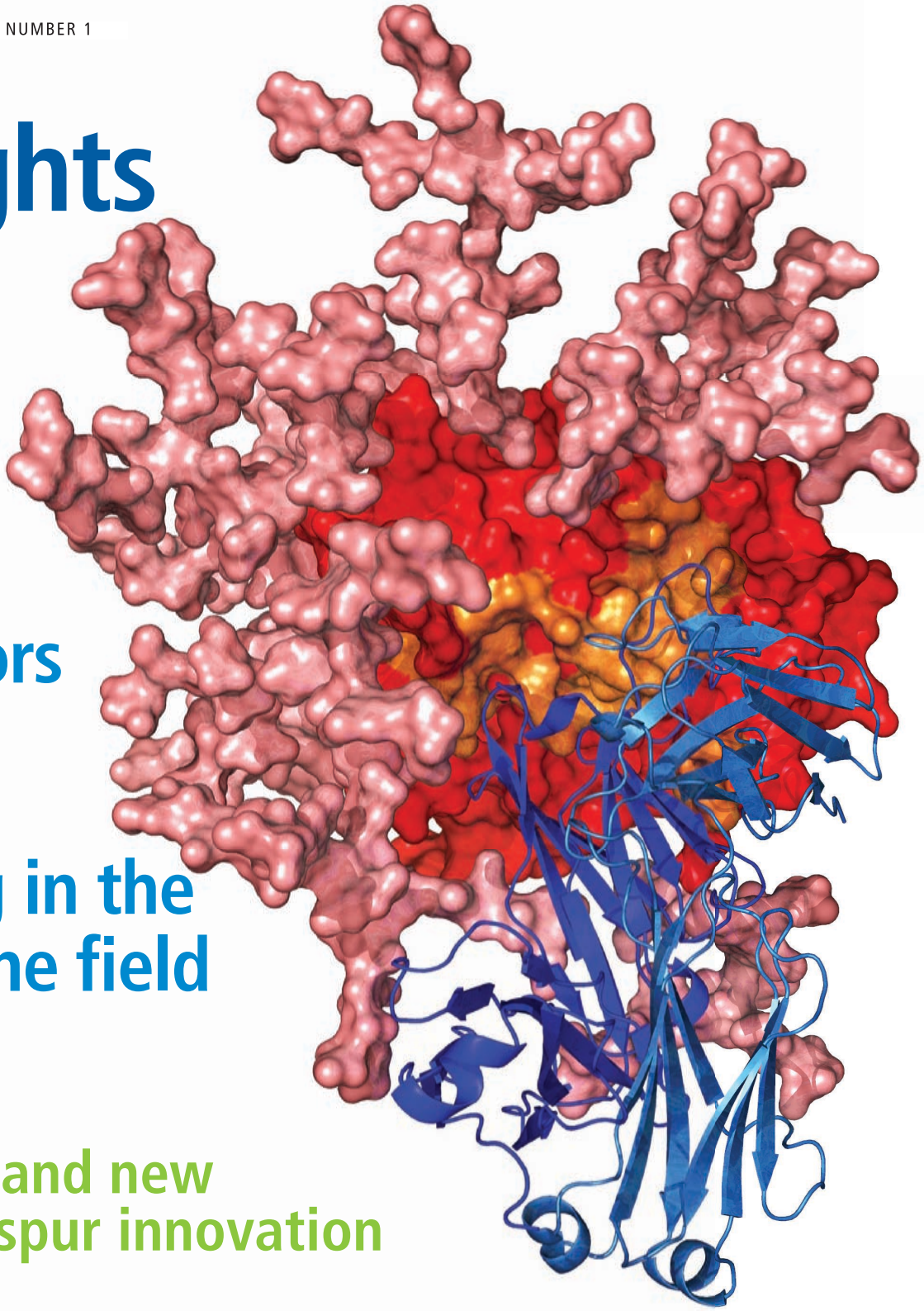


Highlights from CROI

Young investigators describe hurdles to succeeding in the AIDS vaccine field

PLUS:

More money and new programs to spur innovation

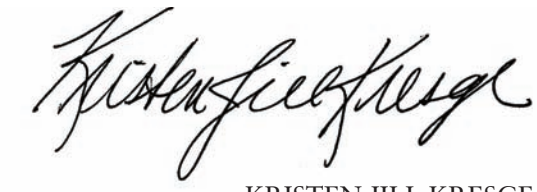
EDITOR'S LETTER

In past years, the opening session of the Conference on Retroviruses and Opportunistic Infections (CROI) has coincided with what would be considered by many to be much bigger events—the Super Bowl or the annual Academy Awards Ceremony. But oftentimes, the conference itself has had a "big event" feel, generating its fair share of publicity. At the 3rd annual CROI in 1996, data showed that the first protease inhibitor, when used along with two other antiretrovirals (ARVs), could substantially boost antiviral activity and control HIV's furious replication. This, of course, opened the door to effective combination therapy for the treatment of HIV/AIDS. Over the following years, other waves of excitement would sweep over this annual scientific conference as new strides were made in the treatment of HIV infection.

What was remarkable at this year's 16th annual CROI was not a new advancement in the ability to treat this disease, but rather how undeniably well different combinations of the now more than 20 ARVs work in controlling HIV replication. Granted, after 28 years of battling HIV, there is still a long way to go. Throughout developing countries, access to life-saving ARV therapy still reaches only a fraction of individuals in need, and despite decades of research, condoms and circumcision remain the only effective means of protection against sexual transmission of HIV, the most common route of infection. But as Robert Siliciano of Johns Hopkins University School of Medicine remarked in his delivery of the 14th Bernard Fields Memorial Lecture, "It is now possible to completely stop HIV replication," with ARV therapy. "It is actually *highly active* antiretroviral therapy," he said.

This has led researchers to pursue with a renewed vigor the possibility of eradication—curing an infected individual of HIV infection. By all accounts, this will be no easy task. Latent reservoirs of HIV-infected $CD4^+$ T cells persist in unknown hiding spots in the body (think of them as the virus's network of terrorist sleeper cells). And now, researchers think there is at least one additional mysterious reservoir of infected cells that is contributing to a continued ongoing low-level viremia seen even in HIV-infected individuals that are on effective ARV therapy.

Given the complexity and challenges relating to eradication, it is no surprise that some of the greatest excitement at this year's CROI was related to HIV prevention strategies. Results from a Phase IIb trial of the microbicide candidate PRO 2000 offered the first positive microbicide trial results so far, and more promising data from nonhuman primate studies continued fueling optimism that the success of ARVs may extend to their use in pre-exposure prophylaxis. Meanwhile vaccine researchers are mining elite controllers for clues about what a partially effective AIDS vaccine might look like, and some researchers now think that T-cell responses similar to those seen in elite controllers will likely be induced by vaccine candidates in the near future. Wouldn't all that be better than the Super Bowl?



KRISTEN JILL KRESGE

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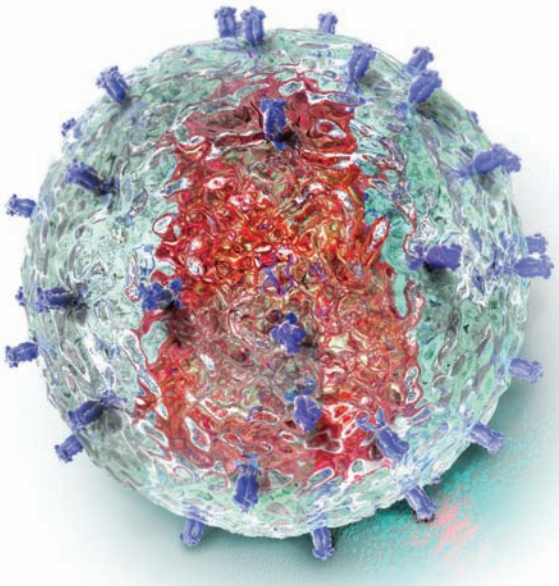

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[ON THE COVER]

The HIV-1 gp120 envelope glycoprotein (red) is protected from antibody recognition by multiple mechanisms, including self-masquerading glycan (pink). The b12 antibody (blue), however, finds a site of vulnerability (orange) to exploit and effectively neutralizes HIV-1. This site is the initial site of HIV-1 contact with the CD4 receptor and a focus of current vaccine efforts.

Image courtesy of the Structural Biology Section, Vaccine Research Center, NIAID/NIH and rendered in PyMOL and POV-Ray by Jonathan Stuckey.

Canvassing CROI

The successes of ARV therapy and promising results with new HIV prevention strategies stoke excitement at recent scientific meeting

By Kristen Jill Kresge and Regina McEnery

At the opening session of the 16th Conference on Retroviruses and Opportunistic Infections (CROI)—which was held this year from February 8-11 in Montreal, Canada—the 3rd N'Galy-Mann Lecture was given in tandem by Glenda Gray and James McIntyre, executive directors of the Perinatal HIV Research Unit at the University of the Witwatersrand in Soweto, South Africa. They spoke about the challenges and opportunities associated with conducting HIV research in South Africa and provided a historical recounting of many of the successes that have been transformative in this community, including the provision of antiretroviral (ARV) therapy to HIV-infected individuals and the use of ARVs to prevent mother-to-child transmission of the virus.

Indeed it seems many hopes in combating HIV these days are pinned to ARVs, whether it is in expanding access among HIV-infected individuals worldwide, developing microbicide gels based on existing ARVs, or using them as a means of pre-exposure prophylaxis (PrEP) to block HIV infection. It is also clear that ARV therapy has its limitations. Putting aside the immediate side effects, researchers are now gaining more insights into longer-term complications associated with treatment, which were the focus of several sessions at CROI. And a pair of studies provided evidence to suggest that complete suppression of HIV replication, which is routinely measured in blood, does not necessarily correspond with lack of virus shedding in semen, suggesting HIV transmission may still be possible even if individuals are on ARV therapy. It is now also becoming more apparent that eradicating HIV from infected individuals is unlikely to be accomplished with ARVs alone.

All of this suggests that research on new HIV prevention strategies, including microbicides, PrEP, and vaccines, will continue to be top priorities. And this year's CROI showcased some promising results from both clinical trials and animal studies evaluating microbicides and PrEP, providing a burst of enthusiasm around new HIV prevention strategies. "It's an exciting time in the prevention field," said Sharon Hillier, vice chairman of the Department of Obstetrics, Gynecology, and Reproductive Sciences at the University of Pittsburgh. Researchers are also still mining for clues from the STEP trial, as well as from individuals who can successfully control HIV infection, to gain insights into the types of immune responses that may be critical to vaccine-induced control of HIV.

The cause of persistent viremia

Robert Siliciano, professor of molecular biology and genetics at Johns Hopkins University, delivered

the Bernard Fields Memorial Lecture this year and spoke about new ways of understanding and evaluating the efficacy of ARV therapy, which when taken properly, can often completely stop new cycles of viral replication. However, residual viremia still occurs even in individuals on completely suppressive ARV therapy. This ongoing, incredibly low-level viremia, which when quantified with super-sensitive assays is approximately one copy of HIV RNA/ml of blood, is coming from at least two sources, according to Siliciano. One is the long-lived, and long ago described, reservoir of latent HIV-infected $CD4^+$ T cells. While activated T cells die rather quickly, HIV's genome can be integrated into long-lasting memory T cells on their way back to a resting state very early in the course of HIV infection, what Siliciano calls the "perfect recipe for persistence." He estimates that only one million of these intrinsically stable, resting $CD4^+$ T cells harbor latent HIV, but because of their slow decay rates, "it would take over 70 years to eradicate a reservoir of a million cells," Siliciano said.

Another still undetermined reservoir is now also believed to be contributing to the persistent, yet minute viremia. "The residual viremia is complicated," said Siliciano. He has observed that residual viremia in individuals on suppressive ARV therapy is dominated by a small number of viral clones that are not found in resting $CD4^+$ T cells. The goal of rooting out and eliminating these pools of virus-infected cells is still the focus of much research, but so far at least one strategy—treatment intensification—in which additional potent drugs are added to an individual's existing ARV regimen doesn't seem to be the answer. "We will never reduce residual viremia any further with ARV drugs," said Siliciano.

Two other studies presented at CROI looked at whether suppression of HIV in the blood corresponds with suppression of the virus in other compartments. Several studies have established a strong relationship between HIV viral load, as measured in blood, and heterosexual transmission rates. These studies indicate that individuals on ARV therapy with very low or undetectable viral loads are less likely to transmit the virus. However, some studies now suggest that in some individuals on suppressive ARV therapy, there is still ongoing viral shedding in semen, indicating that HIV transmission may still be possible.

In the first study, researchers from the University of Toronto followed 25 HIV-infected individuals who had never been on ARVs and used the branched DNA assay, which is better at detecting HIV RNA in semen, to measure their HIV viral loads in blood and seminal plasma samples following initiation of highly active antiretroviral therapy (HAART). They found that a significant proportion—12 of the 25 individuals—had isolated HIV shedding in semen over a six-month period after initiation of HAART, which resulted in an undetectable viral load in blood (fewer than 50 copies/ml). In four of these individuals with undetectable viral loads in blood, levels of isolated semen HIV shedding were greater than 5,000 copies/ml in seminal plasma.

Researchers also studied 12 individuals who had been on a suppressive HAART regimen for at least four years and found that four of them also had isolated HIV shedding in semen. Prameet Sheth, a PhD student at the University of Toronto who presented this study, said the virus that was shed was potentially infectious—HIV isolated from the individual with the highest level of seminal shedding (16,000 copies/ml of seminal plasma) was capable of infecting activated $CD4^+$ T cells in *in vitro* studies. "Our study shows that even though HAART will be able to reduce sexual transmission of HIV on a population level, there is still an individual risk that exists despite long-term HAART," said Sheth.

Researchers did not find any association between the ARVs used and the level or frequency of HIV shedding in seminal plasma in these individuals, and they observed a wide differential in penetration of ARVs in both seminal and blood plasma.

In a second study, researchers at the Hospital Pitié-Salpêtrière in Paris collected blood and semen samples from 145 HIV-infected, ARV-treated males who were participating in a reproduction program that allows men to have their sperm washed of HIV for transplantation into their uninfected female partners. Over a six-year period they collected 264 paired blood and semen samples and found undetectable HIV viral loads (fewer than 40 copies/ml of plasma) in both 85% of the time. Nine of the paired samples, only about 3%, showed detectable levels of HIV in both blood and semen. However, Anne-Geneviève Marcelin, who presented the study, reported that seven men or 5% of the study population had detectable HIV levels in seminal plasma even though there was no detectable virus in blood. Marcelin said these results suggest a "small, residual risk of transmission is still possible during unprotected sexual intercourse."

Another perennial question regarding HIV transmission is whether the virus that is transmitted and establishes infection is typically cell-free virus in plasma, detectable by viral RNA, or cell-associ-

We will never reduce residual viremia any further with ARV drugs.

– Robert Siliciano

ated virus in lymphocytes, detected by proviral DNA. To determine which of these viral reservoirs in male genital secretions is the primary source of HIV infection, David Butler, a post-doctoral student at the University of California in San Diego, studied four HIV transmission pairs, all of whom were men who have sex with men (MSM). Blood samples were collected an average of 59 days following the estimated date of HIV infection, and semen samples were collected from the infecting partner an average of 72 days after transmission. Genetic sequencing was used to analyze both the transmitted and infecting virus, and in all four cases Butler concluded that the virus that was transmitted and established infection was cell-free virus originating in the infecting partners' seminal plasma. He is now planning to study a greater number of transmission pairs.

Even though HAART will be able to reduce sexual transmission of HIV on a population level, there is still an individual risk that exists despite long-term HAART.

– Prameet Sheth

First hint of microbicide efficacy

Some of the more encouraging data at CROI came from a triumvirate of clinical and nonhuman primate studies with new HIV prevention strategies. The first study, known as HPTN 035, evaluated the safety and efficacy of the microbicide candidate PRO 2000, a topical gel composed of 0.5% of a synthetic polyanionic polymer that non-specifically acts to block attachment of HIV to host cells.

This Phase IIb study enrolled 3,099 women at seven clinical trial centers in Africa and the US and evaluated the efficacy of PRO 2000, as well as a second topical microbicide called BufferGel, which contains an agent designed to boost the natural acidity of the vagina in the presence of seminal fluid.

The study also had two control arms—one received a placebo gel and the other, which was unblinded, received only condoms and no gel. A no-gel arm was included in the trial over concerns that the placebo might have antimicrobial properties that could protect against HIV.

The results of this study showed that women who were randomly selected to receive both PRO 2000 gel along with condoms had 30% fewer HIV infections than those who received the placebo gel and condoms. At the conclusion of this three-year trial, there were 36 HIV infections among women in the PRO 2000 group, compared to 54 in the BufferGel group, 51 in the placebo gel group, and 53 in the no-gel group.

However, Salim Abdool Karim, a clinical infectious disease specialist who led the PRO 2000 study, cautioned that the results were not statistically significant compared to either the placebo gel or no-gel groups. "This could be a chance finding," he said, adding that additional evidence would be necessary to "conclusively determine whether PRO 2000 is an effective microbicide."

When researchers analyzed the data based on adherence, they found that women who reported using the gel at the last coital act at least 85% of the time, had an overall 44% reduction in HIV infection compared to women who received the placebo gel. And in women who reported using the gel that often without regularly using condoms, there was a 78% reduction in HIV infection compared to the placebo group.

There was a palpable level of excitement following Karim's presentation, with many audience members rushing to the microphones to congratulate the researchers on the conduct and results of the trial. Karim said this excitement was understandable given the recent results from two trials of other microbicide candidates. Carraguard, made from a seaweed derivative, was found last year not to reduce the risk of HIV acquisition in a three-year, Phase III study of 3,200 women in South Africa. And a Phase III trial of cellulose sulfate that had enrolled 1,333 women was discontinued in December 2007 after early data suggested that the microbicide candidate might be contributing to an increased risk of HIV infection.

"We are at the end of a series of disappointments," Karim said. "We need something that gives us hope. The HPTN 035 trial results represent that hope." A Phase III study of PRO 2000 conducted by the Microbicide Development Programme in the UK is nearing completion in South Africa, Tanzania, Uganda, and Zambia. This trial has enrolled 9,000 women, and results, which are expected in late 2009, will provide additional data on whether PRO 2000 is effective at blocking HIV transmission.

New animal data on PrEP

Other excitement came from two nonhuman primate studies, conducted by the US Centers for Disease Control and Prevention (CDC), which provided additional evidence for the effectiveness of PrEP. Studies in nonhuman primates have shown that ARVs administered systemically prior to exposure to a simian immunodeficiency virus (SIV)/HIV hybrid known as SHIV can prevent infection, although the success of this intervention seems to vary based on both the challenge model and the ARVs (see *PrEP Work*, *IAVI Report*, Nov.-Dec. 2008).

One study at CROI evaluated the efficacy of intermittent oral PrEP use—a strategy referred to as iPrEP. In this study, CDC scientists administered the human equivalent doses of oral Tru-

vada—a combination pill of two ARVs, tenofovir and emtricitabine (FTC). Four groups of six macaques received two doses of Truvada, one prior to and one following rectal SHIV challenge. The first dose was administered as long as seven days before or as soon as two hours prior to challenge, and the second dose was administered either two or 22 hours after challenge. These animals were then compared to 32 untreated controls.

All animals were challenged once a week with SHIV over a 14-week period, with a dose of Truvada administered before and after each of the 14 challenges. It took a median of two challenges to infect the untreated control animals. However, three of the six animals in the groups that received Truvada either two hours before and 22 hours after, or seven days before and two hours after challenge were protected against SHIV infection throughout the 14 weeks.

The best results were seen in the group that received Truvada either 22 hours before and two hours after, or three days before and two hours after challenge. In these two groups, five of the six animals were completely protected against SHIV infection over the 14-week period. J. Gerardo García-Lerma, the CDC researcher who presented these findings, reported that comparable levels of both tenofovir and FTC were seen in the infected and uninfected animals. He also said researchers observed a blunted level of acute viremia in the macaques that were infected despite PrEP, as compared to controls.

All of the ongoing PrEP trials are testing the efficacy of daily dosing, but there is interest in iPrEP because of the concern that daily adherence could prove to be a major barrier to PrEP effectiveness. Intermittent PrEP use would also dramatically slash the cost of providing this intervention. Just how the drugs are given intermittently could be driven by exposure or based on a fixed-dosing schedule.

Results were also presented from a topical PrEP study that compared the effectiveness of gels containing either 1% tenofovir or a combination of 1% tenofovir/5% FTC against repeat, low-dose vaginal SHIV challenge in female pigtailed macaques. Two groups of six macaques received either the 1% tenofovir gel or the 1% tenofovir/5% FTC combination gel. These groups, as well as two animals who received no gel and nine who received a placebo gel, were challenged twice a week over the course of the 10-week study.

The two animals who received no gel were infected after three and five SHIV challenges respectively, while eight of the nine animals who received the placebo gel were infected after a median of four challenges. However, both groups of six animals who received either the tenofovir or tenofovir/FTC combination gel were completely protected against SHIV infection after 20 challenges.

Concentration levels of the drugs in blood were measured in the treated animals 30 minutes after each gel application. Charles Dobard, the CDC researcher who presented this study, reported that only about 0.3% of the drug was absorbed systemically. This could be an advantage of topical PrEP, in that less systemic drug absorption could lead to fewer potential side effects and make it less likely that drug-resistant strains of HIV would develop in individuals who become HIV infected despite using the gel.

There are currently six clinical trials of PrEP involving nearly 21,000 volunteers. The VOICE study, which involves 4,200 women in Africa, is comparing the safety and acceptability of oral PrEP to a topical microbicide formulation. The first data on the effectiveness of PrEP from clinical trials will be available next year.

Mining for vaccine clues

Meanwhile, researchers are continuing to mine data from the STEP trial, which showed that Merck's adenovirus serotype 5 (Ad5)-based vaccine candidate known as MRKAd5 had no effect on HIV infection and may have enhanced susceptibility to HIV infection in certain sub-groups of volunteers, including those with pre-existing antibody immunity to Ad5. One possible explanation for the increased susceptibility to HIV infection is that individuals with higher Ad5 antibody levels would also have higher levels of Ad5-specific T cells, which following vaccination, would expand and become activated, creating more target cells for HIV. But a study presented at CROI cast doubt on this hypothesis. Using flow cytometry, researchers measured levels of Ad5-specific T cells from volunteers in an earlier Phase I trial with MRKAd5 administered at the same dose and schedule as in the STEP trial. They analyzed samples from 25 volunteers prior to vaccination—some of whom subsequently acquired HIV—and found a similar magnitude of Ad5-specific T-cells among all trial volunteers, as measured using IFN-γ ELISPOT assay, regardless of their pre-existing Ad5 neutralizing antibody levels. And after vaccination, there was no significant difference in the level of Ad5-specific $CD4^+$ T cells between individuals who remained uninfected or seroconverted during the course of the study. Although this suggests Ad5-specific T cells are unlikely the cause of an increased susceptibility to HIV, researchers were

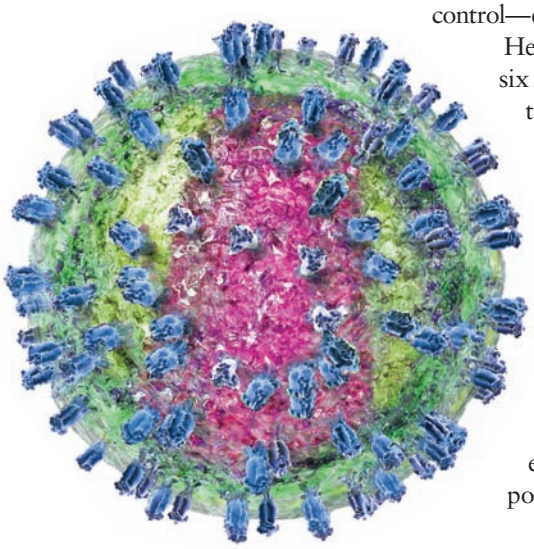
We are at the end of a series of disappointments. We need something that gives us hope. The HPTN 035 trial results represent that hope.

– Salim Abdool Karim

unable to rule out preferential trafficking of activated Ad5-specific T cells to the mucosal sites as a mechanism for increased risk of HIV infection.

All volunteers in this Phase I trial who seroconverted became infected after a single vaccination. Yet participants with no pre-existing Ad5 immunity developed both antibody and Ad5-specific cellular responses following the first vaccination and researchers still did not observe an enhanced susceptibility to HIV infection among these volunteers after subsequent vaccinations. This would suggest that it is unlikely that Ad5 immunity increases susceptibility to HIV infection following Ad5-based vaccination, said Natalie Hutnick, a molecular biologist from the University of Pennsylvania who presented the findings.

Another study, presented by David Heckerman, senior director of eScience at Microsoft Research, described what he called "a hidden success in the STEP trial." Heckerman, along with colleagues at the Ragon Institute including its director Bruce Walker and Florencia Pereyra, set out to identify what best predicts viral control in HIV-infected individuals. Using a predictive model they developed, researchers analyzed 148 HIV controllers—individuals who maintain viral loads of less than 2,000 RNA copies/ml blood without treatment—and 102 chronic progressors. Certain previously identified human leukocyte antigen (HLA) alleles are associated with control of HIV, yet some individuals with these alleles still have high viral loads, Heckerman said. This led researchers to hypothesize that it is not the HLA allele but rather the specific cytotoxic T lymphocyte (CTL) epitopes they target that are responsible for control of virus. They found that, among these individuals, recognition of optimally defined CTL epitopes were the best predictor of viral control—even better than HLA class I alleles.



Heckerman and colleagues then identified six of what they referred to as "good or protective" HIV epitopes in these viremic controllers that were significantly associated with viral control. Some of these epitopes correspond to HLA alleles that were not previously thought to be protective. Researchers then analyzed post-immunization, pre-infection immune responses in a group of 19 participants from the STEP trial, who subsequently became HIV infected, to see if targeting of these six specific epitopes was associated with a lower set-point viral load among vaccinated volunteers. Of the 19 participants, not a single person had responses to more than one of these six epitopes, but those who responded to at least one had lower viral loads than those who didn't.

Ten of the 19 individuals studied had the A*02 allele, but only four responded to LV10 on Nef, one of the protective epitopes they identified. Heckerman suggested that this could be because their immune systems were distracted by targeting other epitopes. The four participants with A*02 that did respond to LV10 had lower viral loads. Heckerman concluded that the design of a successful immunogen may therefore hinge on inclusion of good epitopes as well as exclusion of others that could just distract the immune system.

Clinical data on protein vaccine

Data was also presented from a Phase I/II dose-escalation study of the vaccine candidate F4/AS01, developed by GlaxoSmithKline Biologicals (GSK), which consists of a recombinant fusion protein (F4) comprised of four clade B HIV antigens—Nef, reverse transcriptase from Pol, and p24 and p17 from Gag. The vaccine candidate was administered along with the company's proprietary AS01 adjuvant to 180 volunteers at three different doses (10 µg, 30 µg, and 90 µg). Marguerite Koutsoukos, project leader of the HIV vaccine program at GSK, reported that all volunteers who received two intramuscular injections of the vaccine candidate at the lowest 10 µg dose developed $CD4^+$ T-cell responses to at least three antigens in the vaccine candidate and 80% had responses to all four, as measured by intracellular cytokine staining for expression of interleukin (IL)-2 and at least one other marker of activation, including either expression of TNFα, IFN-γ, or CD40L. The majority of F4-specific $CD4^+$ T cells secreted IL-2 alone, or in combination with TNFα, IFN-γ, or both, and were persistent. "A substantial $CD4^+$ T-cell response was maintained throughout the entire study period," said Koutsoukos.

Very low $CD4^+$ T-cell responses were observed in volunteers who received the F4 vaccine without the adjuvant, and no $CD8^+$ T-cell responses were observed in any volunteers. GSK is now evaluating the candidate in a Phase I trial in HIV-infected individuals to explore the potential of the candidate in a therapeutic setting. According to Koutsoukos, since the candidate only induces a $CD4^+$ T-cell response, and not a CD8+ T-cell response, the company would consider testing the candidate prophylactically in "a more complex regimen including other strategies." ■

In With the New...

The AIDS vaccine field considers ways to encourage innovation and recruit new minds to the effort

By Regina McEnery

Peter Kwong clearly remembers the day a seminar helped guide his career path to AIDS vaccine research.

It was 1991 and Kwong, working toward a PhD in biology at Columbia University, was among 25 students who gathered to hear pioneering Australian biologist Peter Coleman describe how he had used crystallography and the relatively new technique of structure-based drug design to define small-molecule inhibitors from the three-dimensional structure of neuraminidase, a protein found on the outer layer of the influenza virus.

Coleman's pioneering research in structural biology would eventually lead to a new class of antiviral drugs against influenza, but in the early 1990s it was still conjecture whether crystallography—which primarily relies on X-rays to determine the shape and structure of proteins—was going to be useful for the pharmaceutical industry. Kwong was impressed with the notion that you could use atomic-level characterization of proteins and eventually started wondering whether structural biology could also be useful in vaccine design, specifically for HIV.

He decided to tackle this question and now, as head of the Structural Biology Section at the Vaccine Research Center (VRC) at the National Institute of Allergy and Infectious Diseases (NIAID), part of the US National Institutes of Health (NIH), Kwong is using X-ray crystallography to understand one of the rare broadly neutralizing antibodies against HIV called b12 (see cover image). In work originally featured on the cover of the journal *Nature*, Kwong's lab used X-ray crystallography to illustrate how b12 blocks HIV from entering $CD4^+$ T cells by precise targeting of the initial contact site between virus and cell receptor (*Nature* **445**, 732, 2007). This work is important because the lack of immunogens capable of provoking a strong immune response against HIV remains one of the biggest barriers to the discovery of an effective AIDS vaccine.

Kwong and his collaborators Bill Schief and David Baker at the University of Washington are building epitope scaffolds—another tool of structural biology that acts as a template for monoclonal antibodies—to try and teach the immune system to create antibodies that recognize the ever-changing face of HIV. "We're basically performing magic," says Kwong. "But then everything in science is magic until you figure it out."

HIV has arguably been studied more strenuously and comprehensively than any other pathogen in history. But since 1983, when French researchers and 2008 Nobel Laureates Luc Montagnier and Françoise Barré-Sinoussi discovered the retrovirus, there has not been a Eureka moment that has opened the door to an effective vaccine.

Several new grant programs, including many at the US National Institutes of Health (NIH), are designed to encourage innovation in AIDS vaccine research and attract new investigators to the field. Listed below are some additional NIH grant/award programs that apply to innovation but are not specific to the AIDS vaccine field.

PIONEER AWARD PROGRAM: Supports individual scientists of exceptional creativity who propose pioneering or transformative approaches to major challenges in biomedical and behavioral research. Initiated in 2003, each award provides US$2.5 million over five years. Sixteen awards were granted in 2008.

NEW INNOVATOR AWARD: Seeks to stimulate highly innovative research and support promising new investigators. Targeted for young researchers who have not received a traditional NIH research grant. Established in 2007, it provides $1.5 million over five years per recipient.

TRANSFORMATIVE R01 PROGRAM (T-R01): In response to concerns that traditional R01 grants may discourage submission of risky research proposals, NIH created T-R01 grants to support exceptionally innovative, unconventional, and high-risk projects with a potential for high impact. Launched in 2008, the NIH expects to invest $250 million over the next five years on this program, beginning with 60 awards in 2009.

More information about these programs is available from the NIH (www.nih.gov).

Whether or not Kwong's experiment leads to the discovery of an HIV immunogen capable of inducing antibodies against HIV, his research is one of the many innovative approaches being utilized to overcome a number of daunting biological challenges in AIDS vaccine development. Following some recent setbacks, notably the failure of Merck's adenovirus serotype-5 (Ad5)-based vaccine candidate known as MRKAd5 in the STEP trial, the field is trying to invigorate research efforts by pursuing new ways to attract more young researchers like Kwong, and encourage more innovative thinking.

The search for new blood and fresh ideas face a number of practical hurdles, though, that threaten not just the pace of AIDS vaccine science but the entire research arena. The percentage of new investigators throughout academia competing for their first US government-funded general research grant, known as an R01, has declined from 35% in 1965 to 25% in 2003. Meanwhile, the average age of principal investigators rose from 35-40 in 1983 to 50 in 2003, according to José Esparza, a senior advisor on HIV vaccines at the Bill & Melinda Gates Foundation.

With fewer young, less-established researchers competing for R01 grants, the pace of scientific breakthroughs, such as those that will lead to an AIDS vaccine, will slow considerably, Esparza and others contend. To ensure this doesn't happen, agencies and foundations that fund AIDS vaccine research are creating new ways to encourage young scientists to enter the field and are developing new funding streams to encourage more innovative thinking.

The hunt for innovation

Leading the charge to spur innovation is NIAID, which devoted US$497 million of its $1.5 billion HIV/AIDS budget to vaccine research in 2008 and has made the development of a safe and effective AIDS vaccine a top priority. Last March, NIAID held a day-long summit attended by 200 researchers to discuss shifting priorities in AIDS vaccine research and the myriad of challenges still facing vaccine development (see *Balancing AIDS vaccine research*, *IAVI Report*, March-April 2008).

At the summit, which was sparked by the results of the STEP trial, some researchers urged NIAID to place a higher priority on basic discovery research because of the outstanding questions about how best to develop a vaccine. NIAID Director Anthony Fauci agreed. But with five years of flat funding to NIAID, Fauci decided to shift money allocated for clinical development to basic discovery to support two new grant programs aimed at generating new ideas that may help advance the basic understanding of how to develop a vaccine that prevents HIV infection or controls disease progression. "Money is not the answer, it is ideas we need," says Peggy Johnston, NIAID's director of the Vaccine & Prevention Research Program. "We need to get new blood, new people involved in AIDS vaccine research."

The new Basic HIV Vaccine Discovery Research R01 grant program will commit $10 million to support 20-30 research projects that can engage investigators in AIDS vaccine development and related fields, individually or in a collaborative interdisciplinary manner, to substantially improve the basic understanding of the immune system's response to natural infection and vaccination; dissect immune mechanisms of protection; identify effective immunogens and approaches toward manipulating the immune response; discover new mechanisms and pathways that could be targeted by vaccines; and conduct *in vitro* studies in animal models and in humans to examine how HIV pathogenic mechanisms relate to vaccine design.

The new Highly Innovative Tactics to Interrupt Transmission of HIV, or HIT-IT, R01 grant program will fund 10 proposals that target the technical and scientific hurdles facing the field by providing support for HIV pathogenesis studies on the biology of HIV transmission and human genetics. Collaborations among virologists, immunologists, molecular and cell biologists, and other relevant areas are encouraged among HIT-IT grantees and so is risk-taking. "Reviewers will be advised that unavoidable risk is acceptable as long as the probability of success is greater than zero," the grant description reads. HIT-IT research proposals were due last November but the grant winners have not yet been announced.

These R01 grants are just one plank in a platform that reflects the shifting priorities in the AIDS vaccine field. While NIAID, the biggest public funder of AIDS research, is leading the charge, large

Money is not the answer, **it is ideas we need.** We need to get **new blood, new people involved** in AIDS vaccine research. – *Peggy Johnston*

philanthropic organizations like the Gates Foundation along with IAVI have also been trying to spark innovation with an array of projects and grants.

Last year, the Gates Foundation, the largest private funder of HIV/AIDS research, awarded $100,000 grants over five years to 105 researchers through its Grand Challenges Explorations program, which funds novel ideas that cross a number of areas of importance to the foundation's mission, including AIDS vaccine research.

Grand Challenges Explorations grants are small seed grants and, like HIT-IT, they target high-risk, out-of-the-box proposals that traditionally would have a hard time attracting private and public funding. The review process is less restrictive and the turnaround time for funds is quicker than traditional research streams such as the NIH's extramural grant programs. "This program is designed to open up the gates a little, no pun intended," says Andrew Serazin, the Gates Foundation's program officer in Global Health Discovery. "We don't expect full proof of concept in that first year, but you need to show us that first step in the right direction." If a grantee manages to do just that, they could be eligible for $1 million more in funding over two years, says Serazin.

The Gates Foundation received about 4,000 applications and 31 of the 105 grants awarded fall under the category of HIV prevention and eradication. A number of other HIV-related projects fall under a more general vaccine category that involves "platforms" that could be applicable for control of a number of diseases, says Serazin.

IAVI, meanwhile, has a two-year-old Innovation Fund that provides seed capital to help bring novel, early-stage technologies to the field of AIDS vaccine research. The Innovation Fund, which is partially supported by a grant from the Gates Foundation, has made six awards since its creation in August 2007 and nine more are expected over the next two years. Its most recent recipient, South African Elevation Biotech, is attempting to enhance the structural stability of HIV's envelope protein in complex with a broadly neutralizing antibody and then use this to design antigens that can induce broadly neutralizing antibodies against the virus.

While the Innovation Fund does entertain and fund projects from academic researchers, the program is primarily aimed at identifying novel technologies within industry, says Kalpana Gupta, IAVI's director of new alliances and initiatives. Gupta says antigen discovery and immunogen design are of high interest to the Innovation Fund.

Economic uncertainty

This new roadmap toward innovation is being laid out during a time of great economic uncertainty, but Fauci says the $14.5 million pledged for the HIT-IT and Vaccine Discovery Research programs, which will both kick in this summer, is secure. Moreover, he said the agency is "committed to not only maintaining but increasing HIV vaccine research," particularly in basic science. Fauci says that means redirecting money from other areas of NIAID's AIDS budget considered "less pressing."

"There is obviously a lot of interest in HIV vaccine research," says Fauci. "A lot of that is coming from philanthropic groups. But with the economy in free fall, the question remains whether people will be more reluctant to give money to philanthropic groups." Also of concern is whether the economic downturn will dampen philanthropic

Survey Says…

As the AIDS vaccine field focuses increasingly on basic discovery research, innovation and engaging a new generation of researchers is becoming a high priority. In response to this, IAVI, the AIDS Vaccine Advocacy Coalition, and The Global Vaccine Enterprise surveyed a group of 75 young investigators who attended the AIDS Vaccine 2008 conference in Cape Town, South Africa, and conducted interviews with more veteran researchers to assess the obstacles to engaging young investigators and retaining promising researchers already involved in AIDS vaccine work.

The primary obstacles identified by respondents were limited access to funding, particularly for those young investigators who lack a significant publication record, and negative perceptions of the field due to recent trial outcomes. Young investigators also expressed concern about not receiving proper credit for their work in large consortia, due to their junior status and the hierarchical nature of such settings.

Some recommendations suggested by respondents for improving the environment for young researchers included: continuing and improving mentorship programs; fostering opportunities for young investigators to publish their findings; continuing evaluation and improvement of peer review processes; and developing funding mechanisms to strengthen research capacity in low- and middle-income countries to engage new and young researchers as well as retain and support those already involved.

The majority of those interviewed saw the need to engage young investigators, especially from fields other than vaccine research, as necessary to sustain the vitality of the field. Additionally, bringing these eager young minds into the field may prove critical to spurring the innovative ideas and approaches necessary to advance AIDS vaccine research and development. *—Genevieve Lynch, contributing writer*

support for high-risk projects that are likely to deliver more blanks than "magic bullets." This may not be the case, at least for one philanthropist who recently made a $100 million commitment to AIDS vaccine development (see box, below).

The next generation

Another way to encourage innovation is to attract young researchers to the field (see Perspective article, page 14). Because early career scientists are perceived to have a certain naive curiosity that fosters exploration, they are also considered a critical component in the pursuit of innovative approaches. Seasoned researchers generally agree that the discovery of a safe and effective vaccine will likely fall to a new generation of scientists, so they are now focusing on attracting these new minds to the cause.

The Global HIV Vaccine Enterprise—an international alliance of researchers, funders, and advocates committed to accelerating the development of an HIV vaccine—has made this issue one of its primary areas of focus and recently it established the Young and Early Career Inves-

$100 million Gift Creates New AIDS Vaccine Research Institute

AIDS vaccine scientists agree a renewed emphasis on basic discovery is what is needed to solve some of the obstacles impeding AIDS vaccine development. That pursuit received an enormous boost this month after technology magnate Phillip Ragon, the founder and owner of a company that provides database software to hospitals and other industries, announced a US$100 million gift to Massachusetts General Hospital (MGH) in Boston to explore how the immune system combats disease, with an initial focus on developing an AIDS vaccine.

The gift is unprecedented for MGH, which is using the money to form the Phillip T. and Susan M. Ragon Institute, a unique collaboration of engineers, biologists, and doctors drawn from MGH as well as Harvard University and Massachusetts Institute of Technology (MIT).

The Ragon Institute—named for the donor and his wife—will be headed by Bruce Walker, an immunologist and director of the Partners AIDS Research Center, which is now part of the Ragon Institute. "It is a long-standing passion that I have, trying to help the poor and developing countries," says Ragon.

Ragon, who has a degree in physics from MIT, became drawn to the field of AIDS vaccines after meeting Walker and hearing about his research. "He started telling me about his activities," recalls Ragon. "I said I hear everything you say, but I still don't understand it."

So about two years ago Walker suggested that Ragon, whose company happens to have offices in South Africa, visit AIDS clinics there. Seeing the human face of the 28-year-old epidemic affected Ragon deeply. "It was really quite shocking," he says. "I began to talk with Bruce about what I could do to help," and the Ragon Institute evolved from those early discussions.

"What this money means is that we can launch new collaborations in new areas with people with new perspectives, and do that immediately," says Walker. "To me the thing that has made the most difference in my career has been flexible funding. What we are going to be able to do is track a lot of talented people and give them that license with flexible funding—the license to be innovative and creative and to take some bold chances."

Walker's laboratory has already done extensive research on a subgroup of HIV-infected individuals called elite controllers, who maintain viral loads of <50 HIV RNA copies/ml plasma without antiretroviral therapy. Walker is now tracking a cohort of 1,000 elite controllers and this area of research is considered a promising avenue in AIDS vaccine research.

This new $100 million award will also elicit the expertise of scientists from fields not necessarily associated with HIV. One of these researchers is MIT scientist Darrell Irvine, a polymer scientist and immunologist who has worked on cancer vaccines. He is attempting to build the biological version of a smart car—an efficient, nimble vehicle constructed from nanoparticles that can safely deliver DNA vaccines to their intended targets.

"Many people would argue that DNA vaccines could be ideal," says Irvine. "They are cheap to manufacture and synthetically produced so they would be well-defined. You have good quality control with them and you eliminate issues of vector-specific immunity like you have with viral vectors."

The problem is that DNA vaccines are also the equivalent of a 'gas guzzler.' "DNA vaccines work in mice because you can inject a ton of DNA in a relatively large volume of solution, which promotes transfection," says Irvine. "You cannot scale up those amounts in large animal models and humans."

Nanoparticles can deliver molecules with a high level of precision to specific receptors inside cells, so Irvine's laboratory is working on several different formulations that would improve immunogenicity either by directly stimulating the immune system or enhancing DNA expression. The nanoparticles are comprised of lipids and bioresorbable polymers because of their track records with other vaccines and drug delivery applications unrelated to HIV.

Christopher Love, a chemical engineering professor at MIT and another member of the Ragon Institute, is developing new assays that can monitor the immune response of single cells, allowing them to pinpoint precisely how the responses differ. His work is particularly applicable to research on elite controllers because being able to decipher what induces immunological control could help lead researchers to a vaccine that can do the same.

The Ragon Institute is also partnering with IAVI to conduct preclinical evaluation of AIDS vaccine concepts developed at the Institute. —*RM*

tigators (YECI) Initiative to identify ways to attract young researchers to the vaccine field. The Enterprise has held three meetings since the NIAID summit seeking input from young/early career researchers; the most recent was at the 2008 AIDS Vaccine Conference in Cape Town.

"We are not a funder," says Alan Bernstein, the executive director of the Enterprise. "It is not our responsibility or mission to mandate those things directly, but I think our job in this case is to highlight a problem or opportunity and come up with possible ways of addressing it and then present that to funders." Bernstein said the role of the YECI Initiative is two-fold—to diagnose the problem of why young investigators are not joining the AIDS vaccine effort, and to develop specific recommendations for the Enterprise's scientific advisory board. "I would like this issue of attracting and retaining new researchers to become part of our scientific strategic plan that we hope to have ready by this fall," says Bernstein.

Two early career scientists, Dan Barouch, associate professor of medicine at Beth Israel Deaconess Medical Center in Boston, and Thumbi Ndung'u, associate professor at the University of KwaZulu Natal in Durban, South Africa, are chairing a YECI committee that has been charged with figuring out how to bring new researchers on board.

The Center for HIV/AIDS Vaccine Immunology (CHAVI) and the HIV Vaccine Trials Network (HVTN), both funded by NIAID, are also reaching out to early career scientists, most notably those interested in non-human primate (NHP) research. "The ultimate goal is to build a team of new and established scientists committed to advance our understanding of NHPs to predict immunogenicity and efficacy of candidate vaccines in humans, to develop novel models of preclinical evaluation of candidate vaccines, and to define new concepts in correlates of protection from infection or immune control after acquisition," according to the grant summary.

Barouch said the STEP trial findings, ironically, have given young researchers a huge opportunity. "The future has never looked more promising because the failure of the Merck [candidate] shows there is a lot more research to be done," says Barouch. "There are a lot of problems to be solved and it's clear that the development of an HIV vaccine is not going to occur in the next few years. The field of investigators has come to the realization that they will have to pass the torch to the next generation. The scientific problems are there, and it will need young, talented, and creative investigators to solve them."

Bernstein says he hopes that the recommendations of the YECI committee will provide traction in the AIDS vaccine field, but also well beyond that. "These issues are not unique to HIV vaccine research," says Bernstein. "Young people have particular challenges these days in biomedical research. If we don't renew ourselves as a scientific community, we will be in trouble."

But to meet these challenges, particularly in countries hardest hit by the epidemic, it will require a long-term investment to prevent the kind of brain drain that has prevented many African countries from developing their own research infrastructure and holding onto their scientists, says Ndung'u, a Harvard-trained virologist whose research institute in Durban was built primarily with funds from the Doris Duke Foundation.

He said salaries in Africa are low and with a few notable exceptions, such as South Africa, most countries lack infrastructure and trained personnel to support basic AIDS vaccine science. "It takes time to build a good research institution," says Ndung'u. "A lot of the grants that have been given to investigators to do work in Africa, I don't think those grantees were held to the fire in terms of making sure there is a pathway that is developed and sustained."

The field of investigators has come to the realization that they will have to pass the torch to the next generation. The scientific problems are there, and it will need young, talented, creative investigators to solve them. – *Dan Barouch*

And in developed countries with good research infrastructure, the money is simply getting tighter. "It is getting tougher and tougher to get into the big laboratories because they don't have the money," acknowledges Galit Alter, who worked with Marcus Altfeld at Partners AIDS Research Center and now has her own research laboratory there.

Alter said a professor at McGill University, where she completed her undergraduate and graduate training, literally ordered her, at the age of 19, to join his HIV lab. She was skeptical about her abilities but agreed. "The most important thing that the NIAID summit did, I think, was encourage investigators not to give up," says Alter. "Even though funding is tight there really is a reason to stay in it. It's survival of the fittest. Those who survive will be the creators." ■

Challenges Facing Young Investigators

Three junior researchers describe the hurdles to succeeding in the AIDS vaccine field

By Galit Alter, Jason Brenchley, and Jacques Fellay*

Since the news in September 2007 regarding the STEP trial, the HIV research community has re-introduced basic bench science as a renewed priority, with the definition of the correlates of immune protection as the primary target. At the Summit on HIV Vaccine Research and Development held by the National Institute of Allergy and Infectious Diseases last March, support for young investigators was deemed as a pivotal step toward ensuring that innovation would continue and furnish the momentum and enthusiasm to move the AIDS vaccine field forward following the recent setbacks. Here we analyze the route to success for young investigators that are pertinent to the present state of the field in the context of evolution.

Evolution of young investigators

Evolution is defined as a "change in the inherited traits of a population" that is achieved in at least three different manners: variation, reproduction, and selection. This concept is highly pertinent to the development of a young scientist. Based on the above rules for success, a young investigator must *vary* from their mentor to establish a new area of research in which they must learn to collaborate, *reproduce*, and develop their own lab in a location where they can establish their roots. They also must overcome both financial and creative *selection* imposed by the scientific community.

Step 1: Variation

Fundamental to the process of evolution is variation, upon which selective forces can act. A striving HIV research community is inherently a fast-evolving organism, and as such, it requires variation of its own kind: new ideas, creative technology, provocative experiments, and innovative concepts.

Support for young investigators is intended to build a new generation of scientists that can bring fresh and imaginative ideas to the field. Nevertheless, it is critical that we remember that youth is not—and has never been—a certificate of brightness. However, young investigators are unique in that they possess the advantage of inexperience. Being somewhat naive affords the luxury of unorthodox thinking and allows one to take unusual approaches to addressing questions. This "naive curiosity" allows young investigators to extend into novel areas, breaking down the "walls" or bridging biological sciences to other domains such as mathematics, physics, engineering, and chemistry. These extensions bring new dimensions and novel per-

spectives to the HIV research field which stem from the imagination of these new additions to the field.

Yet, uniqueness often can be impractical, particularly when trying to obtain funding. Novel concepts are usually not immediately accepted by the scientific community as inherently critical and therefore are difficult to fund. Funding for high-risk work is often far more difficult to acquire than money to perform work in areas that are "hot" or directly relevant to vaccine design. However, with high risk can come high reward. Some of the best scientific publications have stemmed from research projects that were, at the time of inception, very high risk. It is also true that young scientists often lack pragmatism, and, as a consequence, ideas for projects that many consider "risky" are not lacking. However, it is important to appreciate the risk. While risky projects may result in high-profile papers, they also can become exercises in futility. A lab invested too heavily in risky projects may have funding difficulties and young investigators being considered for tenure may not be favorably reviewed with multiple failed projects under their belts. Junior scientists must carefully balance risky projects, which may result in highly visible publications, with more secure, more fundable projects that can result in more guaranteed, albeit less high-profile, publications.

These days, young investigators can find unconventional support by interdigitating their novel programs in larger scientific networks to form symbiotic relationships and support their growing laboratories. One model currently employed by the field is the use of large consortia to promote collaboration and advance the science efficiently, as individual collaborators bring different types of expertise to a project. These consortia play a dominant role in scientific progress and therefore young investigators are being strongly encouraged to participate. These science consortia provide the critical mass that is indispensable to perform large-scale studies, which require both rich collaborative networks and expensive technology, and also are an integral part of many laboratories' financial backing.

Furthermore, large consortia offer support for the high-risk ideas of young scientists. Here are several examples of how our work has been influenced by these consortia. Through the Center for HIV/AIDS Vaccine Immunology (CHAVI), Galit Alter has gained visibility and been funded to perform high-risk work in the development of a new platform to quantify antibody dependent cell-mediated cytoxicity (ADCC). Jacques Fellay is also working with CHAVI on HIV host genomic projects. The Ragon Institute (formerly known as Partners AIDS Research Center or PARC) has also begun to offer innovation awards that are targeted toward young investigators interested in initiating high-risk, "out-of-the-box" collaborations to develop new technologies that may move the field forward. Through the Ragon Institute, Alter has now partnered with researchers at Massachusetts Institute of Technology to develop high-tech imaging tools to gain an in-depth appreciation for the enigmatic role of natural killer cells in HIV infection. Thus CHAVI and the Ragon Institute have taken a momentous initiative to encourage and provide small, catalyst-style grants to new investigators to support innovative ideas that pertain to vaccine design.

Collaborations are also important for young investigators because they increase their visibility within their respective fields. For example, while working as a post-doctoral fellow Jason Brenchley became involved in a consortium led by Michael Lederman of Case Western Reserve University in Ohio called "The Bad Boys of Cleveland" (BBC). This consortium began as a small group of researchers interested in the role and causes of immune activation in the chronic phase of HIV infection. These researchers would meet every nine months to discuss current data and plan future experiments. These meetings significantly increased the visibility of Jason Brenchley, generated many active collaborations, led to five co-authored papers (one as a first author and one as last author), and introduced him to several premier researchers in the field. These introductions ultimately led to his being able to recruit a very talented post-doctoral fellow into his own lab. The BBC is now funded by an US National Institutes of Health (NIH) P01 grant and the productive collaborations continue.

The NIH also recently launched larger grants that are directly aimed at supporting young scientists as they transition from their mentored to their independent phases, the K99/R00. These young investigator grants are a vital resource in the tenuous period during the early career transition to independence. The K99/R00 has played a pivotal role in the early career development of Galit Alter and afforded her with the financial support to tran-

It is critical to remember that youth is not—and never has been—a certificate of brightness. However, young investigators are unique in that they possess the advantage of inexperience.

sition to independence and build her laboratory. With this grant, she was able to recruit a post-doctoral fellow and begin to engage in the development of a novel technological approach to defining the role of innate immune receptors on the evolution of the T-cell synapse. Similarly, CHAVI and the HIV Vaccine Trials Network will also offer a "track to independence award" targeted at young investigators interested in simian immunodeficiency virus (SIV) research, and will hopefully catalyze a renewed movement in the area of primate research, which is imperative. These types of awards are absolutely vital in providing the financial stability to upcoming young investigators to engage in independent research programs that will likely flourish in their future lab interests.

Step 2: Reproduction

While the role of reproduction in the evolution of a young investigator may not be evident, academia forms an everlasting ecosystem due to the cyclical processes that sustain it. Thus through apprenticeship, young investigators learn from their mentors during graduate and post-graduate work, after which they themselves must provide the support to grow their own generations of mentees. The struggle in reproduction is therefore three-fold: separation and survival from the mentor, finding young mentees to build a lab, and finding a nurturing environment in which to build a lab.

It is impossible to argue that training is not the most critical catalyst for success. The skills one learns from a mentor mold the young investigator. We have all been privileged to work with exceptionally talented mentors that have certainly had an immeasurable impact on our development as investigators. Our greatest obstacle is then to diversify ourselves from these remarkable role models and to generate independent areas that are equally successful. The pressure is on, but similar to our mentors who rose to the occasion when they left their impressive mentors, it is clear that those that overcome this obstacle are the ones that have a chance to make it in this ultra-competitive world.

One of the most critical resources in the scientific community is the mentee, both in the form of students and post-doctoral candidates. These individuals form both the labor and the neural network that are responsible for the rapid evolution of the career of a young investigator. The hurdle is attracting these young mentees away from the established investigators that offer some security of success. However, there are certainly advantages for mentees who choose to conduct their training with young mentors, as these mentors at this early phase in their careers are highly involved and intensely invested in the success of their mentees. At this early stage in the career of a young investigator, the generation of high-quality manuscripts is absolutely vital. Whilst a highly invested young mentor may be attractive to some trainees, this problem is both a cultural hurdle for the academic community as well as a problem with advertising for young mentors who have available positions in their laboratories.

Geography is also at play in the reproduction process. At the crossroad between scientific expectations and life experiences, every young researcher will be confronted, often repeatedly, with the daunting task of deciding where to conduct research. Indeed, a scientist early on in their career has the opportunity to experience both the freedom and the loneliness of a migratory bird, free to roam where the grass is always greener. However, with independence comes the necessary decision of where to set roots.

The responsibility falls more heavily on young investigators from developing countries. The pressure to return to their homeland is much greater, due to the need in their nations for capacity building. Despite the luxuries in science these individuals may experience in developed countries, they face especially tough personal and societal demands when making the decision to stay or go back. The differences are innumerable, starting with scarcer financial support systems, less intellectual capacity, difficulties in obtaining reagents, less chance of up-to-date technological equipment, etc. Despite the HIV research community's clear appreciation for these hurdles faced by young investigators in the developing world, a dearth of grants are available for those brave enough to make the journey home. However, the next generation of AIDS researchers should not only replenish the existing army of experienced investigators, significantly expand the number of successful scientists working in developing countries.

Step 3: Selection

Selection pressures in the world of HIV research are not driven by chance or circumstance. They are clearly determined by the scientific agenda of the community. Thus grant review panels and journal reviewers are profoundly involved in determining the fate of a young investigator. The conundrum lies in the fact that a virtual agenda is defined annually through conferences, publications, and brainstorming sessions that help shape the path forward.

Naturally, due to their exemplary track records, the allocation of funds to experienced investigators is "safe" and is believed to have a greater likelihood to generate high-impact publications. However, young investigators must compete fiercely, and as mentioned above, risky propositions are not always favored for the fledglings. Therefore new investigators must be mindful to develop programs that are relevant to the current interests of the community, and yet sufficiently novel to appeal to their peers.

The idea that chance might play a part in the success of a young scientist is definitely a debatable topic. Timeliness seems to be a recurrent success tip: should investigators that develop exciting new topics at a time when the scientific climate favors that subject be construed as lucky, or just clever? Fundamentally, the flexibility to maneuver through the scientifically relevant and novel areas of research with stealth and success is definitely the trait that has served the most successful scientists well. Timing might be due to luck, but the art of flexibility might also lend itself to being able to stay at the leading edge of the field.

As stated above, some of the best scientific publications stem from research projects that are derived from ideas that arose outside the domains of the proverbial box. Often young researchers have not been in their respective fields long enough to actually know the confines of such a box, and many of their ideas therefore represent novelties in nature that, if successful, aid in scientific evolution. For example, one of the hallmarks of chronic HIV infection is pleiotropic activation of the immune system. While a post-doctoral fellow, Jason Brenchley led a project with the hypothesis that the damage to the gastrointestinal (GI) tract that occurs during acute HIV infection would allow microbial products to translocate from the lumen of the GI tract into peripheral circulation. These microbial products would then be a cause of the immune activation. This hypothesis was met with some pessimism in the field, but the data supported the hypothesis and was ultimately published in a high-profile journal. This "novelty in nature" has subsequently been confirmed by several other groups and has been shown to have a role in AIDS dementia, failure to reconstitute $CD4^+$ T cells after initiation of antiretroviral therapy, and perhaps atherosclerosis. Moreover, several novel therapeutic interventions that aim to reduce microbial product-mediated immune activation are currently in trial.

Future considerations

HIV research requires both long-term commitment as well as a sense of urgency. Scientific and political leaders have made it a priority to build a solid "next generation" of scientists that can quickly contribute to the vitality of HIV research by bringing fresh and imaginative ideas. In direct response to this pressing need, the Global HIV Vaccine Enterprise has launched the Young and Early Career Investigators (YECI) Initiative to contribute to the development of the Enterprise's 2009 Scientific Strategic Plan by articulating the importance of young investigators as drivers of innovation and by proposing the structural and cultural changes required to engage and retain new scientific talent and to integrate innovative ideas and new technologies into HIV vaccine research. Co-chaired by Dan Barouch and Thumbi Ndung'u, the YECI Committee is comprised of scientific investigators from around the world who are age 40 or younger, or who are within 10 years of receiving their terminal degree or related clinical training. The Enterprise's YECI Committee will increase dialogue between young and established researchers and provide innovative and constructive recommendations that address the challenges young investigators face in both developed and developing countries.

The main question that this committee and the field have to address is how do we best attract young researchers to the field? And what type of support, both financial and otherwise, is needed to keep young investigators involved in HIV vaccine research? When trying to support the next generation of scientists, we have to ask ourselves the following questions: How can we increase the visibility of the next generation of thinkers, provide new opportunities to support high-risk initiatives of these new minds, revolutionize the mechanism used to evaluate success in light of the new scientific climate, and provide a support system to help recruit mentees for young investigators? Appropriate answers to these challenges should offer benefits well beyond the newest generation of HIV scientists to the whole HIV research community. ■

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Galit Alter

Jason Brenchley

Jacques Fellay

It Eradicated Smallpox, BUT HOW?

Researchers are finally collecting clues about the life-long protection afforded by the smallpox vaccine, the gold standard of vaccines

By Andreas von Bubnoff

One riddle currently confronting AIDS vaccine researchers is identifying the immune correlates of protection against HIV. But they are far from alone on this type of quest. For many vaccines the correlates of protection elude researchers even after they have been used for decades. Once a vaccine works, there is little interest in figuring out why, even though there are benefits to understanding just how an effective vaccine affords protection. "We should really know how the things that work, work," says Shane Crotty, an associate professor for vaccine discovery at the La Jolla Institute of Allergy and Immunology.

Smallpox, caused by variola virus, was eradicated in the late 1970s with a vaccine that was a live preparation of the genetically related vaccinia virus. Crotty refers to the smallpox vaccine as the gold standard because it is the only vaccine that has ever led to the eradication of a disease. Yet, for several reasons, the correlates of protection for this vaccine are still unknown. When smallpox was eradicated, many of the modern methods to measure immune responses weren't yet available. At that time, researchers could not measure T-cell responses, says Mark Slifka, associate professor at the Vaccine & Gene Therapy Institute at Oregon Health & Science University. The human data on how the smallpox vaccine works are mostly from observational studies, and because there are no naturally occurring smallpox infections anymore, it would be impossible to do a randomized clinical trial today of a smallpox vaccine to study the correlates of protection. It would also be unethical to perform a placebo-controlled clinical trial because of the existence of an effective vaccine, Slifka says.

Renewed interest in trying to understand the smallpox vaccine is in part driven by the need to develop a new vaccine with fewer side effects, which could be used to guard against a potential bioterrorism attack with the remaining stockpiles of smallpox, Crotty says.

The vaccinia smallpox vaccine, called Dryvax, would be considered too dangerous to use for some because it can cause serious side effects in immunocompromised people, including people with AIDS, according to D. Huw Davies, a project scientist at the University of California in Irvine. "The side effects mean that [Dryvax] wouldn't be approved today," Davies says. Another smallpox vaccine called ACAM2000, which was recently approved, is a safer, cloned version of Dryvax manufactured according to more stringent modern Good Manufacturing Practice standards. But this vaccine still causes side effects in immunocompromised people, adds Davies. Researchers are now working on safer smallpox vaccines such as those based on modified vaccinia Ankara (MVA), which is derived from vaccinia but is unable to replicate in human cells and also lacks genes that suppress the host immune system.

Identifying the correlates of protection for the smallpox vaccine in humans may never be possible, but researchers are starting to collect clues about the way it protects by studying vaccinated individuals, people who have survived an infection, and by using animal models. They have found that the original smallpox vaccine primarily works on the basis of neutralizing antibodies, the way most vaccines are thought to protect, and that the antibody responses are surprisingly variable and redundant. At the same time, they are trying to identify certain markers in the antibody response that they hope to use to predict whether a safer, alternative vaccine will be protective.

Searching for the correlates

Progress in understanding how the smallpox vaccine works has been slow in coming. "It's been thought for quite some time that the smallpox vaccine does work on the basis of neutralizing antibodies, but it was really just [a few] years ago that that was directly shown," says Crotty, referring to a 2005 study by Genoveffa Franchini's group at the National Cancer Institute that provided evidence in animal experiments that antibodies are required for protection (*Nat. Med.* **11**, 740, 2005). "That experiment nailed it," Crotty says.

In the study, researchers vaccinated monkeys with the human smallpox vaccine and then inhibited either the humoral or the cellular immune responses to determine which part of the vaccine-induced immune response was required for protection against intravenous monkeypox challenge. They found that only inhibiting the antibody response eliminated the protection afforded by the vaccine. "They were able to show in a relevant challenge model that antibody-mediated protection is the main component for protection," Slifka says. The study also showed that transferred human antibodies could protect unvaccinated animals against the challenge virus, he adds, suggesting that antibodies are both necessary and sufficient for protection.

This confirms observations in humans, Slifka says, referring to a 1941 study that showed that transfer of serum from smallpox survivors could protect infected people from death (*Bulletin de l'Institut d'hygiene du Maroc* **1**, 59, 1941). In another study, antibody alone protected the majority of children with a genetic defect in their T-cell responses from dying from vaccinia infection after a smallpox vaccination (*Pediatrics* **18**, 109, 1956). Slifka says the fact that in the absence of cellular immunity the immune serum from vaccinated people protected most of the children suggests that while T cells play a role, antibody alone may almost be completely sufficient for protection against smallpox.

While antibodies are clearly important for protection, it's unclear which antigens they need to be directed against or which concentration of antibodies is required. According to Slifka, some studies suggest that high antibody titers appear to be a marker of protective immunity (*Am. J. Trop. Med. Hyg.* **21**, 214, 1972; *Bull. WHO* **52**, 307, 1975). However, these are observational studies that don't include a control group. T-cell responses, which may also have contributed to protection, were also not measured in these studies.

To learn more, Slifka is now studying the antibody and cellular responses in a cohort of smallpox survivors and people who received a smallpox vaccination to see if the vaccine induces an immune response similar to that in natural infection.

Others like Davies and Crotty have started to systematically study the antibody response to smallpox vaccine using microarray chips that contain most of the approximately 200 smallpox proteins. In a typical person vaccinated with Dryvax, they have identified antibodies to 20-30 proteins, only about a dozen of which are surface proteins (*Proc. Natl. Acad. Sci.* **102**, 547, 2005; *J. Virol.* **82**, 3751, 2008). "We get immunoreactivity to membrane proteins as well as proteins that are not in the membrane of the virus, and even proteins that don't end up in the virus at all," says Philip Felgner, a coauthor of these studies and the director of the applied proteomics research laboratory at the University of California in Irvine. Antibodies to non-surface antigens are probably directed to proteins released from necrotic infected cells, according to Davies.

> There is **no single protein** that a person always has to have a response to **in order to get protection.**
>
> *– Philip Felgner*

These studies show that antibody responses to the vaccinia vaccine are surprisingly variable—only half of the antibody responses in two people are typically directed toward the same smallpox proteins. Also, the dominant response in two vaccinated people will likely be to a different smallpox protein.

"There is no single protein that a person

always has to have a response to in order to get protection," says Felgner. The cellular immune responses to the vaccine are probably even more variable in humans than antibodies, says Felgner, whose lab used the same preparations of the complete set of smallpox proteins to study T-cell responses to the vaccine by stimulating peripheral blood cells from vaccinated people.

The antibody responses to smallpox also appear to be redundant. Even if antibodies that in and of themselves are sufficient for protection are removed from serum from vaccinated people, this serum can still protect against infection (*J. Virol.* **82**, 3751, 2008). This suggests that there is not a single mechanism for protection.

It seems that as long as an antibody covers the surface of the virus, it will protect, Crotty says. And it doesn't matter which of the smallpox proteins on the surface an antibody binds to. "What you really need is an antibody that covers the surface of the pathogen sufficiently so that the pathogen can't bind to the target," he adds. "[It's like] throwing a net over the virus." Large complement proteins might also play a role, assisting antibodies in binding to each other to cover the virus.

Predicting protection

While there doesn't seem to be a single, clearly defined immune response induced by the smallpox vaccine, Felgner has used the smallpox protein microarray in animal experiments to identify markers in the antibody response that might predict protection. His group compared the immune responses of vaccinated rabbits that were protected from challenge with ones that weren't protected despite vaccination and identified three markers that were associated with protection. Felgner is currently also analyzing data from non-human primate studies.

Researchers will use these markers to evaluate samples from a Phase I clinical trial of the MVA vaccine against smallpox to see if it can protect as well as Dryvax, says Felgner. The US Food and Drug Administration (FDA) may then consider using this data, along with the animal data, as the basis for licensure of the MVA vaccine. If approved, it would add MVA as a safer alternative to Dryvax and ACAM2000. In cases like this, where there are no humans infected with a given pathogen, the FDA has a "two animal rule," Felgner says, which means that evidence from two different animal models is sufficient for vaccine licensure.

Now that the immune response elicited by the smallpox vaccine has been rather clearly described, Crotty says, the next big question about the original smallpox vaccine is how it can give such long-lasting protection. "Why is it that you can give one immunization with this vaccine and you get a fantastic protective antibody response and it lasts for life?" he asks. ■

Lessons for AIDS Vaccines?

The principles learned from a vaccine that protects against smallpox are unlikely going to apply directly to development of an AIDS vaccine. There are many differences between smallpox and HIV. Unlike HIV with its one surface protein, smallpox virus is very large, with about 200 genes and dozens of surface proteins. And also unlike HIV, smallpox doesn't mutate much.

Given these differences, smallpox may not be the best example to guide development of an AIDS vaccine. "We have been applying the rules of conventional vaccinology to HIV since it emerged in 1983," says D. Huw Davies, a project scientist at the University of California in Irvine, "but this has largely failed us." While antibodies are likely important for protection to both smallpox and HIV, something very different from conventional vaccines needs to be developed against the rapidly evolving HIV, Davies adds.

Still, there are some general lessons. If there is anything to be learned from understanding the smallpox vaccine, "it's that neutralizing antibodies are so key for the protection," says Shane Crotty, an associate professor for vaccine discovery at the La Jolla Institute of Allergy and Immunology. "It's yet another piece of information that suggests that you probably need to be able to make neutralizing antibodies." What's more, the success of the smallpox vaccine shows that in principle, it is possible to develop a vaccine that can induce life-long immunity and long-lasting T-cell and antibody memory, says Mark Slifka, associate professor at the Vaccine & Gene Therapy Institute at Oregon Health & Science University.

AIDS vaccine researchers are also generating candidates with vectors derived from vaccinia virus. Some are using replication-incompetent strains such as modified vaccinia Ankara (MVA), while others use replication-competent strains. "Most workers are using replication-deficient strains because of safety," says Bernard Moss, chief of the laboratory of viral diseases at NIAID (see *Go forth and multiply*, *IAVI Report*, May-June 2008).

But worldwide use of the smallpox vaccine has taught researchers enough about the benefits and risks to be able to use replication-competent vaccinia virus, says Julia Hurwitz, a member in the department of infectious diseases at St. Jude Children's Research Hospital. "Less is known about the newer, non-replicating vaccine vectors," she adds. Her group has conducted a Phase I safety trial with a replicating vaccinia virus vector based on the smallpox vaccine (*Eur. J. Clin. Microbiol. Infect. Dis.* **23**, 106, 2004).

Zhiwei Chen, an associate professor and director of the AIDS Institute at the University of Hong Kong, is developing an attenuated but replication-competent vector derived from a vaccinia strain called Tiantan, which was used for the eradication of smallpox in China. This vector carrying the gene for the spike protein of the severe acute respiratory syndrome (SARS) virus induces between 20- to 100-fold higher levels of neutralizing antibodies to the spike protein than the replication-incompetent MVA strain after intranasal or oral administration in mice. "[A] replicating vaccine may offer better immunogenicity and induce better memory response," says Chen, who presented the findings at a recent conference on mucosal vaccines in Porto, Portugal (see *Mucosal Vaccines: Insights from different fields*, *IAVI Report*, Nov.-Dec. 2008). He eventually plans to use this vector to develop an AIDS vaccine candidate. —*AvB*

Vaccine BRIEFS

Four New AIDS Vaccine Trials Launched in Recent Weeks

Four new early-stage AIDS vaccine trials were launched in recent weeks. A Phase IIa trial in North and South America and a pair of Phase I trials in the UK and India will test two different prime-boost regimens of DNA and modified vaccinia Ankara (MVA) vector-based vaccine candidates. Another Phase I trial in the US is evaluating the safety and immunogenicity of an adenovirus serotype 35 (Ad35)-based candidate.

The trial furthest along, a Phase IIa trial testing the safety and immunogenicity of two vaccine candidates developed by US-based GeoVax, began enrolling volunteers in January at 13 clinical trial centers in the United States and Peru. The trial known as HVTN 205 will involve 225 volunteers and is being conducted in collaboration with the US National Institutes of Health and the HIV Vaccine Trials Network (HVTN).

Volunteers randomly selected to receive the vaccine candidates in HVTN 205 will receive two doses of a DNA vaccine candidate encoding HIV clade B Gag, Pol, Env, Tat, Rev, and Vpu, followed by two doses of an MVA-based vaccine candidate carrying HIV clade B Gag, Pol, and Env proteins.

Harriet Robinson, senior vice president of research and development at GeoVax, says the vaccine candidates showed "fabulous control" against SHIV, a hybrid HIV/ simian immunodeficiency virus (SIV), in preclinical studies in non-human primates. The candidates did not fare as well against SIV challenge but still showed a 10-fold reduction in viral load after six months compared to unvaccinated control animals, says Robinson.

In December 2008, IAVI in conjunction with St. Stephen's AIDS Trust at the Chelsea and Westminster Hospital in London initiated a Phase I clinical trial involving 32 volunteers to evaluate the safety and immunogenicity of a different DNA/ MVA prime-boost regimen. This regimen involves the candidate TBC-M4, which utilizes an MVA vector to deliver clade C HIV *env*, *gag*, *rev*, *reverse transcriptase*, *tat*, and *nef* genes. TBC-M4 was developed in collaboration with the National Institute of Cholera and Enteric Diseases in India and was tested previously in a Phase I trial conducted there.

In the Phase I trial in the UK, administration of TBC-M4 will be preceded by a DNA-based vaccine candidate called ADVAX, a plasmid DNA candidate encoding HIV clade C *env*, *gag*, *pol*, *nef*, and *tat* genes. ADVAX was developed at the Aaron Diamond AIDS Research Center in New York City in collaboration with Rockefeller University and IAVI. The ADVAX vaccinations will be administered with a needle-free device called Biojector 2000 to see if this delivery system induces stronger immune responses than a syringe injection.

In this trial investigators will evaluate blood samples from the volunteers using a viral suppression assay to determine whether the $CD8^+$ T cells produced in response to the TBC-M4/ADVAX vaccine candidates are capable of inhibiting HIV. "What we would like to do is see if the $CD8^+$ T cells after vaccination stop the virus from growing," says Jill Gilmour, senior director of clinical research at IAVI.

Since ELISPOT results are not necessarily an accurate predictor of whether a vaccine can prevent or control HIV infection, Gilmour says it's important to find other tests, such as the viral suppression assay, that could potentially provide better insights into the immunogenicity of vaccine candidates. The viral suppression assay being used in the UK trial is an optimized version of one developed by Bruce Walker, director of the Ragon Institute in Boston (see *Newly Established Institute Promotes Innovation in AIDS Vaccine Research*, page 12).

Another Phase I, prime-boost trial of TBC-M4/ADVAX in India, known as P001, was also recently announced by the Indian Council of Medical Research. This trial will enroll volunteers at sites in Pune and Chennai and will evaluate different doses and vaccination regimens of the two candidates. In the UK trial, volunteers randomized to receive the vaccine candidates will either receive two doses of ADVAX via Biojector followed by an injection of TBC-M4, or three injections of TBC-M4. Volunteers will be followed for six months after receiving their last vaccination. In the India trial, volunteers will receive either two doses of ADVAX by traditional syringe injection followed by two injections of TBC-M4, or three injections of TBC-M4.

IAVI is also planning to begin enrollment of volunteers in a Phase I trial of its Ad35-based vaccine candidate encoding the GRIN insert (HIV clade A Gag, Pol [RT and Int], and Nef), as well as HIV clade A Env. This candidate was manufactured by the French biotechnology company Transgene. The trial will enroll 42 volunteers at New York State's University of Rochester Medical Center who will receive either two intramuscular injections of the vaccine candidate or placebo at three different doses. Clinicians will first administer the lowest dose and will review the safety data before proceeding to the next higher dose. —*Regina McEnery*

Research BRIEFS

Researchers Employ Systems Biology Approach to Predict Adaptive Immune Response to Yellow Fever Vaccine

Researchers have for the first time used a systems biology approach to predict the immune response to a vaccine. A study led by Bali Pulendran, a professor of pathology at Emory University, used microarray analysis to measure gene expression changes in the innate immune response to the yellow fever vaccine to predict the level of the adaptive T- and B-cell immune response with up to 90% and 100% accuracy, respectively (*Nat. Immunol.* **10**, 116, 2009).

The team vaccinated a group of volunteers with yellow fever vaccine, one of the most effective vaccines ever developed, and then used microarray analysis to measure gene expression changes as an indication of the innate immune response, which occurs within hours to days after vaccination, and is believed to regulate the adaptive antibody and T-cell response, which happens days or weeks later.

There are now overwhelming data that the innate immune system programs the adaptive immune system, according to Pulendran. For example, studies in mice suggest that eliminating the early innate immune response by eliminating certain genes severely compromises the adaptive immune response. And in 2006, Pulendran's group showed that the yellow fever vaccine induces a number of toll-like receptors that are part of the innate immune system and this in turn was essential for the later $CD4^+$ and $CD8^+$ T-cell responses (*J. Exp. Med.* **203**, 413, 2006).

"If the innate immune system is acting within a few hours of pathogen entry and if it is programming the adaptive immune [response], can we use this early innate signature as a biomarker to predict which vaccinee will have a strong antibody or T-cell response?" asks Pulendran.

To find out, his group vaccinated 15 volunteers who had never been exposed to yellow fever virus or vaccine and used microarrays to measure gene expression changes in almost all of their genes. This avoided any possible bias that could come from focusing on biomarkers that are already thought to be important.

The researchers measured gene expression changes at several time points up to three weeks after vaccination, and the level of the yellow fever-specific B- and T-cell responses 60 days after vaccination. The same measurements were done in a second group of volunteers vaccinated one year later in an independent trial. In each group the researchers identified genes that had expression changes that best correlated with a high or low adaptive immune response later on. The gene expression signatures identified in one of the trials could predict with up to 90% accuracy whether vaccinees in the other independent trial would go on to develop a strong T-cell response; the accuracy was up to 100% for the antibody response.

"It's the first study I am aware of that has used genomic data in a predictive fashion in two independent human studies," says Paul Thomas, an assistant member in the immunology department of St. Jude Children's Research Hospital, who is not connected to the study.

For now, it remains an open question as to whether the same signatures will apply to different vaccines. Pulendran has done preliminary studies that suggest that injectable flu vaccine as well as FluMist, which is given intranasally, both induce expression changes in genes that are very different from the ones induced by the yellow fever vaccine.

Still, the situation is different with flu. Unlike yellow fever vaccine, many people have likely previously encountered the influenza virus, according to Pulendran. "We are looking at a secondary response there, whereas with yellow fever we are mostly looking at a primary response," he says. Also, the injectable flu vaccine is a purified protein and not a live-attenuated virus like the yellow fever vaccine. And FluMist, while live-attenuated, is given intranasally, suggesting that different types of cells are likely exposed to it. So, Pulendran says, it's still possible that other live-attenuated vaccines have a similar signature.

> It's the first study… that has used genomic data in a predictive fashion in two independent human studies.
>
> – *Paul Thomas*

Vaccine manufacturers of emerging vaccines to HIV, tuberculosis, or malaria could use this approach in small trials to identify gene expression signatures that predict long-term immunogenicity, according to Pulendran. In larger trials, they could then use it to identify people who are the most likely to be protected.

Next, Pulendran plans to study the biological role of some of the genes that showed up in the signatures. One gene that's one of the best predictors of the T-cell response is known to be involved in the response of cells to stress, Pulendran says. "Why such a gene is predicting the cytotoxic T-cell response so well is a mystery," Pulendran says.

A second recent study led by Rafick-Pierre Sékaly, a professor at the University of Montreal, also used microarray analysis to find that yellow fever vaccination consistently induces the expression of a group of transcription factor genes in three independent groups of vaccinees (*J. Exp. Med.* **205**, 3119, 2008). Sékaly says the signature could be used to guide the development of vaccine candidates and adjuvants. —*Andreas von Bubnoff*

Elite Controllers Found to Have More Lethal CD8+ T Cells

Even after many years of research, it's still not well understood how rare HIV-infected individuals called long-term nonprogressors (LTNPs) control HIV replication and remain healthy without antiretroviral (ARV) therapy. Now, a study of elite controllers (ECs), which are a subset of LTNPs who control their viral loads to below 50 copies/ml of blood, has found that one key may lie in an enhanced ability of their CD8+ T cells to divide and kill HIV-infected CD4+ T cells (*Immunity* **29**, 1009, 2008).

Researchers cultured CD8+ T cells taken from ECs for six days in the presence of HIV-infected CD4+ T cells. They found that during that time, the cells divided and upregulated the protein perforin, which pokes holes into target cells, and the protein granzyme B, which kills target cells. This, they believe, explains why their CD8+ T cells could kill the HIV-infected CD4+ T cells much more efficiently than CD8+ T cells taken from progressors, which divided less vigorously and made less of these proteins. "We think going through the cell cycle causes [the CD8+ T cells] to upregulate perforin and granzyme B," says Stephen Migueles, the lead author of the study.

"[In our research] we really did not previously have an effector function of HIV-specific CD8+ T cells that so dramatically segregated with the [ECs]," says Mark Connors, chief of the HIV-specific immunity section at the National Institute of Allergy and Infectious Diseases, part of the US National Institutes of Health, and senior author of the paper.

ECs have the same number of HIV-specific CD8+ T cells in their blood as untreated progressors but Migueles and Connors showed in 2002 that these cells can divide better than CD8+ T cells from progressors. The new study shows that on a per-cell basis, the CD8+ T cells from ECs transferred granzyme B into a greater fraction of HIV-infected CD4+ target cells than CD8+ T cells taken from a progressor. "It's not that there are more CD8+ [cells] present in the nonprogressors, it's that each cell kills more efficiently," Migueles says.

The study also suggests that ARV therapy cannot repair the inability of CD8+ T cells in progressors to divide and upregulate perforin and granzyme B. ARV-treated progressors had fewer HIV-specific CD8+ T cells than ECs with equally low viral loads, and their cells behaved like CD8+ T cells from untreated progressors. This is unlike other functions like CD4+ T-cell proliferation, which improve after ARV therapy, Connors says. "This is really an impairment of the HIV-specific CD8+ T cells [in progressors] that is not fixed by ARV therapy," says Connors.

For now, it's still unclear what it is about ECs that makes their CD8+ T cells divide and kill so much better. "That's the million dollar question," Migueles says.

One consistent feature observed more often in ECs than in progressors is an allele in the major histocompatibility complex called B57. This is a host protein that HIV-infected cells such as CD4+ T cells use to present small pieces of HIV proteins on their surface to activate CD8+ T cells. But B57 alone is neither sufficient nor required for people to become ECs. "Obviously B57 has something to do with it but it can't be the whole story," Connors says. "There is some other interaction that we don't yet understand that allows [ECs] to do this."

Next, the researchers want to understand what's different about CD8+ T cells taken from ECs once they divide and gain their ability to kill target cells. Migueles says they are looking at the genes that are not working correctly in the cells that are not dividing.

The study also found that the CD8+ T cells from progressors can be converted *in vitro* into ones that behave like cells taken from ECs through a combination of stimulation and rest. "If we can hit them really hard with very potent stimuli and get them to divide, they actually upregulate the killing machinery and kill very efficiently just like [cells taken from] nonprogressors," Migueles says. "It's far away from a treatment," Connors adds, "but it is theoretically restorable."

"It's a great study," says Guido Silvestri, an associate professor of pathology at the University of Pennsylvania who was not connected to the research. "This observation advances our mechanistic understanding of how these individuals can achieve immune control of HIV and thus avoid AIDS." —*Andreas von Bubnoff*

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>
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