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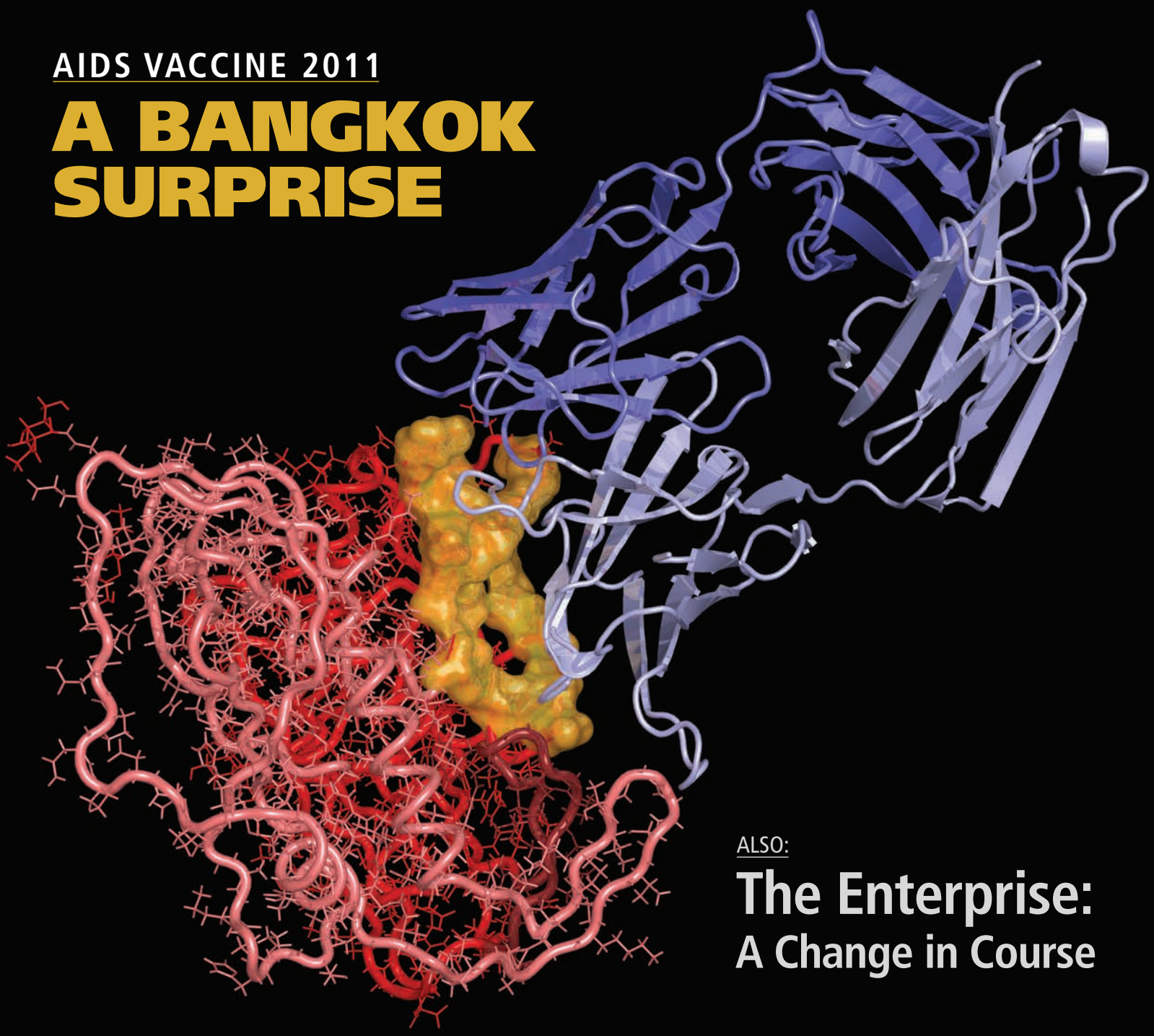
AIDS VACCINE 2011

A BANGKOK SURPRISE



ALSO:

The Enterprise:
A Change in Course

EDITOR'S LETTER

The past two years have been fruitful in AIDS vaccine research.

One of the biggest developments was of course the first evidence of vaccine-induced protection against HIV to emerge from clinical trials. The results of the landmark RV144 trial in Thailand surprised many in the field. And as reported in this issue, RV144 continues to yield surprising results. At this year's annual AIDS Vaccine Conference in Bangkok, researchers reported the results of a two-year collaborative effort to identify immune correlates that could explain the 31.2% protection afforded by the prime-boost vaccine regimen tested in RV144 (see page 4). Further investigation of the two antibody correlates they identified will now be one of the major areas researchers will be pursuing in coming years. Following the conference in Bangkok, some have even suggested that this follow-up work could lead to a first-generation partially effective vaccine.

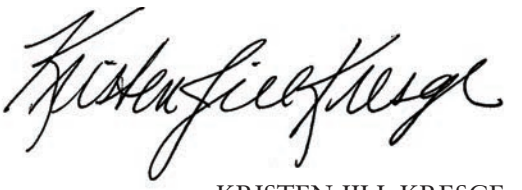
The other major development in the past two years has been the isolation of dozens of new antibodies against HIV that are both broadly neutralizing and very potent. Following these discoveries, researchers have begun the challenging work of using structural biology to elucidate the targets of these antibodies on the virus, and the development of first-generation vaccine antigens that are now being tested in small animal models to see if they can induce these highly desired broadly neutralizing antibodies (see pages 4 and 22). This is another major frontier of AIDS vaccine research today.

But while these two areas are the most burgeoning, other researchers are still considering somewhat more radical approaches to HIV vaccine development, such as allovaccination (see page 14).

Meanwhile, the Board of Directors of the Global HIV Vaccine Enterprise has released a new vision for the organization. Given the dramatic changes in the field, the tight funding environment, and the lack of leadership, the Enterprise will have a streamlined focus in the future (see page 9).

Also in this issue, we highlight the creation of the new nonhuman primate consortia to explore the earliest stages of mucosal infection (see page 19), the discovery of a new type of T cell with stem-cell like properties (see page 23), and other news from HIV prevention research (see pages 20 and 21).

Fruitful indeed!



KRISTEN JILL KRESGE

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International AIDS Vaccine Initiative

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The International AIDS Vaccine Initiative (IAVI) is a global not-for-profit organization whose mission is to ensure the development of safe, effective, accessible, preventive HIV vaccines for use throughout the world. Founded in 1996 and operational in 25 countries, IAVI and its network of collaborators research and develop vaccine candidates. IAVI was founded with the generous support of the Alfred P. Sloan Foundation, The Rockefeller Foundation, The Starr Foundation, and Until There's A Cure Foundation. Other major supporters include the Bill & Melinda Gates Foundation, the Foundation for the National Institutes of Health, The John D. Evans Foundation, The New York Community Trust, the James B. Pendleton Charitable Trust; the Governments of Canada, Denmark, India, Ireland, Japan, The Netherlands, Norway, Spain, Sweden, the United Kingdom, and the United States, the Basque Autonomous Government (Spain), the European Union as well as the National Institute of Allergy and Infectious Diseases and The City of New York, Economic Development Corporation; multilateral organizations such as The World Bank and The OPEC Fund for International Development; corporate donors including BD (Becton, Dickinson & Co.), Bristol-Myers Squibb, Continental Airlines, The Gilead Foundation, GlaxoSmithKline, Google Inc., Pfizer Inc, and Thermo Fisher Scientific Inc.; leading AIDS charities such as Broadway Cares/Equity Fights AIDS; and many generous individuals from around the world. For more information, see www.iavi.org.

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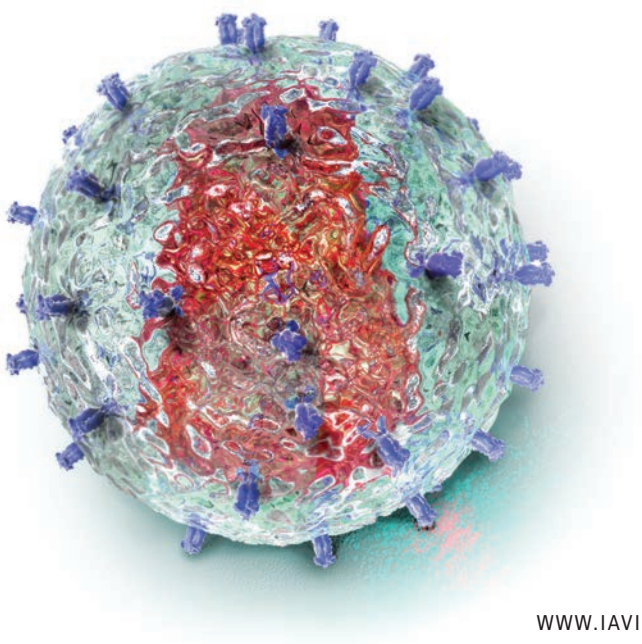

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[ON THE COVER]

This image shows the crystal structure of HIV-1 gp120 (red) in complex with human antibody VRC03 Fab (blue) and the epitope (orange) it shares with VRC01 and VRC-PG04. The 3D rendering is based on data reported in the *Science* cover article focusing on the evolution of HIV-1 neutralizing antibodies (Wu et al., 333 (6049): 1593-1602).

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

A BANGKOK *Surprise*

Results of the immune correlates analysis of RV144 and advances in broadly neutralizing antibodies topped the developments reported at the annual AIDS vaccine conference

By Kristen Jill Kresge

If you polled researchers in the HIV vaccine field three years ago and asked them what they thought would come out of the RV144 trial in Thailand, the most popular answer would likely have been nothing. The prime-boost combination of Sanofi Pasteur's canarypox vector-based candidate ALVAC-HIV (vCP1521) with AIDSVAX B/E, a genetically engineered version of HIV's gp120 surface protein, was considered unlikely to work and many scientists thought the field would reap greater benefits by developing better candidates than by testing this regimen in the largest trial to date, involving more than 16,000 Thai volunteers (*Science* **303**, 316, 2004).

Even after the results of RV144 were released two years ago showing the prime-boost regimen provided 31.2% protection against HIV infection—the first evidence of vaccine-induced protection—several researchers were still skeptical. The modest efficacy inspired many to raise questions about whether the protective effect was real or just a statistical fluke.

But now there may be fewer skeptics. After a two-year effort to elucidate the possible immune responses that correlated with the protection seen in RV144, the trial has once again yielded surprising findings. At the AIDS Vaccine 2011 conference that took place Sep. 12-15 in Bangkok, Thailand, Barton Haynes, who led the scientific steering committee that oversaw the collaborative and thorough RV144 correlates search, reported that two antibody responses were found to be significantly correlated with the risk of HIV infection among vaccine recipients in RV144. This finding generated several hypotheses and helped dispel doubts about whether the modest efficacy exhibited by the vaccine regimen was real. "The findings lend credence to the vaccine efficacy seen in the RV144 trial," said Haynes.

The first surprise, even for Haynes, was that any correlates at all were identified. Given the lack of support for RV144 when the trial began, investigators scaled back sample collection in the trial. This made the analysis somewhat akin to searching for a needle in a haystack. The second surprise was that while one antibody response was inversely correlated with HIV infection risk, the other was directly correlated with infection risk, suggesting this antibody response reduced the protective effect of the vaccine candidates.

While the correlates results are intriguing, it is still unclear exactly whether the antibody responses were directly responsible for the modest protection. Researchers are now aggressively investigating this.

Meanwhile, the blizzard of more potent and broadly neutralizing antibodies against HIV and elucidation of their targets on the virus has propelled research into the design of immunogens capable of eliciting such broadly neutralizing antibodies. Some of the advances in determining the structure of the targets of the broadly neutralizing antibodies (bNAbs) and the first generation of such immunogens were also presented in Bangkok.

The hunt for correlates

The RV144 correlates presented at AIDS Vaccine 2011 were the result of a collaborative process by a team of researchers established soon after the results were reported in 2009 at the AIDS Vaccine meeting in Paris (see *Raft of Results Energizes Researchers*, *IAVI Report*, Sep.-Oct. 2009). Since that time, under Haynes's supervision, the team conducted a series of pilot studies, eventually settling on six primary and approximately 30 secondary assays that were used in case-controlled studies to try to determine what immunological measurements predicted HIV infection risk among RV144 volunteers over a three-year period.

The six primary assays that were selected measured the following immune responses: binding immunoglobulin (Ig)A antibodies in plasma; IgG antibody avidity to the A244 gp120, the antigen used in the vaccine candidates; antibody-dependent cellular cytotoxicity (ADCC); neutralizing antibodies against a six-tier panel of HIV isolates; binding IgG antibodies to the first and second variable loops of HIV Envelope, known as V1 and V2, scaffolded onto a gp70 from murine leukemia virus; and $CD4^+$ T-cell responses as measured by secretion of several cytokines/chemokines, including interferon-γ, interleukin-2, and tumor necrosis factor α, among others.

The statistical analysis plan was developed and carried out by Peter Gilbert and colleagues at the Statistical Center for HIV/AIDS Research and Prevention (SCHARP), based in Seattle. The analysis had 80% power to detect an approximately 50% reduction in HIV infection rate and controlled for variables including gender and baseline behavioral risk in the multi-variate analysis. An independent team of statisticians validated the statistical analyses by Gilbert and colleagues.

For the case-controlled studies, the correlates team analyzed samples from 41 HIV-infected vaccine recipients, 205 uninfected vaccine recipients, and 40 placebo recipients. The samples used in the analysis were collected at week 26 of the trial—two weeks after all six vaccinations (four ALVAC primes and two AIDSVAX boosts) were administered—when the immunogenicity peaked.

The results of the assays identified two so-called correlates of risk. The first statistically significant correlate was IgG antibodies that bind to the V1/V2 loops of HIV Env. The presence of these antibodies correlated with a 43% reduction in HIV infection rate. For volunteers with high levels of IgG binding antibodies to V1/V2 compared to those with medium- or low-levels of these antibodies, there was a 75% reduction in HIV infection rate. Volunteers with high levels of V1/V2 antibodies appeared to be protected, while those with lower levels received little or no protection from the vaccine regimen, Haynes said.

The second immune response identified as a statistically significant correlate of risk was plasma IgA antibodies that bind HIV Env. These IgA antibody responses were directly correlated with a 54% increase in HIV infection rate among vaccinated volunteers, suggesting these antibody responses reduced the protective effect of the vaccine regimen. There was, however, no evidence that these IgA responses were associated with an enhanced risk of HIV infection. When researchers compared HIV-infected vaccinees to placebo recipients, they found that the HIV infection rates among these groups were the same.

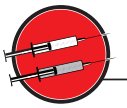
To investigate this correlate further, researchers did epitope mapping of the IgA responses to gp120. They identified the C1 peptide on gp120 as the binding site of the antibodies, which has been shown to be the target epitope of ADCC, according to Haynes. This led him to suggest that IgA antibodies that bind to C1 may block ADCC, a mechanism by which antibodies facilitate the destruction of HIV-infected cells (see *Antibodies: Beyond Neutralization*, *IAVI Report*, Jan.-Feb. 2010). This phenomenon has been observed in cancer, according to Haynes, who says that IgA antibodies can block ADCC responses against tumors.

Further exploration of this mechanism is now underway. Researchers are conducting an exploratory analysis to see whether low or high levels of IgA antibodies in plasma had an effect on ADCC responses, which were also measured in one of the six primary assays. Haynes also reports that additional studies will be conducted to see if plasma IgA interferes with any other immune responses.

Trial investigators did not collect any mucosal samples in RV144, so only plasma IgA antibodies can be analyzed. Secretory IgA at the mucosa, where it predominates, is in the form of a dimer, while only four percent of plasma IgA is dimeric. Haynes says plasma IgA also has a different potency than its mucosal counterpart. "It's an open question about what implications this finding may have for mucosal immunity," said Haynes, adding that collection of mucosal secretions will definitely be incorporated into the RV144 follow-up studies.

Researchers also have several other studies planned or already underway to further investi-

RV144 in Detail



THE VACCINE CANDIDATES

Prime

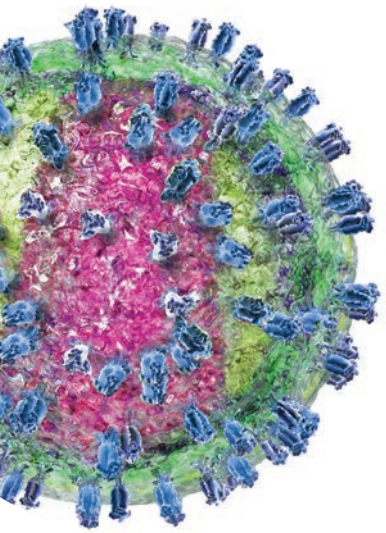
ALVAC-HIV (vCP1521)

A live, recombinant, non-replicating canarypox viral vector vaccine encoding clade B *gag/pro* and clade E *env*
(*Vaccine Developer: Sanofi Pasteur*)

Boost

AIDSVAX gp120 B/E

A genetically engineered version of HIV gp120 (*env*) from clades B and E
(*Vaccine Developer: Genentech; its spin-off, VaxGen, tested AIDSVAX previously; intellectual property rights now owned by Global Solutions for Infectious Diseases*)



gate the correlates. "They [the correlates] give us an important lead on improving on these responses," said Haynes. "Now we have informed hypotheses and directions that come from a trial."

Haynes's lab will be conducting passive administration studies of V2 monoclonal antibodies identified as a correlate in RV144 in nonhuman primate (NHP) studies to see if they are protective following challenge with a simian immunodeficiency virus (SIV)/HIV hybrid known as SHIV. Additional *in vivo* studies will also be used to investigate if IgA antibodies are capable of blocking ADCC.

There are also plans to re-evaluate the samples collected in the VAX003 and VAX004 trials—two Phase III trials conducted with only AIDSVAX. Both of these trials showed that AIDSVAX alone provided no protection in either men who have sex with men (MSM), injection drug users (IDUs), or high-risk women, but once again the samples from these trials may provide useful clues. The correlates findings from RV144 have sparked interest in whether similar immune responses were induced in VAX003 and 004 vaccine recipients but were perhaps overshadowed by the viral diversity or the quantity of virus the high-risk volunteers in these two trials were exposed to. "Challenge dose may overwhelm immunity," said veteran vaccinologist Stanley Plotkin, citing the Polio vaccine as an example.

Genoveffa Franchini, chief of the animal models and retroviral vaccine sections at the National Cancer Institute, has seen evidence for this in NHPs. Using a low-dose challenge model, Franchini can replicate the RV144 results in a monkey model with about 30% of macaques protected against SIVmac251 challenge following vaccination with a similar regimen based on SIV. However, if the dose is increased, the protective effect is lost. "If you're using too much virus you can't see vaccine efficacy," said Franchini. There are also similarities in the antibody responses elicited in NHPs. The animals protected against low-dose challenge appear to have higher anti-gp120 antibody levels, and the antibody avidity to the V2 loop appears to be important, though Franchini says more experiments are necessary to show whether this is the mechanism of protection in the macaque studies.

In addition to challenge dose, the mode of transmission may also be important. One of the unique aspects of RV144 was the trial population—volunteers were largely heterosexuals at low risk of acquiring HIV. Studies of the earliest stages of HIV infection have shown that heterosexual transmission is predominantly (about 80% of the time) the result of a single transmitted or founder virus that establishes infection (see *HIV Transmission: The Genetic Bottleneck*, *IAVI Report*, Nov.-Dec. 2008). Whereas, in MSM and IDUs, researchers have reported that on average a much higher number of founder viruses are transmitted. Katharine Bar of the University of Alabama at Birmingham summarized the research findings in MSM and IDUs. She said that two studies have shown that in approximately 40% of MSM, multiple founder viruses established infection, while two studies in IDUs have provided conflicting results. In one study, approximately 60% of volunteers in a small cohort of IDUs were infected by multiple founder viruses (as many as 16 variants), while in another small study the percentage of IDUs infected with multiple transmitted founder viruses was the same as MSM.

Bar said that in VAX003, 44% of HIV-infected volunteers were infected with multiple variants, which she said sets a higher bar for vaccine protection than in heterosexual cohorts, such as the population enrolled in RV144. "AIDSVAX may have had a modest vaccine effect that did not rise to the level of overt vaccine protection," she said. In contrast to the VAX003 population, Morgane Rolland of the University of Washington reported in Bangkok that 75% of HIV infections in RV144, among both vaccine and placebo recipients, were the result of a single transmitted founder virus.

What next?

No one is quite sure exactly what the correlates findings mean for the development of an HIV vaccine. Giuseppe Pantaleo, chief of the division of immunology and allergy at the Centre Hospitalier Universitaire Vaudois in Lausanne, Switzerland, is confident that the RV144 trial will continue to inform the field. "The RV144 correlates work is clearly going to guide us on the future of HIV vaccine development," he said. Jerome Kim, deputy director of science at the US Military HIV Research Program, a key collaborator on RV144, was more cautious. "Any results may be unique to this vaccine," he said. "We have to bear that in mind as we look to the next step in HIV vaccine development."

Other researchers think that a more effective vaccine will have to induce bNAbs against the virus. "Even though we're getting advances toward non-neutralizing antibodies, there's still a big gap in potency between these and broadly neutralizing antibodies," said Robin Shattock, professor of mucosal infection and immunity at Imperial College London.

Meanwhile, other researchers see the correlates findings as a way to improve upon the 31% efficacy seen in RV144, perhaps even increasing the efficacy

to a high enough level that it might lead to a first generation AIDS vaccine. "Even a partially effective vaccine, if it reduced transmission, would have an overall effectiveness that would be quite high," said Plotkin. And this pathway to a partially effective vaccine means finding one that works through non-neutralizing activities. "I believe in neutralizing antibodies, but there's more than one way to skin a cat," said Robert Gallo, founder and director of the Institute of Human Virology (IHV) in Maryland.

The first step in improving on the RV144 results is extending the duration of the immune responses. In RV144, efficacy after one year (six months after the full vaccine regimen was administered) was as high as 60%. Although measuring efficacy at this time point was not part of the pre-specified trial analysis, it has been intriguing to many researchers and suggests that improving the durability of the immune responses induced by this vaccine regimen might dramatically increase the efficacy.

Nicos Karasavvas of the Armed Forces Research Institute of Medical Sciences quantified the decline in IgG antibody responses that occurred in the RV144 trial based on the results of a peptide microarray evaluation. This evaluation showed that IgG antibody responses to cyclic V2 peptides dropped significantly by 28 weeks after the last injection. "They declined very rapidly with time," said Karasavvas. A 10-fold decrease in antibody responses occurred between two weeks after the final injection and 28 weeks after the final vaccination was administered.

To improve upon the durability of the immune responses, researchers are planning follow-up studies to RV144 with an additional AIDSVAX boost. The hope is this will extend the efficacy seen in RV144 beyond one year.

Gallo has had similar problems with duration of immune responses in his work at IHV. "Antibodies to Envelope don't last," he said. In experiments with colleagues at IHV, Gallo says they have seen sterilizing protection against a repeat, low-dose SHIV challenge in NHPs vaccinated with their full-length single chain immunogen without induction of conventional bNAbs, but the protection is lost in about four months (see *Vaccine Briefs*, *IAVI Report*, May-June 2011).

Another approach to improving on the RV144 efficacy is using a different canarypox vector. One of the alternative vectors being considered for RV144 follow-up trials is NYVAC, a canarypox vector developed by the EuroVac consortium. Pantaleo presented data in Bangkok on the immune responses induced by the second-generation NYVAC vector expressing trimeric gp140. This vector has been evaluated in combination with an HIV Env boost, and this induced better immune responses when an additional boost was administered at 12 months than a DNA prime and NYVAC boost, according to Pantaleo. He also presented on a second-generation, replication competent NYVAC vector that he says induced even better antibody and ADCC responses than the replication deficient form.

Yet another approach to improving on the RV144 results is changing the immunogen. According to Haynes, the correlates results are already leading to the design of new immunogens. Part of this work involves gaining a better understanding of the gp120 antigen AE.A244 that was tested in RV144. This was a relatively unique immunogen, according to Kim, and part of this uniqueness stems from a gD protein from herpes simplex virus that is tagged on to part of the A244 antigen. This antigen was originally developed at the biotechnology company Genentech and the gD protein was included because of its ability to pull antibodies out of serum.

Inclusion of this protein in the A244 gp120 antigen results in a 10-fold increase in binding affinity with CH01 and PG9, two of the recently identified bNAbs that target the V2 and V3 loops of HIV Env. While binding to these quaternary antibodies was increased, the gD molecule on A244 has a marginal effect on gp120 binding to linear epitopes. The A244 antigen also binds to and recognizes the germline sequences of these antibodies, according to Kim. "Any vaccine antigen has to bind to and recognize germline sequence and A244 does this," he says. But just what role the A244 antigen played in the protection afforded by the RV144 vaccine regimen is unknown. Haynes's lab is currently exploring this.

I believe in neutralizing antibodies, but there's more than one way to skin a cat.
–Robert Gallo

Nabbing more bNAbs

The flurry of discoveries of new bNAbs has become more like a blizzard. Among the latest additions to the antibody armamentarium are a collection of 17 antibodies isolated from four individuals from IAVI's cohort of chronically HIV-infected individuals (*Nature* **477**, 466, 2011)—the same cohort that first led to the isolation of PG9 and PG16, two of the more potent bNAbs identified in the past few years (*Science* **326**, 285, 2009).

The target of many of the 17 new antibodies is different, however, from that of PG9 and PG16. "All of them seem to be glycan dependent," said Ian Wilson, the Hansen professor of structural biology at The Scripps Research Institute (TSRI) in La Jolla, California. Another antibody, 2G12, one of the original handful of bNAbs that researchers had to work with, is also glycan dependent, but according to Wil-

son these new antibodies are more complex than 2G12 and also are "extremely potent." He says that several antibodies in this new family are 10 times more potent than recently isolated bNAbs, and 100 times more potent than other antibodies that were described much earlier. The potency and neutralization breadth of the PGT antibody family were assessed using a panel of 162 HIV pseudoviruses, representing all HIV subtypes currently in circulation. While a few of the PGT antibodies are not as broadly neutralizing, many of them are quite potent. For example, the PGT128 antibody neutralizes about 70% of HIV isolates, as compared to approximately 80% of isolates for PG9 and PG16, but PGT128 can neutralize 50% of isolates at a concentration of 0.1 µg/ml, illustrating its potency.

Wilson and colleagues, in collaboration with Bill Schief, now a principal scientist at IAVI's Neutralizing Antibody Center based at TSRI, then determined the crystal structure of the Fab (Y-shaped) portion of the PGT128 antibody in complex with an engineered glycosylated gp120 outer domain construct containing a truncated V3 loop, work that has since been published (*Science* 2011 doi:10.1126/science.1213256). This crystal structure revealed that PGT128 engages two glycans, as well as the terminal end of the V3 loop. "This is a pretty extensive epitope," said Wilson. Susan Zolla-Pazner, a professor of pathology at New York University, said that while the PGT family of antibodies is certainly more potent and broadly neutralizing than other antibodies, the phenomenon of V3-targeting antibodies also binding to glycans had been documented previously (*J. Gen.Virol.* **73**, 3099, 1992; *J. Virol.* **76**, 9035, 2002).

Wilson said that when the PGT128 antibody was modeled onto the HIV Env trimer using electron microscopy, it showed that "this epitope is pretty accessible." This, along with the fact that PGT128 is so potent, suggests that this epitope may be a good target on which to design vaccine immunogens.

But do they protect?

The next frontier in developing bNAb-based vaccine candidates is designing vaccine immunogens capable of inducing these bNAbs in HIV-uninfected individuals. At the VRC, the focus is primarily on designing immunogens based on the CD4-binding site, which is the target of bNAbs such as VRC01. "We want the most minimal immunogen that has the CD4 site but nothing else," said Jeffrey Boyington, a staff scientist at the VRC. To accomplish this, Boyington and colleagues are adding their CD4-binding site immunogen into the E2 domain of a Chikungunya virus-like particle (VLP).

When this fusion construct was tested in rabbits, researchers found the sera could neutralize some tier 1 HIV isolates after two or three injections. In experiments in rhesus macaques, researchers found injecting the animals with gp140 trimers followed by a boost with the modified Chikungunya VLP construct resulted in elicitation of neutralizing antibodies with greater specificity to the CD4 binding site, according to Boyington. Although these antibodies can only neutralize tier 1 isolates, it provides a proof of principle, and now there are many more tools and immunogens in the pipeline, Boyington said. "We have a long way to go but I think there's a path ahead of us," said Barney Graham, chief of the clinical trials core laboratory at the VRC.

While immunogen design work continues, Graham and colleagues are preparing for trials to test passive immunization of the bNAb VRC01 to answer the question of whether this antibody is capable of blocking HIV infection. Studies in NHPs have shown passive immunization of VRC01 is able to block SHIV infection. At a dose of 20 mg/kg, VRC01 prevents infection following a high-dose rectal SHIV challenge in all of the monkeys studied, while at a lower dose of 5 mg/kg of VRC01, only half (two of four) of the monkeys were protected, suggesting dose of antibody is critical to the level of protection.

The VRC plans to conduct a series of Phase I and II trials of passive immunization of VRC01 to establish the proof of concept that this antibody is capable of blocking HIV infection. The goal is also to define the specificity, potency, and function of VRC01 to provide targets for vaccine-induced protection.

The trials include a Phase I study in HIV-infected and uninfected adults, a Phase I safety and pharmacokinetic study in infants in the US, a Phase I safety study in infants in developing countries, and a Phase IIb study to see if VRC01 can prevent HIV infection in infants. The first Phase I study in adults is expected to start in late December, and if all goes as planned, the Phase IIb study in infants would start in mid-2013.

Originally, the VRC also planned to conduct a Phase IIb trial in adults; however, this study is on hold for now because it would require producing 30kg of VRC01, as compared to 3kg for all of the other Phase I and IIb studies combined. "The problem is in the manufacturing," said Graham, who acknowledged that this trial would be the most informative. Researchers are now looking at several different ways to engineer the antibody so less would need to be delivered. They are also trying to improve the manufacturing capacity. ■

THE ENTERPRISE Changes Course

Eight years after researchers outlined their vision for an HIV vaccine enterprise, the organization is streamlining its focus

By Regina McEnery

About 10 years ago, a handful of leaders in the field of AIDS vaccine research began considering the idea of creating an organization that would bring greater coordination, collaboration, and transparency to the field. Developing a safe and effective AIDS vaccine had proven to be a hugely difficult task, and scientists were in agreement that research and development needed to be accelerated.

Out of this idea for an organization that could serve as a guiding force for the field came the Global HIV Vaccine Enterprise—a concept officially proposed in *Science* in 2003 by 24 players in AIDS vaccine research. The following year, six working groups were created to make recommendations on key areas highlighted in the *Science* article. This led to the establishment of an interim Enterprise Secretariat that was housed at the Bill & Melinda Gates Foundation, and the publication of a scientific strategic plan for the field the following year.

The Enterprise was a grand metaphor, invoking the image of a spaceship boldly steering the HIV vaccine field. But now, after both the organization and the field have undergone many changes, the future direction of the Enterprise has been reconsidered. A seven-person board of directors, after a concerted review, released a letter on October 26th describing a re-envisioned Enterprise that "will both complement the efforts of stakeholders and address the collective needs of the field."

The Enterprise will now focus on three key priorities: coordination, collaboration, and resource optimization. The main activities of the Enterprise will now include organizing the annual AIDS Vaccine Conference, convening relevant parties on strategic issues, and organizing an annual funders' forum to optimize current resources and mobilize new funding. A small Secretariat led by a to-be-named director will oversee implementation of these activities. "The board is working very hard to rejuvenate the Enterprise with a model that is more agile, focused, streamlined, and relevant to the field," says Jose Esparza, senior advisor on vaccines for the Bill & Melinda Gates Foundation, who has served as interim president of the Enterprise board since late 2010 when Peter Piot, director of the London School of Hygiene & Tropical Medicine, resigned the post.

While scientists and advocates support greater collaboration and coordination within the field, there has always been a lack of consensus on how the Enterprise should be structured, what its role should be, and what kind of leader would best suit the organization's needs, as well as those of the field. These questions have intensified since the departure of its first executive director, Alan Bernstein, in June.

Indeed, interviews with 15 researchers, advocates, and policy makers in the field illustrate how difficult it has been to reach consensus on these questions. Esparza acknowledges that there is no shortage of opinions about what the Enterprise should or should not be, and he believes that this collision of ideas has created confusion among people in the field. Still, he says, the dialogue is fruitful and necessary, particularly at a time when research dollars are in short supply and every dollar should be used with maximum efficiency.

The Enterprise is being modified at a time when funding for AIDS vaccine research is on the decline, dropping by 11% since 2007, when it reached a high of US$960 million. By contrast, it is a particularly fruitful time for AIDS vaccine research, fueled by the unexpectedly promising findings from the

RV144 trial, the first trial to show any vaccine-induced protection against HIV, and the recent discovery of broadly neutralizing antibodies and the structural elucidation of their targets on the virus.

The genesis of the Enterprise

The Global HIV Vaccine Enterprise was the brainchild of Richard Klausner, who headed the US National Cancer Institute for six years before joining the Gates Foundation as its executive director for global health in 2002. The foundation had recently made a substantial contribution to AIDS vaccine development in January 2001 with a $100 million, five-year challenge grant to IAVI that was announced at the World Economic Forum in Davos, Switzerland, to correct what Bill Gates considered an "unbelievable market failure" in developing an AIDS vaccine (see *Gates Foundation Pledges "Challenge Grant" to IAVI*, *IAVI Report*, Dec. 2000-Jan. 2001). The grant—significantly higher than the $25 million grant the foundation had awarded to IAVI in 1999—put IAVI on track to launch clinical trials of three of its most promising vaccines (see *Gates Takes On AIDS*, *Science*, Jan. 29, 2001). Klausner joined the foundation 16 months later and felt there was a need for an organization that could provide a more systematic way of developing and evaluating AIDS vaccine candidates.

Those interviewed for this article who were part of the earliest discussions about the Enterprise model say it was Klausner who coined the term "Enterprise" and who suggested that it be modeled after the $3 billion Human Genome Project, a collaboration of more than 2,000 scientists from academia, industry, and government sectors begun in 1990 by the US National Institutes of Health and the US Department of Energy. A hallmark of the government-led Human Genome Project and its network of collaborators, which included the Broad Institute in Cambridge and the Sanger Centre at Washington University in St. Louis, was a call to share data openly.

Deciphering the human genome, however, was essentially an engineering problem, while the development of a successful AIDS vaccine candidate was, and remains, a more basic science challenge. Researchers still lacked understanding about which immunological mechanisms correlated with protection and how to induce these HIV-specific immune responses with a vaccine. "We're basically talking about defining the black box of the human immune system and a virus that has defeated it 59 million times or more," says Carl Dieffenbach, director of the Division of AIDS (DAIDS) at the US National Institute of Allergy and Infectious Diseases (NIAID) and an honorary board member of the Enterprise.

IAVI's founder and former chief executive officer Seth Berkley, who was involved in some of the earliest discussions about the Enterprise, felt a better model might have been the Atlanta-based Task Force for Child Survival and Development, which was formed in 1984 as a collaboration between the World Health Organization (WHO), the United Nations Children's Fund, the United Nations Development Program, the World Bank, and the Rockefeller Foundation, to achieve universal child immunizations by 1990.

"These groups met quarterly to talk about how they were going to improve immunizations in the world," says Berkley, who left IAVI in June to head up the GAVI Alliance, a Geneva-based global partnership launched in 2000 to increase access to immunizations that evolved from the Task Force. "They coordinated rather than competed, they worked together," says Berkley, who recalls pushing for the task-force model. But the idea never gained traction among those involved in creating the Enterprise.

Lawrence Corey, who was principle investigator of the Seattle-based HIV Vaccine Trials Network (HVTN) when Klausner first approached him about the concept of the Enterprise, thinks the analogy to the Human Genome Project may have been a "bit overblown." But, he says, scientists involved in the creation of the Enterprise were largely in agreement that the field needed to change in some ways.

"There was recognition that the pipeline was not very robust," says Corey, who is now president and director of the Fred Hutchinson Cancer Research Center in Seattle. "The people we had worked in traditional ways, in individual groups, trying to do it all. There was an egocentric aspect to the programs, such that 'I would invent an HIV vaccine.' There was no sense of sharing of data and no sense of saying I tried that, don't walk down that path," Corey says.

Some of this sentiment arose in 2003 after VaxGen's vaccine candidate AIDSVAX proved to be ineffective in men who have sex with men, injection drug users, and women in two large Phase III efficacy trials, VAX003 and VAX004, enrolling nearly 8,000 volunteers total. It was with these results still fresh that scientists published the article in June 2003 laying out the need for a Global HIV Vaccine Enterprise (*Science* **300**, 2036, 2003). The concept of the Enterprise outlined in *Science* was an amalgamation of suggestions from various players who had signed on to the article. The article described the need for creative, new public and public-private partnerships to drive the vaccine discovery effort,

the creation of HIV vaccine development centers to increase the diversity of approaches and types of vaccines entering clinical trials, HIV vaccine consortia to address some of the basic immunological questions impeding the development of a vaccine, and more standardized approaches to pre-clinical and clinical laboratory assessment.

The publication in *Science* was followed by a meeting in August 2003 at the Airlie House in Virginia, where about 60 AIDS vaccine scientists, funders, advocates, and policy makers laid out a vision for an alliance of independent agencies and research groups that would participate in the implementation of a shared strategic plan. This alliance was to consist of representatives from government research agencies like the US National Institutes of Health, HIV vaccine research groups like IAVI, the pharmaceutical and biotech industries, developing countries, and international organizations such as the WHO and the Joint United Nations Programme on HIV/AIDS (UNAIDS). A year later, six working groups were formed to evaluate and make recommendations for the field. That same year, the Group of Eight (G8) countries endorsed the idea of the Enterprise at their annual summit.

"I think there was a sense there wasn't a place for the basic research entities to come together," says Chris Collins, who as AVAC's executive director in 2003 co-authored the *Science* paper. "They wanted to try to find ways to have discussion about standardized measures and a forum to share information, to have a place to have a discussion about rational research. It wasn't clear how that would happen without creating a new entity," he adds.

From the start, the Gates Foundation was the biggest benefactor of the Enterprise. Prior to 2007, the Foundation provided in-kind support for the small administrative management needs of the Enterprise Secretariat and funding for the meetings of the working groups. Since 2007, when Bernstein took up the reins at the Enterprise, the Gates Foundation contributed approximately $20 million. DAIDS, the Enterprise's second-largest funder, has given almost a million dollars per year to the Enterprise since 2007. The Canadian government has also contributed some funding.

"In truth, I think the Gates Foundation was clearly interested in this area and this was one way to help channel their potential involvement in addition to what they were already doing, which was primarily funding IAVI," added Collins, who is now vice president and director of public policy at amFAR, the Foundation for AIDS Research, in Washington, D.C.

The Enterprise takes root

The initial planning for the Global HIV Vaccine Enterprise was swiftly followed by release of its inaugural Scientific Strategic Plan (see *An Enterprising Solution Takes One Step Forward*, *IAVI Report*, Dec. 2004-March 2005; *PLoS Med.* **2**, e25, 2005). The plan called for a near doubling of the annual investment in vaccine research. The strategic plan also called for the standardization of assays, including greater access to clinical trial specimens for immunological analysis, common reagents, and for assays to be validated for studying vaccine technologies. It highlighted the need for new tools to detect rare, broadly neutralizing antibodies through the large-scale screening of human blood and the pursuit of novel T-cell inducing candidate vaccines. The plan also emphasized the need to attract new talent to the field by creating new funding opportunities, and to bring new funders on board whose missions and plans were aligned with those of the Enterprise.

"The Enterprise was never going to have grant-making or research decision-making authority," recalls longtime AIDS vaccine activist Bill Snow, a founding member of AVAC who was not involved with the writing of the plan but later served on the Global HIV Vaccine Enterprise Coordinating Committee. "Its main goal was to create this shared plan that people could work off of and identify areas that weren't getting enough attention."

But Giuseppe Pantaleo, executive director of the Swiss Vaccine Research Institute, believes the decision to not give the Enterprise the authority to award grants and fund research limited its influence. "Since the Global HIV Vaccine Enterprise was not intended to be a funding agency, a lot of people at the beginning had limited interest," says Pantaleo. "You attract a lot of attention if you have a lot of funding."

Pantaleo says the Enterprise, as it was structured, was also potentially interfering with the established role and leadership of other governmental and non-governmental institutions and organizations that made their own decisions on funding allocations and research priorities.

Still, many researchers credit the Enterprise discussions and strategic plan with spurring a groundswell of financial support for AIDS vaccine R&D. The same year the Enterprise released its strategic plan, NIAID announced it would provide $300 million over seven years to fund the Center for HIV/AIDS Vaccine Immunology (CHAVI). A year later, the Gates Foundation awarded the initial round of grants totaling $287 million through its newly created Collaboration for AIDS Vaccine Discovery (CAVD).

[ENTERPRISE TIMELINE]

2003

Article in *Science* calling for greater collaboration in HIV vaccine research

Meeting held to develop vision for the Global HIV Vaccine Enterprise

2004

Six working groups (140 participants from 17 countries) develop roadmaps and recommendations for the field

Interim Enterprise Secretariat established and housed at the Bill & Melinda Gates Foundation

2005

First scientific strategic plan published calling for a near doubling of worldwide investment in vaccine research and coordination among researchers

NIAID awards seven-year, US$300 million grant establishing CHAVI

2006

Adel Mahmoud selected to head Enterprise but withdraws before assuming post

Gates Foundation awards $287 million to the CAVD

2007

The Enterprise takes on organizing annual AIDS Vaccine Conference

Alan Bernstein becomes first director of the Enterprise and establishes Secretariat in New York City

Enterprise establishes Young and Early-Career Investigators Committee

2010

Enterprise releases 2010 Scientific Strategic Plan

2011

Bernstein steps down as director of the Enterprise; new vision for Enterprise released

NIAID: National Institute of Allergy and Infectious Diseases; CHAVI: Center for HIV/AIDS Vaccine Immunology; CAVD: The Collaboration for AIDS Vaccine Discovery

Opinions are mixed on how much CHAVI or the CAVD owe their existence to the early work of the Enterprise. Corey believes the Enterprise did give rise to CHAVI and CAVD and says "anyone who thinks otherwise is not part of the history." Snow agrees. "There is no question in my mind that neither CHAVI nor the CAVD would have happened in quite that way without this sense of agreement that individual grants were not big enough to solve some of these complex problems." Others believe the field was already moving in a more collaborative direction by the time the Enterprise was taking root.

Even so, the push for greater coordination and communication among scientists, while widely viewed as important, wasn't always easy or welcome. "Everyone wants coordination and no one wants to be coordinated, at times not even Larry," says Corey.

Berkley feels too much coordination in the field could have sidelined good projects that at the time seem like outliers but later on prove to be game-changers, such as RV144 trial. "Different approaches and different ideas are needed," he says. Dieffenbach agrees. He wonders where the field would be had scientists not pushed forward with RV144. "History has proven them correct but at the time there was this feeling that the only thing we have in the field is this pox-protein component that doesn't induce immunity in people," says Dieffenbach. "The field needs to continue to accept scientific risk and pursue the full range of HIV vaccine concepts, including broadly neutralizing antibodies and novel immunogens that may elicit their production, as well as follow-up on RV144."

Trying to find its niche

Though the field was attracting new funding and working more collaboratively, the Global HIV Vaccine Enterprise had difficulty finding its feet. People familiar with its history say the Enterprise's mission was hampered by a lack of leadership, a growing lack of respect among some scientists and advocates, and ongoing struggles to identify niches that other stakeholders hadn't filled but were nonetheless important to the field. Some people had difficulty distinguishing the difference between the Enterprise concept that 15 organizations had signed on to in 2003 and the Enterprise Secretariat.

The organizational structure of the Enterprise was also criticized by participants as being overly bureaucratic. The Secretariat—which consisted of the executive director and a small staff that at its peak reached 10 people—initially reported to a small three-person board of directors. There was also a larger Enterprise Council, containing nearly two dozen members from the alliance of organizations that made up the Enterprise, which served as a venue for information exchange and coordination among the key parties, according to Esparza.

Richard Jefferys, basic science, vaccines and prevention project coordinator for the Treatment Action Group in New York City likened the Enterprise's role to that of "herding cats," and says the Enterprise's attempts at getting laboratories to adopt standardized assays, while welcome, nonetheless duplicated efforts already taken up by the DAIDS-sponsored Partnership for AIDS Vaccine Evaluation (PAVE).

There was also confusion over the ideal qualifications for the leader of the Enterprise. Adel Mahmoud, former head of Merck's vaccine program, had accepted the executive directorship of the Enterprise in 2006, but several months later, before assuming his new role, changed his mind amid confusion among the Enterprise's Steering Committee over whether the organization needed a scientific leader, a scientific administrator, or an ambassador (*HIV Vaccine Effort Loses Leader*, *Science*, Aug. 15, 2006).

A year later, Bernstein, the founding president of the Canadian Institutes of Health Research, became the first executive director of the Enterprise, and under his direction the Enterprise established its Secretariat in New York City (see *Vaccine Briefs*, *IAVI Report*, Sep.-Dec. 2007).

Pantaleo thought Bernstein's scientific background in oncology, as opposed to immunology, presented challenges for the Enterprise when it came time to write and launch its updated 2010 Scientific Strategic Plan. "He was a great scientist in the cancer field but he was not an immunologist," said Pantaleo. "That to me created a little bit of difficulty."

But Bernstein believes the Enterprise was wise to consider recruiting someone outside the HIV vaccine field. "Fresh perspectives, new ideas and opportunities, and new ways of looking at an old problem are always good in science," he says. And the Enterprise under Bernstein made important inroads in a number of key areas. For instance, the Enterprise made the concerns of young and early career investigators (YECI) a primary area of focus, creating the YECI committee in 2008 and launching the electronic clearinghouse of AIDS vaccine information HIV*e* that is primarily for young investigators.

Jacques Fellay, a YECI co-chair, says other groups like CHAVI and CAVD have now started to more actively promote junior scientists. "Young investigators need more visibility and

exposure and the Enterprise, I think, made it fashionable to do this," says Fellay, adding that he believes the work of YECI through the Enterprise should go forward. "No one is doing it in such a structured and vocal way."

Under Bernstein, the Enterprise also assumed a greater role in the direction of the annual AIDS Vaccine Conference, which will be held next year in Boston and co-chaired by the YECI's former co-chair Dan Barouch, chief of the Division of Vaccine Research at Beth Israel Deaconess Medical Center.

Bernstein also says that during his reign the Enterprise and its Secretariat played a "catalytic role" in the development of regional HIV vaccine research initiatives in Africa—the African AIDS Vaccine Programme, now based in Uganda, and the AIDS Vaccine for Asia Network that was formed shortly after the RV144 results were released.

The Enterprise also brought the topic of systems biology center stage, which some scientists hoped would become one of the organization's signature issues. Under Bernstein's tutelage, the Enterprise tried to encourage the HIV vaccine field to take a more integrative approach to systems biology and its intersection with HIV vaccine research. In 2008, the Enterprise held one of the first meetings to bring together systems biologists and HIV vaccine researchers (see *A Systems Approach to Understanding Vaccines*, *IAVI Report*, July-Aug. 2010).

But the Enterprise's foray into systems biology also seemed to be short-lived, disappointing some scientists. Rafick Sekaly, the scientific director of the Vaccine & Gene Therapy Institute in Florida, attended that meeting and he and others hoped that the Enterprise would push for funding for a globally accessible database where systems biologists could report and share their findings. However, the effort was stalled by delays in the release of the Enterprise's 2010 Scientific Strategic Plan, which highlighted the importance of systems biology, and then shelved completely following Bernstein's departure in June. "There was a huge vacuum after Alan left," said Sekaly.

Bernstein, however, feels the impact of the Enterprise's initiative to highlight the importance of systems biology will become evident. "I believe we have planted a seed that will increasingly bear fruit in the coming years," he says.

Esparza thinks the organization could have facilitated other recent initiatives like the Pox-Protein Public Private Partnership (P5), which was formed recently to follow up on the RV144 results and joins NIAID, the Gates Foundation, the HVTN, the US Military HIV Research Program (MHRP), Novartis, and Sanofi Pasteur. "I think the Enterprise could have facilitated more if we had been prepared organizationally to do it," says Esparza. "To some extent, the tension was between the Secretariat remaining distant and neutral or fully involved supporting the work of the Enterprise partners. I believe that the latter is true, provided that it adds real value to their efforts."

Looking forward

The Enterprise's Board of Directors will release a more detailed framework for the organization by the end of this year. But just as there are many opinions about the accomplishments and shortcomings of the Enterprise, there are also many ideas about what it should be. While some of the ideas about the role of the Enterprise align with its updated vision, others are not included in the current list of key activities. Some within the field think the Enterprise Secretariat should be focused on engaging pharmaceutical companies in HIV vaccine research, which with a few exceptions has not invested heavily so far. Others believe the Enterprise should be a kind of scientific conscience for the field, helping it prioritize its goals and objectives and determine how best to utilize resources in a financially constrained environment. Others suggest it should continue to focus on recruiting new, young investigators to the field through its YECI committee. Some even believe that the Enterprise has outlived its usefulness and should be disbanded.

Pantaleo, who is not on the board, hopes the Enterprise will move forward as a less US-centric organization, which he believes would help boost funding for AIDS vaccine research among European donors. "I have been making this point forever. The problem is that with the exception of the UK, I don't think most of the other European countries or politicians have a really clear impression of what the objectives of the Global HIV Vaccine Enterprise are. They have no clue what it is."

Meanwhile, Nelson Michael, who directs the MHRP and is a member of the Enterprise's board, believes the Enterprise will be reborn as a much more efficient organization. "Now this is just Nelson Michael talking, but running an annual vaccine meeting is obviously a core function. Funding in the field is far more uncertain for all of us, so having a sounding board or forum for funders could be a useful function for the Enterprise." Michael thinks this revamped Enterprise model should be tested. "Let's test drive it and then make a decision downstream," he says. ■

The board is working very hard to rejuvenate the Enterprise with a model that is more agile, focused, streamlined, and relevant to the field.

–Jose Esparza

THE HUMAN PARTS *of HIV*

Some researchers wonder if targeting the human proteins HIV carries might be a promising vaccine approach

By Andreas von Bubnoff

Two decades ago, there was a sense of optimism in the field of AIDS vaccine research after a few groups of researchers found that vaccinating macaques with inactivated simian immunodeficiency virus (SIV), the monkey equivalent of HIV, which had been grown in human cells could protect the majority of vaccinated animals from challenge with SIV that had been grown in the same human cells (*Proc. Natl. Acad. Sci.* **86**, 6353, 1989; *Science* **246**, 1293, 1989; *AIDS Res. Hum. Retroviruses* **6**, 1239, 1990). "We were excited," remembers Michael Murphey-Corb, a professor of microbiology and molecular genetics at the University of Pittsburgh School of Medicine and the first author of one of the studies, which showed that eight out of nine animals were protected (*Science* **246**, 1293, 1989). "It was Christmas time when the paper came out, and people really wanted to believe," she says.

But the optimism didn't last long. In 1991, James Stott and colleagues at the National Institute of Biological Standards and Control (NIBSC) in the UK reported that just vaccinating macaques with human cells protected them from challenge with SIV that had been grown in the same cells. This suggested that the impressive protection that had fueled the optimism in the field might have little to do with a virus-specific immune response, but rather with an anti-cell immune response. This finding led many researchers to abandon this research. "I think it was logical to say let's focus on how to induce a good antiviral response, which we know based on all the vaccines that are available for all other viruses is the way of generating a good protective immunity," says Adriano Boasso, a Wellcome Trust research fellow at Imperial College London who recently co-authored two review articles on anti-cell vaccines.

But a few stalwarts continued studying it and still believe today that an anti-cell vaccine might be an interesting alternative type of HIV vaccine. One of them is Gene Shearer, a senior associate scientist at the National Cancer Institute (NCI), who says he has tried to revive interest in the approach twice without success, but isn't giving up. Earlier this year, he teamed up with Boasso, a former post-doc in his lab, to write two articles that again argue that it is still worth studying the approach (*F1000 Med. Rep.* **3**, 12, 2011; *The Scientist*, June 2011). "We are now 20 years later and still don't have an effective AIDS vaccine and continue to do the same things over and over again," says Shearer, who closed his lab and is now semi-retired. "So I came back and thought maybe we should reopen this idea and see if anybody is interested in it."

Proponents of the anti-cell vaccine approach say that an anti-cell vaccine would have at least one clear advantage. Because it is based on immune responses to the host cells HIV grew in and not to HIV proteins, it would avoid the problem of HIV constantly mutating to escape the immune response. "It clears away the problem of antigenic variation," says Stott. "That is a huge, huge advantage." But there are still many open questions about whether this vaccine approach is effective or feasible. For example, people vaccinated this way might not be able to receive organ transplants. There are also concerns that a vaccine that induces immune responses to host cells might induce autoimmune responses. This makes some researchers skeptical as to whether an anti-cell vaccine could

be approved. "Autoimmunity is always a concern," says David Montefiori, a professor in the department of surgery at Duke University Medical Center. "It's not necessarily insurmountable, but it would take a long time to prove safety."

The negative control

Stott's finding on anti-cell immunity came about because he was vaccinating macaques in a slightly different way from the other research groups at the time. Instead of inactivated and purified SIV that was grown in human cells, Stott and colleagues used crude preparations of whole inactivated SIV-infected human cells (a $CD4^+$ T-cell line called C8166) to vaccinate the macaques. He reported that this approach protected all of the vaccinated macaques from challenge with SIVmac251 that had been grown in the same human cells (*Lancet* **336**, 1538, 1990).

In another round of experiments, he vaccinated four macaques twice intramuscularly with inactivated SIV-infected human C8166 cells, and again, the majority (three of four macaques) was protected from intravenous challenge with SIVmac251 that had been grown in the same human $CD4^+$ T-cell line. But when Stott vaccinated four macaques with uninfected human cells from the C8166 cell line—a negative control to prove that the virus components in the cells were responsible for protection—he found to his surprise that two of the four macaques were protected. "[This] was clear evidence that the protective component was actually not the virus part of the infected cells, but the host part," says Stott.

Stott also found that the serum of the animals contained antibodies to the C8166 cell line. The titer of these anti-cell antibodies was significantly higher in the protected animals, Stott says. Reexamination of 49 animals that had been previously vaccinated with inactivated SIV grown in human cell lines or with inactivated SIV-infected human cells and then challenged with human-cell-grown SIV showed the same correlation between anti-cell antibody titer and protection. This suggested that an antibody response to the human cells appeared to be what protected the animals.

Researchers were shocked. At a meeting in Warwick, UK, there was "quite a strong amazed reaction because it did put the whole field in turmoil because everyone was very positive about getting a vaccine at the time," remembers Mark Page, a principal scientist at NIBSC who was working in Stott's group at the time. "Clearly this turned things upside down." At a conference in the US, Stott says, many people "were actually very skeptical as to whether this was really the case and whether there wasn't some technical [mistake]."

Stott's findings, which were eventually published (*Nature* **353**, 393, 1991), led most researchers to abandon this line of research and instead focus on the induction of virus-specific immune responses. Murphey-Corb eventually abandoned the strategy, partly because she found it difficult to study it further, but also because she got promising results when using a vaccine approach that uses SIV DNA. "I gave up not because I didn't believe in it. I abandoned the inactivated whole virus vaccine approach because I found a more acceptable one," she says.

But not everyone stopped studying this vaccination strategy. Further evidence that anti-cell antibodies were protective came when Martin Cranage and colleagues reported that macaques vaccinated with human-cell-grown SIV were protected from challenge with human-cell-grown SIV, but not from challenge with monkey-cell-grown SIV (*Nature* **355**, 685, 1992). They also found that vaccination with human-cell-grown HIV protected against a challenge with human-cell-grown SIV (*AIDS Res. Hum. Retroviruses* **9**, 13, 1993). This further supported the notion that anti-virus immune responses were not responsible for protection, Cranage says, because antibodies to HIV Envelope, which was in the vaccine, don't recognize SIV Envelope, which was in the challenge virus.

Instead, anti-cell immune responses seemed to be responsible for the protection, likely against host cell proteins like major histocompatibility complex (MHC; known as human leukocyte antigens, or HLA, in humans), because in 1992, Larry Arthur reported that when HIV and SIV bud off an infected cell, they take part of the host cell membrane and its host cell proteins, including HLA or MHC, with them (*Science* **258**, 1935, 1992).

Further studies by Stott and Arthur revealed that, indeed, immunizing macaques with mouse cells expressing HLA proteins or with purified human HLA proteins was sufficient to protect them from SIV that was grown in human cells that expressed the same HLA proteins (*J. Virol.* **69**, 3117, 1995).

Additional support that anti-cell antibody was likely responsible for protection came when Stott, and Murray Gardner's group found that the protection could be transferred with serum from animals that had antibody to the host cell component to naive animals. Gardner also found that protection could not be transferred from animals that had antibody against the virus (*AIDS Res. Hum. Retroviruses* **11**, 843, 1995).

Page says he also has unpublished evidence for the involvement of complement, a protein cascade that gets activated once it binds to the Fc, or tail region of an antibody that is bound to an antigen. Once activated, complement activates a membrane attack complex that punches holes in the virus envelope. Page found that complement binds to anti-host cell antibodies on the virus and then latches onto the virus and lyses it. This suggests that anti-HLA antibodies might protect the vaccinated animals from infection by binding to the HLAs on incoming virus and preventing virus entry into cells (neutralization), or by activating the complement system.

A different mechanism?

Although many researchers believe that anti-HLA antibodies were responsible for protection in Stott's experiments, Montefiori has proposed that it's more likely that antibodies to a different host cell protein were involved (*AIDS Res. Hum. Retroviruses* **11**, 1429, 1995). He and his colleagues found that the anti-cell antibodies in the serum of animals vaccinated with whole inactivated SIV were not important for neutralization of SIV (*Nature* **354**, 439, 1991). This suggested that the animals were protected by a mechanism that doesn't involve neutralization of the virus.

Instead, Montefiori and colleagues found antibodies in the vaccinated animals that bound to complement regulatory proteins (CRPs), human host cell proteins the virus takes with it as it buds from the surface of host cells to protect itself from lysis by the complement system. This suggests that anti-CRP antibodies in the vaccinated animals might keep the CRPs from protecting the virus, rendering the virus susceptible to lysis by the complement system. "That was our hypothesis," Montefiori says.

Consistent with this hypothesis, Montefiori and colleagues showed that complement killed the virus when the CRP antibodies were present, whereas the antibodies alone didn't have any neutralizing effect on the virus (*Virology* **205**, 82, 1994). "In the Jim Stott experiment, I believe the monkeys made antibodies to complement regulatory proteins on the virus that blocked the function of those proteins and thereby rendered the virus susceptible to complement-mediated lysis," Montefiori says. "I am entirely convinced that this was the mechanism of protection." Still, he concedes that to prove that the CRP model is correct, it needs to be shown that blocking all human CRPs can protect monkeys from challenge with virus that was grown in human cells, without neutralizing the virus in a conventional neutralization assay. "This would be strictly a complement-dependent mechanism," Montefiori says. "But the experiment to prove that has never been done."

The CRP mechanism also seems to operate in humans, Montefiori adds, because when researchers studied a woman who had been injected with her husband's white blood cells to treat spontaneous recurrent abortions, they found that her serum could neutralize HIV in a complement-dependent manner even though there were no HIV-specific antibodies. Instead, there were antibodies to the cells that HIV had been grown in (*Science* **263**, 737, 1994).

Stott says Montefiori's model can't explain why just vaccinating macaques with purified HLA protein is sufficient to protect them from SIV that carries that same HLA. But Montefiori says that when he looked in monkeys that had been vaccinated with human-cell-grown SIV, he found no evidence that anti-HLA antibodies had anything to do with neutralization of the virus, perhaps because in this case, the levels of anti-HLA antibodies were too low to neutralize.

Other protective responses

Anti-HLA and CRP antibodies aren't the only potentially protective responses that are induced by exposure to components of whole cells. Lehner and colleagues, for example, found that immunizing macaques with SIV grown in human cells also induces antibodies to the CCR5 receptor that can block HIV entry into its target cells (*Eur. J. Immunol.* **29**, 2427, 1999). Additionally, Shearer and colleagues showed that stimulating human white blood cells with supernatants of cells with different HLAs induces a ribonuclease called eosinophil-derived neurotoxin (EDN) that inhibits HIV replication (*AIDS* **17**, 481, 2003).

Lehner and colleagues also found HIV replication inhibited *in vitro* in T cells taken from couples who regularly have unprotected sex, especially women who are often exposed to foreign HLA in the ejaculates of their partner (*Lancet* **363**, 518, 2004; *PLoS ONE* **4**, e7938, 2009). In addition, they found that the $CD4^+$ T cells of women who were injected with their husbands' white blood cells to treat them for spontaneous recurrent abortion have increased expression of APOBEC3G (which mutates the viral genome during reverse transcription) and inhibited HIV replication (*Eur. J. Immunol.* **39**, 1956, 2009). They also found that such women show lower levels of CCR5 receptor expression and elevated levels of anti-CCR5 antibodies (*Nat. Med.* **5**, 1004, 1999; *Clin. Exp. Immunol.* **129**, 493, 2002).

The fact that anti-cell vaccination induces many different innate and adaptive immune responses

suggests, Shearer says, that an anti-cell vaccine might be able to block the binding of the virus to its target cells at two different steps—at the HLA level by anti-HLA antibodies, and at the CCR5 coreceptor level by anti-CCR5 antibodies or innate factors such as beta chemokines that bind the CCR5 coreceptor (see figure, this page). Anti-cell vaccination also induces other soluble innate factors that inhibit HIV replication such as APOBEC3G and ribonuclease. "No traditional AIDS vaccine will do all of the things that this will," Shearer says.

Open questions

Before a human anti-cell based AIDS vaccine can be developed, there are many questions that need to be answered. There is still no solid proof that vaccination with cells or cellular components from the same species can protect from virus grown in these cells. In Stott's initial 1991 study, researchers protected macaques by xenoimmunization, which means they vaccinated the animals with cells from a different species (humans) to protect them from challenge with SIV grown in human cells.

But humans are infected with HIV that comes from other humans. Therefore, if this type of vaccination approach were ever going to be developed into a human vaccine, it would involve alloimmunization, which means using cells or cell components from the same species (humans).

So one major challenge is to show that alloimmunization can protect macaques as well, Shearer says. But that is not so easy. Murphey-Corb says she tried to grow SIV in primary rhesus mononuclear cells for allogeneic vaccination challenge studies but couldn't get clean enough material to do the experiments. Other attempts had mixed results. According to Page, Stott's group vaccinated monkeys with fixed SIV-infected monkey cells and achieved partial protection from challenge with monkey-cell-grown SIV in a study that was never formally published.

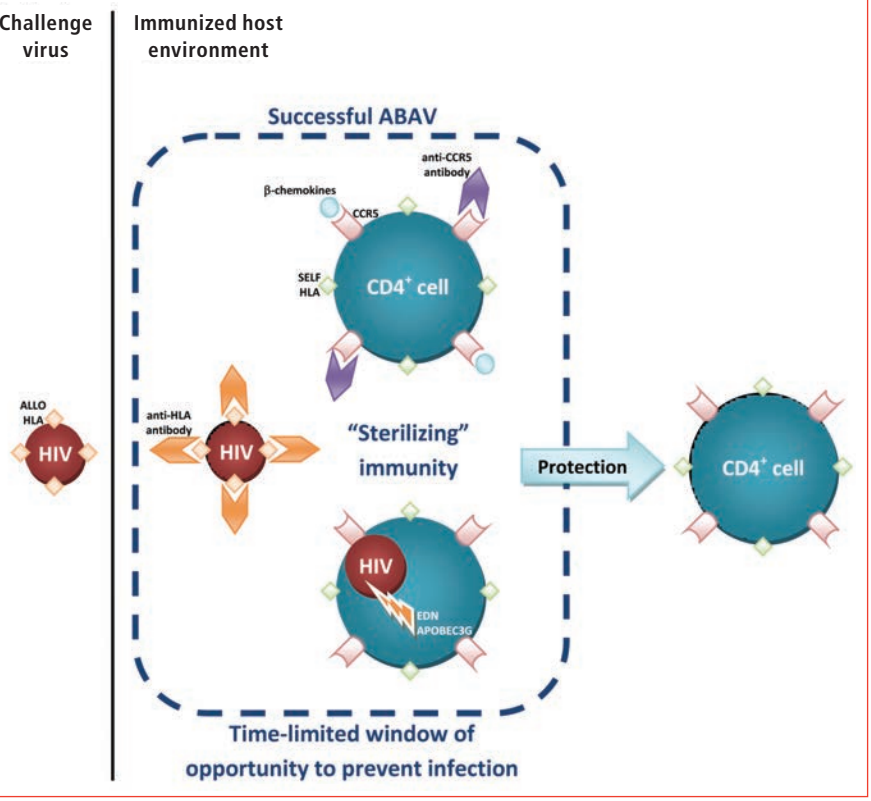
In contrast, Cranage's group did not see protection after alloimmunization of macaques with B cells (*AIDS Res. Hum. Retroviruses* **13**, 923, 1997). More recently, Page and Neil Almond at the NIBSC did another alloimmunization experiment in Mauritian cynomolgus macaques, which do not have very diverse MHCs because they are inbred and have a relatively homogenous genetic background. They also failed to see any protection, perhaps, Stott says, because the Mauritian cynomolgus macaques used to generate the vaccine had very similar MHCs to the vaccinees, and therefore the vaccine might not have generated much of an immune response.

Given these mixed results, Stott says it still needs to be shown that alloimmunization can protect. If he can get funding, Page says he plans to do further alloimmunization studies in Mauritian cynomolgus macaques, taking advantage of the fact that their MHCs are very well characterized.

Another barrier to developing a vaccine that can protect from HIV infection by inducing anti-HLA antibody responses is that the vaccine would need to contain multiple HLA proteins to match all potential HLAs on viruses a vaccinated person might encounter, Boasso says. Studies are needed to find out how many different HLAs

Mechanism of protection of a successful alloantigen-based AIDS vaccine (ABAV)

Upon exposure to HIV particles carrying allogeneic human leukocyte antigen (ALLO-HLA), pre-formed anti-HLA antibodies in the immunized host will block HIV challenge. Anti-CCR5 antibodies and ß-chemokines will inhibit HIV interaction with its coreceptor and, in case HIV successfully enters target cells, intracellular restriction factors such as APOBEC3G and a ribonuclease called eosinophil-derived neurotoxin (EDN) will prevent productive infection. This allogeneic HLA-induced arsenal of antibodies and antiviral factors may efficiently prevent infection ("sterilizing" immunity) and result in full protection. Originally published in *F1000 Med. Rep.* **3**, 12, 2011.



need to be combined in a vaccine to protect against the majority of circulating HIV strains, adds Shearer.

Lehner says that while the type and number of HLAs that are needed to protect from the majority of HLAs is different for different populations, for a given population only a handful of different HLAs might be needed. For Caucasians, analysis of HLA sequences suggests that just four different HLAs could cover 90% of the population, says Lehner.

So far, however, combining several HLAs in a vaccine has not led to the near complete protection researchers observed 20 years ago. Earlier this year, Lehner vaccinated macaques with the four human class I HLA proteins that he says can cover 90% of the Caucasian population, together with one HLA class II protein, HIV-1 gp140, and SIV p27, all linked to dextran (a complex polysaccharide molecule) to keep them together. This protected two out of eight macaques from an intravenous challenge with an SIV/HIV hybrid (SHIV) that was grown in human cells that had at least one HLA class I and one HLA class II protein in common with the HLA proteins used in the vaccine. The remaining macaques had a reduced viral load compared to unvaccinated controls (*J. Virol.* **85**, 6442, 2011).

One possible reason for the incomplete protection is that Lehner used purified proteins and not whole viruses or cells to vaccinate. Lehner says he chose not to use whole cells in the vaccine because that comes with risks, including that they might carry oncogenic viruses. Another possible reason is that not all HLAs in the study were identical in the vaccine and the challenge virus.

Even if all these challenges could be overcome and a human allovaccine was developed, it would have several limitations. For one, it only induces immune responses to HIV particles from another person, Boasso says. Once HIV particles are made by the vaccinee's own cells, it doesn't protect anymore. "As soon as the virus becomes part of you, it's self and every immune reaction induced so far is completely useless. So you would [have to] prevent infection, period."

Another limitation, Page says, is that because people vaccinated with an allovaccine develop anti-HLA antibodies, they would be excluded from donating blood. They also couldn't receive an organ transplant, he says, unless they are plasmaphoresed to remove the anti-HLA antibodies.

A vaccine that induces an immune response to HLAs or other proteins that are similar to proteins on the body's own cells also raises the concern that it might induce inflammation or autoimmunity, Boasso says, adding that HLA is a molecule that is highly immunogenic.

However, there is no clear evidence that exposing people's immune systems to cells or HLAs from a different person actually leads to autoimmunity, he says. For example, there hasn't been any sign of autoimmunity in over 3,000 women who have been vaccinated with their partner's white blood cells as a treatment for recurrent spontaneous abortion. In addition, says Page, women who gave birth multiple times and people who often receive blood transfusions have HLA antibodies without developing autoimmunity.

Also, HIV-infected people who were immunized with inactivated gp120-depleted HIV particles (a therapeutic vaccine candidate called Remune) developed antibodies to the HLA molecules that had been used in the cell line used to grow the HIV particles. But a study by Page and colleagues found that the vaccinees with the HLAs that matched the cells that the virus was grown in didn't mount an immune response (*AIDS* **21**, 375, 2007), suggesting that their immune systems didn't lose their tolerance to self proteins as a result of the vaccination. "That lessened any concerns at the time that you would induce autoimmunity to HLA," says Page, the first author of that study.

Still, Murphey-Corb doesn't believe the US Food and Drug Administration would ever approve a vaccine that carried even a theoretical risk of inducing an autoimmune response. "The concept that you are deliberately going to induce a response to self is going to kill [this] forever in the US in my opinion," Murphey-Corb says. "It's the perception and not the reality."

Stott acknowledges the obstacles but still hopes that the most recent attempt to revive some interest in the allovaccination approach will succeed. "We really should be trying to look at radically different approaches. But because it's radically different, there are a whole lot of hurdles that are going to have to be overcome because it's new territory," says Stott.

This may be difficult, given that the RV144 trial for the first time showed modest protection against HIV, says Montefiori, who has turned away from studying alloimmunization because he couldn't get additional funding. "Since RV144 we have a positive signal that by all accounts doesn't involve anti-cell antibodies or any type of anti-cell immune response," Montefiori says. "So nowadays I think to try to improve on the existing vaccines that are based on the viral proteins alone makes more sense and avoids all of the potential downsides of alloimmunization." ■

Vaccine BRIEFS

Grants Awarded to Establish New Nonhuman Primates Consortia

Researchers from Emory University and the Beth Israel Deaconess Medical Center (BIDMC) were recently selected to lead a five-year, US$60 million effort to use nonhuman primate (NHP) models to better understand events that occur during the earliest stages of mucosal HIV infection. More than 90% of all HIV infections worldwide are sexually transmitted. But, as is likely obvious, studying the earliest stages of HIV transmission is difficult, if not impossible, in humans. So instead, researchers are relying on studying simian immunodeficiency virus (SIV) infection in rhesus macaques.

The US National Institute of Allergy and Infectious Diseases (NIAID) solicited applications for the Consortia for AIDS Vaccine Research in Nonhuman Primates in April 2010 with the goal of establishing a collaborative, multidisciplinary research program to investigate viral and host events at the earliest stages of mucosal infection of NHPs with SIV, and to identify vaccines and vaccine-induced immune responses that can block initial infection, prevent establishment of systemic infection, or significantly reduce the pathogenic effects of SIV. The initial request for applications was for one to two applicants eligible to receive a total of $5 million a year over five years. Then in August 2010, NIAID upped the ante to $8.5 million a year for five years. The grants were then raised again to $12 million a year for five years after NIAID was able to obtain additional funds.

The BIDMC consortium, which comprises nine institutions and will receive about $36 million, is being led by Dan Barouch, chief of the Division of Vaccine Research at BIDMC and R. Paul Johnson, associate professor of medicine at the New England Primate Research Center. The collaborating institutions include Oregon Health & Science University (OHSU), The Scripps Research Institute, the University of Minnesota, the University of Pennsylvania, the Florida-based Vaccine & Gene Therapy Institute at OHSU, the University of Massachusetts Medical School, and SAIC Frederick Inc.

The consortium will work on five projects and six cores designed to elucidate the mechanisms of protection against SIV and an SIV/HIV hybrid known as SHIV in rhesus macaques following administration of vector-based, protein-based, or live-attenuated virus vaccines, as well as monoclonal antibodies.

"The overall goal of our grant is to look at the very early events of acute mucosal SIV infection in monkeys and how different vaccine technologies might be able to block and impede those events," says Barouch. "No one has looked at such an early point at exactly how vaccines work—whether there are antibodies at the site of inoculation, whether there are mucosal T cells at the site of inoculation, what types of immune responses can block or inhibit the virus, or what kinds of immune responses fail to block the virus."

Barouch says the BIDMC consortium will not be developing new vaccine technologies but will be investigating the earliest phases of infection at a level of detail and sophistication that has never been done before.

The Emory consortium, which consists of seven institutions and is receiving $26 million, is being led by Eric Hunter, co-director of the Emory Center for AIDS Research and professor of pathology and laboratory medicine at Emory's School of Medicine. The research will be conducted primarily at the Yerkes National Primate Research Center at Emory. Other collaborators include Louisiana State University Health Science Center, the Mount Sinai School of Medicine, the La Jolla Institute for Allergy & Immunology, and the Nebraska Center for Virology at the University of Nebraska.

The Emory consortium is working on four projects that examine the immunological mechanisms by which adjuvants can enhance SIV-based vaccine candidate induced protection. Hunter says researchers will be looking at two different adjuvants. The first, granulocyte-macrophage colony-stimulating factor (GM-CSF)—a cytokine produced by macrophages, neutrophils, and other immune cells—has already shown promise and is being used in a DNA/MVA-based AIDS vaccine candidate by Georgia-based GeoVax Inc. The other uses toll-like receptor ligands delivered in a novel synthetic nanoparticle formulation. Both are thought to stimulate a more effective immune response through different arms of the innate immune system, though the mechanisms involved are unclear.

Hunter says identifying adjuvants that might enhance the responses of existing vector-based vaccine candidates would be a significant breakthrough for the field, citing the relatively modest 31.2% efficacy observed in the RV144 trial in Thailand as an example. "If one could increase the efficacy of that significantly through adjuvants or modifications in the viral-vector systems, then I think that could really be important in moving the field forward," he says.

While the BIDMC and Emory consortiums will conduct separate projects, they will be looking for areas of synergy, according to both Hunter and Barouch. "The two are distinct, but there will be close interactions," says Barouch. —*Regina McEnery*

Hormonal Contraception Raises HIV Infection Risk, According to New Study

Results from a study of 3,790 serodiscordant couples from southern Africa provides the strongest evidence thus far that the use of hormonal contraception may also elevate the risk of HIV acquisition, in some instances dramatically (*Lancet Infec. Dis.* 2011, doi:1016/S1473-3099(11)70247-X).

The study, led by investigators from the University of Washington, found the use of both oral and injectable hormonal contraceptives was associated with a doubling of the risk of HIV infection among women, as well as doubling the HIV transmission risk from women to men. While women in the study received either oral or injectable forms of hormonal contraception, the long-lasting, injectable methods were the most commonly used in this study population. Sub-group analyses of just the women who received injectable contraception had a significantly increased HIV infection risk, while an analysis of women using oral contraception showed a non-significant increase in HIV infection risk.

This study isn't the first to find a link between hormonal contraception and an increased risk of HIV acquisition. "We went into this analysis knowing the previous data had not provided a clear picture," says study investigator Jared Baeten. "There had been some suggestion of increased risk but not all studies showed this, so we were not sure what we would find."

The World Health Organization (WHO) will convene a meeting in January to consider whether the evidence suggesting hormonal contraception increases HIV infection and/or transmission risk is now strong enough for them to issue a warning to women.

According to the Guttmacher Institute, a New York City-based non-profit that advances sexual and reproductive health through research, policy analysis, and education, about 12 million women in sub-Saharan Africa use injectable contraceptives and eight million use oral contraceptives. Another 11 million use condoms, sterilization, or intrauterine devices as contraception. Baeten says in some HIV prevention studies of vaccines and other interventions like pre-exposure prophylaxis (PrEP), contraception is "often part of the clinical counseling and clinical repertoire of services," and he expects hormonal contraception would continue to be offered in these trials. "I think we would be remiss to say that contraception should not be part of clinical care," adds Baeten.

The serodiscordant couples that were followed in the prospective study were initially enrolled in either the Partners in Prevention HSV/HIV Transmission Study—a randomized, placebo-controlled trial testing whether suppressing herpes simplex virus type 2 (HSV-2) with daily acyclovir prevented HIV transmission among 3,408 serodiscordant couples from Botswana, Kenya, Rwanda, South Africa, Tanzania, Uganda, and Zambia—or a parallel immune correlates of protection study, which involved an additional 485 serodisordant couples from Uganda and South Africa.

Baeten says the cohort they followed was much larger than those from previous studies, making the findings more robust. Focusing on serodiscordant couples, according to Baeten, also allowed them to better track HIV transmission between both men and women. This is thought to be the first prospective study to show increased HIV risk in the male partners of HIV-infected women.

But the study was limited by the fact that contraception use was self-reported and investigators didn't record the specific brand of contraception used, preventing them from being able to draw any conclusions on differences in HIV risk with specific forms of contraceptives. Baeten says preclinical studies have not been able to determine how hormonal contraceptives enhance the risk of HIV, adding that the recent findings were not intended to undermine the importance of contraception. —*Regina McEnery*

New Data on How Tenofovir Protects Against Herpes Simplex Virus

The results of the CAPRISA 004 trial, reported in July 2010, showed that a vaginal microbicide gel containing 1% of the antiretroviral tenofovir reduced the risk of HIV infection among women by 39%, and also reduced the incidence of herpes simplex virus (HSV)-2 by 51% in a subset of 450 women from CAPRISA 004, who were not already HSV-2 infected at the start of the trial (see *Microbicides Finally Gel, Securing Spotlight at the International AIDS Conference*, *IAVI Report*, July-Aug. 2010). Now, researchers have reported a possible mechanism by which tenofovir inhibits HSV-2 (*Cell Host Microbe* **10**, 379, 2011).

In the study, researchers tested a 1% tenofovir gel in tissue samples taken from women infected with HSV-2. These experiments showed that tenofovir gel inhibited HSV-2 replication in cells found in epithelial or connective tissue from women, and decreased HSV-2 replication by as much as 99% in lymphoid and cervicovaginal tissue samples. In tissues from mice infected with HSV-2, 1% tenofovir gel also delayed the formations of lesions and even death. Researchers concluded that the active metabolite of tenofovir inhibited both HSV-2 DNA polymerase and HIV reverse transcriptase. —*Regina McEnery*

Gates Foundation Names New Head of Global Health Program

Trevor Mundel, the global head of development for Novartis Pharma AG in Switzerland, was named the new director of global health at the Bill & Melinda Gates Foundation, overseeing a portfolio that has awarded more than US$14.7 billion in grants thus far.

Mundel, who assumes his new post Dec. 1, will lead the foundation's efforts to develop and deliver drugs, vaccines, and other tools to fight diseases such as HIV/AIDS, tuberculosis, and malaria, and to continue progress toward polio eradication. Mundel replaces Tachi Yamada, who retired in June as head of the global health program and is now senior executive in residence at the Seattle-based venture capital firm Frazier Healthcare.

Mundel has been a senior executive and scientist with Novartis since 2003. While at Novartis, he oversaw some 140 clinical projects, a budget of $3 billion, and more than 7,500 employees. —*Regina McEnery*

Oral Tenofovir Arm of VOICE Trial Discontinued Early

One arm of the multi-arm Phase IIb test-of-concept VOICE trial designed to test the safety, efficacy, and acceptability of one topical and two oral pre-exposure prophylaxis (PrEP) regimens in more than 5,000 women was discontinued in September after the trial's independent data safety monitoring board (DSMB) concluded that the study would be unable to show any difference between a daily dose of the antiretroviral (ARV) pill tenofovir (TDF) and placebo in preventing HIV infection.

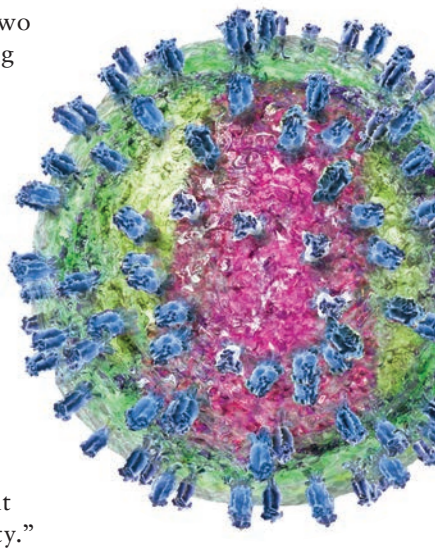
The remaining arms of the trial, one of which is testing daily administration of Truvada (the single pill combination of TDF and the ARV emtricitabine, or FTC), and another testing the topical administration of a 1% tenofovir gel, will continue in order to determine if they are safe and effective HIV prevention measures for women compared to pill or gel placebo groups. Unlike other large-scale PrEP trials that were recently completed or still ongoing, the VOICE study is the first to evaluate both oral and topical PrEP regimens in the same trial.

The US National Institute of Allergy and Infectious Diseases (NIAID), the primary funder of the trial projected to cost US$100 million, noted that the DSMB found no safety concerns with oral TDF, which is currently used to treat HIV. The VOICE trial is sponsored by NIAID, the Microbicide Trials Network, Gilead Sciences (the manufacturer of tenofovir and Truvada), and CONRAD, a Virginia-based research institute developing contraceptive products and options to prevent HIV and other sexually transmitted infections.

The VOICE trial, which is being conducted at 15 clinical sites in South Africa, Zimbabwe, and Uganda, began in September 2009 and completed enrollment of 5,029 women in June. About 1,000 of the volunteers were randomized to the oral TDF arm.

Michael Chirenje, associate professor in the department of obstetrics and gynecology at the University of Zimbabwe and a principal investigator of the trial, says interpreting the recent results from the oral TDF arm would be a matter of speculation at this point. The trial is due to conclude in June and the study unblinded shortly after, at which point investigators will be able to determine whether volunteers in the oral TDF arm were less adherent to the daily dose of tenofovir than women in the Truvada or microbicide arms.

Still, Chirenje says it is perplexing oral TDF did not seem to work any better than placebo in the VOICE study. A previous trial involving serodiscordant couples found the same regimen was 62% effective at reducing HIV infection risk in men and women. One possible reason for the difference in outcomes for these two studies is demographics, according to Chirenje. HIV-uninfected women enrolled in the VOICE study tended to be in their early 20s and unmarried, perhaps making them less inclined to take their pills faithfully compared to the women in the serodiscordant couples study, whose average age was 36 and who were aware their partners were HIV infected.



"Obviously we are all disappointed and perplexed by the recent results," says Chirenje. "But in science, we have to accept reality." Three other trials have found both oral tenofovir and oral Truvada to be effective at preventing HIV infection in serodiscordant couples (see *Treatment Is Prevention*, *IAVI Report* July-Aug. 2011) and men who have sex with men. However, another trial, known as FemPrEP, evaluating oral Truvada in women, was discontinued ahead of schedule after the DSMB concluded that it would be highly unlikely to demonstrate any efficacy (see April 18, 2011 *IAVI Report blog*, *Oral PrEP Trial in Women Stopped Early*). —*Regina McEnery*

Research BRIEFS

Researchers Make Immunogen That Can Bind to Precursors of Broadly Neutralizing Antibodies

Over the past few years, researchers have isolated many new broadly neutralizing antibodies (bNAbs) from chronically HIV-infected individuals. But only a small percentage of HIV-infected individuals can make bNAbs. It also takes years for them to develop, possibly because the precursor B cells that will eventually develop into the cells that produce the mature bNAbs have to first undergo a process called affinity maturation, during which the antibody sequences a person inherits in their germline accumulate mutations that increase the affinity of the antibody for antigens such as HIV Env (see *Vaccines to Antibodies: Grow Up!, IAVI Report*, July-Aug. 2010).

These mutations have been shown to be required for the ability of HIV-specific bNAbs to bind to and neutralize many different HIV strains. The first evidence of this came when Dimiter Dimitrov, a senior investigator at the National Cancer Institute in Frederick, Maryland, and his colleagues found that the unmutated precursors of bNAbs such as 2F5 cannot bind HIV Env (*Biochem. Biophys. Res. Commun.* **390**, 404, 2009). This led Dimitrov to suggest that an HIV vaccine should contain immunogens that can bind the unmutated precursors of bNAbs to kick-start the affinity maturation process because HIV-uninfected people only have B cells that express such precursors (*Viruses* **1**, 802, 2009).

Now, Barton Haynes, a professor at Duke University Medical Center, and colleagues report that they have made such an immunogen by removing part of the sugar groups from the HIV gp140 Env protein (*PLoS Pathog.* **7**, e1002200, 2011). Unmutated precursors of the HIV-specific bNAbs 2F5 and 4E10 can bind this sugar-depleted Env but not the normal Env that has the sugars in place.

In rhesus macaques, the sugar-depleted Env protein was also a better immunogen than the normal Env, inducing higher levels of antibodies that bind to the membrane proximal external region (MPER; the part of HIV recognized by 2F5 and 4E10) a few weeks after immunization. The study is the first, Haynes says, to show that an HIV immunogen "that bound to the unmutated ancestor better or at all just happened to be the one that was a better immunogen."

"This is the first published report of an immunogen capable of binding the putative germline predecessor of two broadly neutralizing antibodies," says Dimitrov, who was not involved in the study. "Even more importantly, they found this newly designed vaccine immunogen, which binds the germline, also shows high immunogenicity in monkeys."

Haynes says the study shows that "there are ways to design immunogens that can bind to germlines that you would expect would be able to trigger and set off this pathway of B cell maturation that we are trying to induce."

While the results are promising, immunization with the sugar-depleted Env didn't induce HIV neutralizing antibodies in the vaccinated macaques, Haynes says, possibly because the immune systems in the macaques only had a few weeks after immunization to respond, too short for affinity maturation to take place. And even if there was enough time for bNAbs to be induced, they might be deleted by the immune system, Haynes adds, because some bNAbs are polyreactive and bind many different antigens including some that are self. "We made the hypothesis years ago that this polyreactivity might predispose these antibodies to be downregulated by the immune system because they cross-react with self," Haynes says.

To show that this is the case, Laurent Verkoczy at Duke University and Haynes did a second study in which they made mice that only expressed the mature bNAb 2F5 in all of their B cells, and found that the immune systems of these mice indeed deleted B cells that expressed mature 2F5 on their surface from the bone marrow (*J. Immunol.* **187**, 3785, 2011). However, when they took the antibody-expressing B cells from the bone marrow of the mice and separated them from the cells that normally eliminate B cells that make polyreactive antibodies, they found that a few of the 100 B cells they characterized in detail made 2F5 antibodies.

This suggests that in most people, even if they develop 2F5 or other types of bNAbs, their own immune systems may eliminate most of them. Why the immune systems of the people who can make these types of bNAbs don't eliminate them is unclear, however. "Some people for whatever reason don't effectively eliminate these cells and they are allowed to eventually go on and mature," Haynes says, adding that they are trying to find out why.

It is also unclear how to keep the immune systems of vaccinees from eliminating bNAbs that are induced by a vaccine. Haynes hopes it might be possible to induce affinity maturation pathways that lead to types of bNAbs that are not subject to this elimination. To test this,

Haynes and colleagues are immunizing animals with immunogens that can bind to unmutated precursors of bNAbs to see if the resulting antibodies are eliminated or not.

In addition, Haynes is working to understand how bNAbs develop from their precursors in HIV-infected individuals to identify ones that are not subject to elimination, and plans to isolate unmutated and partially mutated precursors of bNAbs from the bone marrow of HIV-infected individuals to use them as templates to study additional ways Env can be changed to allow these antibodies to bind. —*Andreas von Bubnoff*

Stem Cell-Like Memory T Cell Identified in Humans

If a person is infected with a virus or pathogen, their immune system responds by generating effector CD4+ and CD8+ T cells that are specific for that pathogen. Many of them become memory CD4+ and CD8+ T cells, which persist long after the infection is over. Memory and effector T cells are derived from naive T cells specific for millions of different antigens, most of which will never be seen. When a naive T cell encounters the right antigen that binds to its T cell receptor (TCR), it starts dividing, and can become either a central memory T cell that circulates in blood and lymph nodes and serves as a source for additional T cells, or an effector memory T cell, which is found in tissues such as the mucosa.

Scientists have long suspected that some memory T cells can persist for an especially long time and multiply and regenerate other types of memory T cells especially well, but the exact identity of these cells was unclear, says Mario Roederer, a senior investigator at the Vaccine Research Center at the National Institute of Allergy and Infectious Diseases.

Now, Roederer and colleagues identified a new type of memory T cell in humans that can regenerate itself and other T cell types better than effector and central memory T cells, making it similar to stem cells (*Nat. Med.* **17**, 1290, 2011). The identification of these so-called stem cell memory (SCM) cells might give researchers a better tool to develop vaccines that can induce long-lasting CD4+ and CD8+ memory T-cell response.

To identify the cells, Roederer and colleagues treated human naive CD4+ and CD8+ T cells *in vitro* with TWS119—a drug that keeps cells from differentiating too much and has been shown to turn naive CD8+ T cells from mice into stem cell-like CD8+ memory T cells (*Nat. Med.* **15**, 808, 2009). They found that these cells expressed unique cell surface markers, differentiating them from known types of memory T cells (effector memory and central memory T cells). Using these markers, Roederer and colleagues were able to identify SCM cells among white blood cells from healthy people.

Of all memory T cell types, SCM cells are most similar to stem cells because they have the best capacity to regenerate themselves and other memory T cell types, Roederer says. "When they are stimulated, they can divide many times and generate a very large population of T cells," he says. "And they can persist for a very long time without needing any antigenic stimulation."

"What's unique is that they found a subset that seems to be more stem-like than the remaining memory cells," says Louis Picker, a professor of pathology, molecular microbiology, and immunology at Oregon Health & Science University, who was not involved in the study. "[Their] function appears to be long-term self renewal of the memory response. This population may underlie the ability of memory responses to last for decades."

The identification of SCM cells could explain previous observations that suggested memory T cells can regenerate themselves and other T cell types surprisingly well, Picker says, referring to a 1997 study where he and his colleagues described a man who had lost all of his T cells as a result of immunosuppressive therapy after a liver transplant. Once the immunosuppressive drugs wore off, the man lived for many years with all of his memory T cells derived from the liver transplant. This means that all of his memory T cells had been regenerated from memory T cells in the donor liver, without any help from naive T cells (*Exp. Hematol.* **25**, 147, 1997).

Because SCM cells can multiply better than other memory T cell types and can develop into CD8+ T cells that can kill other cells, Roederer and colleagues also investigated whether SCM cells could kill cancer cells. They made SCM cells that produced a mesothelin tumor-specific antibody connected to their TCR. Recognition of a mesothelin tumor cell by this antibody could activate the TCR and the SCM cell could then kill the tumor cell. Indeed, they found that injecting these modified SCMs into mice with a human mesothelin tumor caused the tumor to shrink. All of the mice survived, whereas mice that were treated with central or effector memory T cells that had been modified in the same way died after a few weeks. The reason SCM cells were better at killing the tumor is because they generated 10-50 times more cells than the other memory T cell types, Roederer says. "The reason they protected the mice is simply because they were able to expand to much larger numbers and then differentiate into effector cells and then kill the tumor," says Roederer.

Roederer says identification of SCM cells is relevant for the development of vaccines that induce a life-long vaccine-specific CD4+ and CD8+ T-cell memory response in the absence of antigen, which would translate into the ability to control the virus long after vaccination. "To generate life-long immunity, you need to generate these stem cell memory cells that are specific to the vaccine," Roederer says. "They are the ones that can persist for what we think is forever, whereas the central memory and effector memory cells are much more dependent on the presence of antigen, and their numbers wane if antigen is not present."

Currently, Roederer is studying how many SCM cells are generated in rhesus macaques using different vaccine strategies. "I would hypothesize that a vaccine that generates a good number of SCMs would generate better durable long-term memory and so that's the hypothesis that we are going to test," Roederer says. He also plans to look for SCM cells in humans who received different HIV vaccine candidates. —*Andreas von Bubnoff*

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