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CONFERENCE REPORT

KEYSTONE SYMPOSIUM ON HIV VACCINES

PLUS

Why Clinical Trials are a Sound Investment

EDITOR'S LETTER

Conference season is in full swing. In March, the Keystone Symposium on HIV Vaccines was held in Banff, Canada, following on the heels of the 17th Conference on Retroviruses and Opportunistic Infections in February, and the Keystone Symposium on HIV Biology and Pathogenesis in January.

The HIV Vaccines meeting is widely considered to be the most important of the annual scientific gatherings on the topic, and is a highlight on the crowded conference calendars of many researchers. Discussions this year centered on characterization of the new crop of broadly neutralizing antibodies that were discovered over the past year (see *Antibody Fever*, page 4). As researchers scrutinize the structures of these antibodies and their binding sites on the virus, they are gaining valuable insights into how these antibodies are able to neutralize so well. At Keystone, researchers reported for the first time that some of these antibodies appear to be highly developed, having acquired several mutations through a process referred to as affinity maturation. This finding could have important implications for the design of vaccine immunogens based on these antibodies.

Other news related to antibodies also emerged from recently published research, suggesting that in addition to neutralizing free virus particles, antibodies may be able to block the cell-to-cell spread of HIV (see *New Insights on Antibody Inhibition of Cell-associated HIV Spread*, page 16).

In addition to these advances in basic research, the AIDS vaccine field is also gearing up for additional clinical studies based on the prime-boost regimen tested in the RV144 efficacy trial in Thailand, which provided the first evidence of vaccine-induced protection against HIV. Efficacy trials are both costly and complex to conduct, but given the largely surprising results that emerged from recent efficacy studies, many researchers argue that such trials are paramount to advancing HIV vaccine research (see *Investing in Surprise*, page 11). In this issue, we analyze the main factors that contribute to the high costs of clinical research.

We also explore how investigators involved in HVTN 505, an ongoing Phase II trial of a DNA/adenovirus prime-boost regimen, are using social networking sites to boost sluggish enrollment (see *Investigators Tap Social Networking to Pique Interest in Vaccine Trial*, page 18). It turns out there may be some hidden benefits to AIDS vaccine trials going viral.

KRISTEN JILL KRESGE

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's financial and in-kind supporters include the Alfred P. Sloan Foundation, the Bill & Melinda Gates Foundation, the Foundation for the National Institutes of Health, The John D. Evans Foundation, The New York Community Trust, the James B. Pendleton Charitable Trust, The Rockefeller Foundation, The Starr Foundation, The William and Flora Hewlett Foundation; the Governments of Canada, Denmark, India, Ireland, The Netherlands, Norway, Spain, Sweden, the United Kingdom, and the United States, the Basque Autonomous Government, the European Union as well as The City of New York, Economic Development Corporation; multilateral organizations such as The World Bank; corporate donors including BD (Becton, Dickinson & Co.), Bristol-Myers Squibb, Continental Airlines, Google Inc., Henry Schein, Inc., Merck & Co., Inc., Pfizer Inc, and Thermo Fisher Scientific Inc.; leading AIDS charities such as Broadway Cares/Equity Fights AIDS and Until There's A Cure Foundation; other private donors such as The Haas Trusts; and many generous individuals from around the world. For more information, see www.iavi.org.

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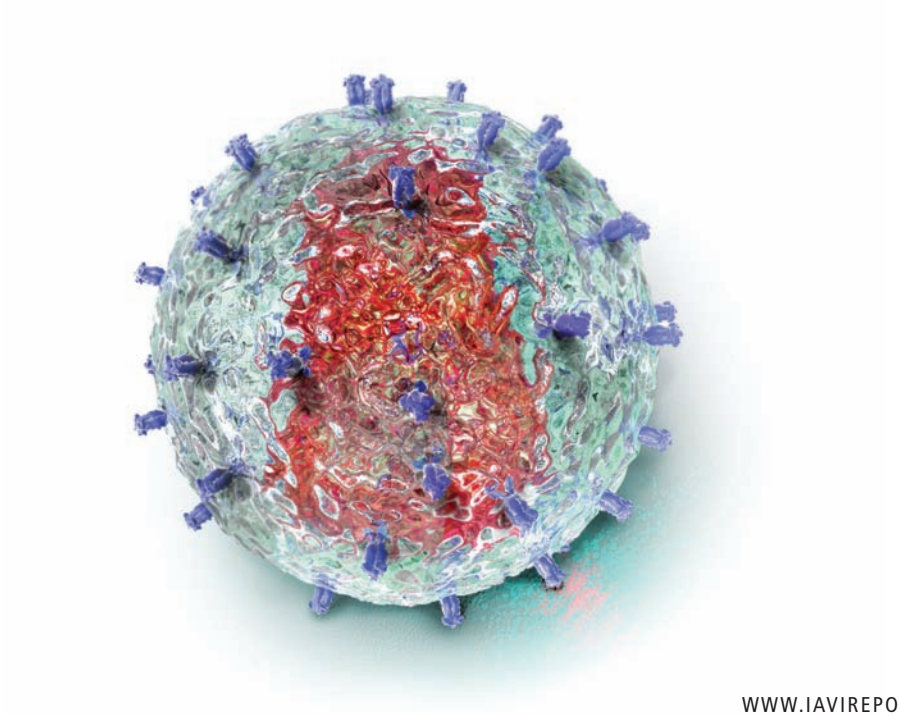

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[ON THE COVER]

Virological synapse-mediated spread of HIV. Surface rendering of membranes in electron tomographic reconstructions reveals the three-dimensional (3D) morphology of a synapse that is formed between an HIV-1 infected (orange) and an uninfected T cell (grey). HIV-1 virions (red) are seen at the interface of the two cells.

Infected-cell samples courtesy of Nicola Martin (Dunn School of Pathology, University of Oxford). Electron tomography and 3D image by Sonja Welsch (Structural and Computational Biology Unit, European Molecular Biology Laboratory). Originally published in Supplemental Movie 3 of *J. Virol.* **84**, 3516, 2010. See *New Insights on Antibody Inhibition of Cell-associated HIV Spread*, page 16.

ANTIBODY *Fever*

Characterization of the slew of recently discovered broadly neutralizing antibodies was one of the advances highlighted at the recent HIV Vaccines conference

By Andreas von Bubnoff

For the first time, the annual Keystone Symposium on HIV Vaccines, which took place from March 21-26 in Banff, Canada, was held in conjunction with a symposium on Viral Immunity rather than with the symposium on HIV Biology and Pathogenesis, which was held from January 12-17 in Santa Fe, New Mexico. Wayne Koff, one of the organizers of the HIV Vaccines meeting and chief scientific officer at IAVI, told the delegates on the first night of the joint symposia that this was done to enable cross fertilization of the two fields. "It's rare that many of us have the opportunity of getting out and seeing what is really occurring in the field outside of HIV," Koff said.

Combining the two meetings was a good idea, said Silke Paust, a postdoctoral fellow at the Ragon Institute and Harvard Medical School. But Paust thought that there could have been more joint sessions. "I think that putting both together was a good idea, just from the mixing of the people that you got that way," she said. "I would like to have them together in the future, but maybe [with] more joint sessions, because sometimes I really wanted to be at both places at the same time."

Even within the HIV Vaccines sessions, there was a lot to digest. Much of the discussion focused on broadly neutralizing antibodies, both the characterization of those recently isolated, as well as a better understanding of how they develop in HIV-infected people. Other topics included antibody-dependent cellular cytotoxicity (ADCC), updates on HIV vaccine trials, the development of new vaccine strategies, further insights into early HIV transmission, and the role of $CD4^+$ T cells in protection.

Characterizing new antibodies

Recently, several new HIV-specific broadly neutralizing antibodies have been identified, including VRC01, which was discovered by researchers at the Vaccine Research Center (VRC) at the US National Institute of Allergy and Infectious Diseases (NIAID), and PG9/16, discovered by IAVI researchers in collaboration with researchers from The Scripps Research Institute (see *Raft of Results Energizes Researchers, IAVI Report,* Sep.-Oct. 2009). Now, one question scientists are attempting to answer is just how these new antibodies neutralize so well.

Some clues come from their structures. Peter D. Kwong, chief of the structural biology section at the VRC, presented data on the structure of the antigen-binding fragment of VRC01 bound to an HIV clade E gp120 monomer. He showed

that VRC01, which recognizes part of the CD4 binding site of gp120, neutralizes so well in part because it mimics CD4 very well. Parts of the VRC01 heavy chain align very well with parts of CD4, and VRC01 binds gp120 at an angle that is only a few degrees different from the angle of CD4 binding to gp120. VRC01 also contacts gp120 in regions that do not change conformation, while it has a gap to accommodate the variable part of gp120. This way, it can accommodate variations in gp120 while retaining its ability to bind.

Another question researchers are grappling with is how an antibody like VRC01 can be elicited by a vaccine. As discussed at the meeting, antibodies like VRC01 have a high degree of affinity maturation. Affinity maturation is a process that starts once a B cell is activated by an immunogen. This process creates mutations in the variable regions of an antibody. Versions of the antibody with higher affinity for the immunogen are then selected, and the population of B cells expressing these altered antibodies expands.

Kwong said that, compared with most antibodies, VRC01 has an exceptionally high degree of affinity maturation. At least 30% of the amino acids in its variable region (more than 60 amino acids) are changed, compared with 5-10% (10-20 amino acids) in most other antibodies. The changed residues include more than half of the ones that come in contact with gp120.

Affinity maturation could in part explain why it seems to take years until broadly neutralizing activity develops in HIV-infected people. For vaccine development, this could mean that to induce antibodies like VRC01, one might have to guide the immune system along the affinity maturation process by sequentially vaccinating a person with several immunogens, each of which binds to intermediate stages of the antibody as it undergoes the affinity maturation process, Bart Haynes, director of the Duke Human Vaccine Institute at Duke University, suggested in his talk.

Kwong is confident that, in principle, this should be possible because affinity maturation is a process that is quite well understood. "This is the first time where we have got an antibody [where] you actually know the mechanism [of how it might be elicited in high titer]," he said, adding that with hundreds of papers published, antibody affinity maturation is a well investigated area, and much of the process is well defined. "That's why I am actually excited about this one."

Another reason he is optimistic is that some of the recently identified broadly neutralizing antibodies are actually observed in high titers in the infected individuals they were isolated from, suggesting that in principle, a vaccine should be able to induce such antibodies. "That's what's so exciting," Kwong said. "Humans can make antibodies that have the same phenotype as the PG antibodies and VRC01."

Data as to how long it takes for broadly neutralizing activity to develop in HIV-infected people were presented by Lynn Morris, head of the AIDS Virus Research Unit at the National Institute for Communicable Diseases in Johannesburg. Morris said that broadly neutralizing antibodies may not be as rare as was once thought. While the definition of breadth varies, about one quarter to a third of HIV-infected people appear to have some level of cross-reactive antibodies after a few years of infection, according to Morris. She presented data from a longitudinal analysis of the CAPRISA cohort of HIV-infected women, mostly sex workers in the province KwaZulu-Natal in South Africa, most of whom are infected with HIV subtype C.

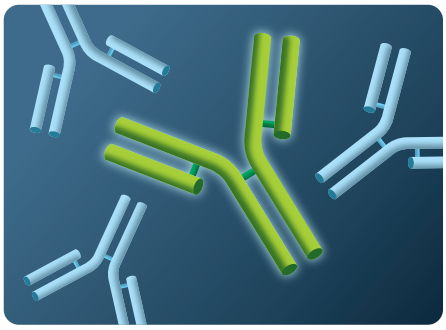
We thought that neutralization breadth was due to the accumulation of lots of different antibodies. But actually it probably isn't—it's probably a single antibody that's affinity maturing. *– Lynn Morris*

By three years after infection, several of the women had developed broadly neutralizing activity in their sera. In most cases, this activity developed gradually. Over time, the serum could neutralize an increasing number of HIV strains in a diverse panel. "I am pretty sure we are the first to show that over time, the development of breadth occurs incrementally," Morris said. In one case, however, the serum became broadly neutralizing almost all at once—about 70 weeks after infection, the individual's serum neutralized just one virus strain, but 10 weeks later, it could neutralize 16 different primary viruses. "That was very suggestive of a single antibody developing in this person," Morris said.

The study also showed that the sera taken prior to the development of broadly neutralizing antibodies could neutralize virus that was taken from the same person a few months earlier, but could not neutralize concomitant virus. How-

ever, once the serum became broadly neutralizing, it could often neutralize concomitant virus as well, suggesting that escape from broadly neutralizing antibodies may be more difficult.

Typically, just one antibody specificity seemed to account for most of the broadly neutralizing activity of the sera. The researchers showed this by using known targets of broadly neutralizing antibodies to deplete the serum of antibody types binding to them and then checking if the sera could still neutralize a diverse panel of HIV strains. For example, in the person in which the serum acquired broad neutralization activity all at once, two thirds of this neutralization was due to antibodies that bind the membrane proximal external region (MPER) of gp41 envelope (*J. Virol.* **83**, 11265, 2009). In another case, in which the neutralization breadth developed gradually, the activity was mostly due to antibody that could be depleted by gp120. This was true at different time points after infection, suggesting that the broadly neutralizing activity was due to the same type of antibody that developed over the years.



Together, these findings suggest that one reason it takes years for the sera in HIV-infected people to develop broadly neutralizing activity is affinity maturation of one type of antibody. This is also consistent with the high degree of affinity maturation observed in broadly neutralizing antibodies like VRC01. "Initially when we saw those data we thought that neutralization breadth was due to the accumulation of lots of different antibodies," Morris said. "But actually it probably isn't—it's probably a single antibody that's affinity maturing."

Dennis Burton, a professor at The Scripps Research Institute, presented a similar analysis of the types of antibodies or antibody specificities that account for the broadly neutralizing activity in 19 HIV-infected individuals whose sera had among the broadest and most potent neutralizing activity from IAVI's Protocol G cohort. The cohort comprises about 1,800 HIV-infected people and includes the individual that was the source of the PG9/16 antibodies. Similar to Morris, Laura Walker, a graduate student in Burton's group, used known targets or properties of broadly neutralizing antibodies to remove the corresponding antibody types from the sera and then determined if the sera could still neutralize a diverse panel of HIV. She found that the neutralization activities were typically due to one or two broadly neutralizing antibody specificities. Major specificities included antibodies binding the CD4 or the CCR5 binding site of gp120, and specificities similar to the PG9/16 antibodies. One donor had a binding specificity similar to the broadly neutralizing antibody 2G12, which binds to glycans on gp120. Four donor sera had specificities that bound to the same glycan, but not directly, suggesting that their antibody specificity was directed to a previously unknown target.

Neutralization is not the only mechanism thought to explain protection afforded by broadly neutralizing antibodies. A 2007 study by Ann Hessell, a staff scientist in Burton's laboratory at The Scripps Research Institute, and colleagues found that eliminating the ability of the broadly neutralizing antibody b12 to bind to Fc receptors, which is necessary for ADCC, makes this antibody less protective in challenge studies in rhesus macaques (*Nature* **449**, 101, 2007; see *Antibodies: Beyond Neutralization, IAVI Report,* Jan-Feb. 2010). At Keystone, Hessell showed that a b12 antibody that lacked fucose residues in the Fc receptor binding region had about a 10- to a 100-fold better ability to bind to the Fc receptor IIIA. *In vitro*, this translated into a 10- to a 100-fold better ability of this modified b12 antibody to mediate ADCC and antibody-dependent cell-mediated virus inhibition (ADCVI). Experiments in rhesus macaques are planned to see if this also translates into better protection from simian immunodeficiency virus (SIV)/HIV hybrid challenge *in vivo*. "We believe that the enhancement of ADCC will lead to protection by a substantially decreased dose of [modified] antibody to achieve the same effect," Hessell said.

Moving beyond RV144

Researchers are still trying to find an explanation for the modest success of the vaccination regimen tested in the RV144 trial in Thailand, an efficacy trial involving more than 16,000 Thai vol-

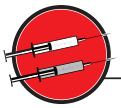
unteers that tested a canarypox vector-based candidate ALVAC-HIV in a prime-boost combination with AIDSVAX B/E (see box below). Nelson Michael, director of the US Military HIV Research Program, presented an update of studies that are designed to address this question. Michael said that six months after the final vaccination, uninfected vaccinees showed no $CD8^+$ T-cell responses, while about one third had $CD4^+$ T-cell responses to Env. Vaccinees that became HIV infected showed CD8 responses to Gag and Env epitopes that were different from the Gag and Env CD8 responses observed in unvaccinated individuals who became infected. "There really is little overlap between the ELISPOT epitope responses to Gag and Envelope in people [with] breakthrough [infections] that received vaccine versus those that received placebo," Michael said. "To us, it's the first evidence that there is an immunologic correlate at the T-cell level to what we saw clinically."

Michael also addressed future plans and said that there are discussions to test a similar vaccine regimen to that evaluated in RV144, but in high-risk populations rather than the general population that was recruited for RV144. The vaccine regimen appeared to be worse at protecting volunteers who had indicated that they had any high-risk activity at any time during the trial compared with people who reported no risk behaviors (see *Prevent and Conquer, IAVI Report,* Jan.-Feb. 2010). But Michael said that does not mean that the vaccine shouldn't be tested in high-risk groups, because it is not clear if the high-risk behavior took place early after vaccination, when the protective effect seems to have been highest, or later when the protective effect was much smaller. "It's hopelessly confounded with the fact that the duration of this [protective] effect was also transient," Michael said.

He said that tests of a similar vaccine regimen in men who have sex with men (MSM) in Thailand or in high-risk heterosexuals in southern Africa are being discussed. Researchers are considering using NYVAC, a poxvirus-based vector that has been developed by the company Sanofi Pasteur, as a prime. While similar to ALVAC, NYVAC might be easier to produce. "There is an impression that NYVAC might be a better vector," Michael said. As a consequence, investigators are discussing whether to do two efficacy studies in high-incidence populations of heterosexuals in southern Africa, one with NYVAC, the other with ALVAC, or to select a single vector now which would then be tested in both high-risk populations in southern Africa and Thailand. At the same time, there are discussions to do additional trials in Thailand in higher-risk individuals, possibly MSM, with additional gp120 booster shots to see if the transient protective effect seen in RV144 is reproducible and if it could be extended by adding additional boosts.

Stepping along

Juliana McElrath, a professor of medicine at the University of Washington, presented results from continuing studies addressing why the adenovirus serotype 5 (Ad5)-based vaccine candidate MRKAd5 tested in the Phase IIb STEP trial failed. One explanation is that the immune response in the vaccinees was not very broad. After mapping HIV-specific T-cell responses to Gag, Pol, and Nef (the antigens used in the MRKAd5 vaccine) in 73 STEP trial vaccinees, these T-cell responses were found to be directed to a median number of one epitope per vaccinee. Last fall at the AIDS Vaccine 2009 conference in Paris, researchers had reported that the median number of epitopes was two (see *Raft of Results*



VACCINE CANDIDATES TESTED IN RV144

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 clade B and E
(*Vaccine Developer: Genentech; its spin-off, VaxGen, tested AIDSVAX previously; intellectual property rights now owned by Global Solutions for Infectious Diseases*)

Energizes Researchers, IAVI Report, Sep.-Oct. 2009). McElrath also reported results from an analysis of the viral loads in some STEP trial volunteers early after HIV infection, before they became seropositive, which suggested that the vaccine had an early inhibitory effect on virus replication, but that the effect then disappeared.

Researchers are also still investigating possible explanations for the trend toward an increased risk of HIV acquisition in some STEP trial vaccinees. One potential mechanism that has been discussed is that the vaccine might have increased the number of HIV target cells. But McElrath said that that does not appear to be the case. In a case-control study of 254 STEP trial volunteers at two time points, week eight (or four weeks after the second immunization) and week 30 (or four weeks after the third immunization), activated $CD4^+$ T cells were not a significant predictor of the risk of HIV acquisition.

As for the other risk factors, initially, researchers observed that vaccine recipients with preexisting antibody immunity to the Ad5 vector used in MRKAd5 vaccine and who were uncircumcised had an increased risk of HIV infection compared to placebo recipients with the same characteristics. In follow-up analyses, the increased risk among uncircumcised men who received the vaccine remains, although it is waning over time, while the effect of preexisting Ad5 immunity is not detectable at later time points (see *Raft of Results Energizes Researchers, IAVI Report,* Sep.-Oct. 2009). Studies in humans are planned to look at the effects of circumcision with vaccination. These will look at cell populations in the foreskin in the context of Ad5 serostatus and co-infection with HSV-2.

Dan Barouch, an associate professor of medicine at Beth Israel Deaconess Medical Center and Harvard Medical School, also addressed whether it is possible that in the STEP trial, preexisting immunity to the Ad5 vector may have created additional HIV target cells following vaccination that then could have migrated to the colorectal mucosa or the foreskin. He presented data from experiments conducted in nonhuman primates (NHPs) that suggest this is not the case. To induce Ad5 immune responses, the researchers infected rhesus macaques intranasally with Ad5 that can replicate in monkeys. They then vaccinated them intramuscularly with a replication incompetent Ad5-Gag/Pol/Nef vaccine that was similar to the one used in the STEP trial.

The Ad5 seropositive macaques did not have more Ad5-specific $CD4^+$ T cells in blood or in the colorectal mucosa when compared with Ad5 seronegative animals. "These new data suggest in a rhesus monkey model that there is no greater increase in mucosal trafficking of either total or Ad5-specific $CD4^+$ T cells in Ad5 seropositive versus seronegative monkeys following Ad5 vaccination," Barouch said. In the blood, these Ad5-

Using Endogenous Retroviruses to Fend Off HIV

One major challenge for HIV vaccine development is that a vaccine needs to protect against the huge number of HIV strains in circulation. Brad Jones, a graduate student at the University of Toronto, reported on experiments that could lead to an HIV vaccine strategy that might be able to circumvent this problem because it doesn't involve targeting HIV itself. Instead, it involves human endogenous retroviruses (HERVs), which are remnants of previous retroviral infections. Their sequences are littered throughout the human genome and make up about 8% of our DNA.

Previous research has shown that HIV-infected people have more HERV sequences in their blood plasma than uninfected people (*PLoS Pathog.* **3**, e165, 2007). This suggests that HIV infection might lead to the activation of HERVs, expression of which could be used as a marker for HIV-infected cells.

For their studies, Jones and colleagues decided to focus on HERV-K, because it is the evolutionarily youngest and most intact type of HERVs and is the most similar to HIV. This might explain previous observations that elements of HIV and HERV-K can interact to facilitate HERV-K expression.

To see if infection of $CD4^+$ T cells could indeed reactivate HERV-K expression, Jones and colleagues isolated HERV-K-specific $CD4^+$ T cells from an HIV-infected elite controller. They found that infecting these cells with HIV *in vitro* led to reactivation of HERV-K protein expression. They also showed that HERV-K-specific $CD8^+$ T cells from the same elite controller could kill these HERV-K-specific HIV-infected $CD4^+$ T cells no matter what HIV strain they were infected with.

This suggests that a vaccine could be developed that induces HERV-K-specific $CD8^+$ T cells that should then be able to kill HIV-infected $CD4^+$ T cells because all of them should express HERV-K, no matter what HIV strain a person is infected with. "I believe this is the first time it has ever been shown that a non-HIV-specific T cell can specifically kill HIV-infected cells," Jones said. "It is a proof of principle for the strategy of targeting a surrogate marker of infected cells rather than the HIV sequence itself." —*AvB*

specific CD4+ T cells showed transient increased proliferation and activation for one week after vaccination. This resolved to baseline levels by week two and therefore does not likely explain the more than 52 weeks of potentially enhanced HIV acquisition in the STEP trial, he added. "Overall, these data would suggest that Ad5-specific cellular immunity does not appear to explain the potential for enhanced HIV acquisition that was seen in the STEP study," Barouch concluded.

The use of alternative Ad vectors such as Ad26 and 35 has been suggested for the development of future candidate HIV vaccines, because of their biological differences to Ad5 as well as lower levels of preexisting immunity. Barouch presented epidemiological data from Africa suggesting that Ad26 and 35 antibody titers are substantially lower than for Ad5, and seroprevalence to Ad26 and Ad35 is also less common than to Ad5. The seroprevalence for these two vectors was less than 1% in 149 healthy two- to nine-month old South African infants. Of 346 South African school children age 6-18, 74% were seronegative for Ad26, and 86% for Ad35, while only 31% were seronegative for Ad5. And of 199 adults age 18-50 from several African countries, 58% were seronegative for Ad26, and 78% for Ad35, while only 16% were seronegative for Ad5. "In total we believe that these seroprevalence data support proposals for further evaluation of these vectors in Phase I trials in sub-Saharan Africa," Barouch said, adding that in preparation for clinical studies, Ad26 and 35 vectors expressing mosaic Gag/Pol/Env antigens are currently being manufactured by the company Crucell. Mosaic antigens are designed to achieve optimal coverage of the many different versions of HIV proteins that are circulating. "We hope to have these ready sometime next year," Barouch said.

While there is concern that vaccines might induce CD4+ target cells, Hendrik Streeck, a junior faculty member at the Ragon Institute, presented evidence that suggests that vaccine-induced CD4+ T-cell responses might actually be important for protection. Streeck showed that secretion of the cytokine interleukin (IL)-21 is only predominantly seen in HIV Gag-specific CD4+ T cells from HIV-1 controllers, but almost not at all in progressors. He also found that IL-21 could increase the ability of CD8+ T cells from progressors to produce perforin and granzyme B, resulting in their better ability to kill HIV-infected cells. This resulted in up to a 1,000-fold upregulation of the ability of these CD8+ T cells to inhibit HIV replication in a viral inhibition assay. "CD4 cells have been completely underestimated," Streeck said, adding that the observation of fewer CD4+ T cells in acute HIV infection doesn't necessarily mean that they are permanently depleted or not important for protection.

Looking for better breadth

Given the limited breadth of immune responses induced by MRKAd5, researchers are trying to develop new vaccine candidates that are capable of inducing a broader response. In NHPs, mosaic vaccines have recently been shown to induce broader and deeper cellular responses than conventional vaccines with inserts encoding consensus sequence or natural sequence antigens, with depth referring to the simultaneous induction of different responses to the same epitopes (*Nat. Med.* **16**, 319, 2010; *Nat. Med.* **16**, 324, 2010; see *Capsules from Keystone, IAVI Report,* Mar.-Apr. 2009).

These mosaic vaccines were primarily developed to induce T-cell responses, but Barouch said that the mosaic vaccines developed in his lab also induce "remarkably potent antibody responses," which are at least non-inferior to the antibody responses generated by other leading modalities such as consensus or natural sequence envelope. "It is a really important aspect that people have overlooked," he said. Bette Korber, a laboratory fellow at the Los Alamos National Laboratory and external professor at the Santa Fe Institute, said that she and her colleagues have developed new strategies to design mosaic vaccines tailored for antibody responses, based on variation in regions of Env that are close together in three-dimensional space.

Overall, these data would suggest that Ad5-specific cellular immunity does not appear to explain the potential for enhanced HIV acquisition that was seen in the STEP study. *– Dan Barouch*

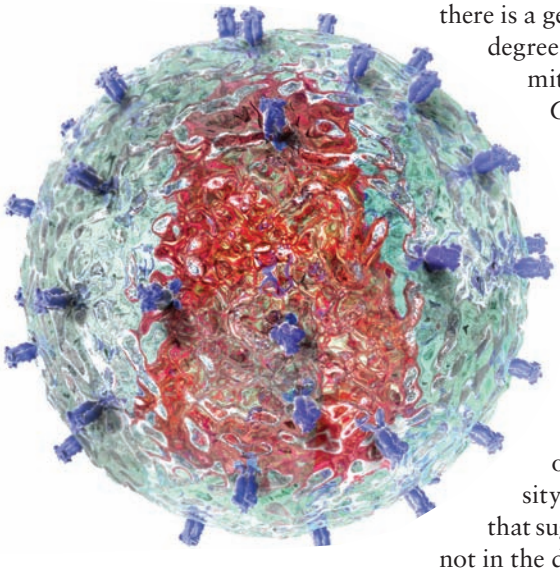
Tomas Hanke, a reader in immunology at the University of Oxford, described another approach to induce broad cellular immune responses. It involves delivering a single DNA construct that contains the 14 most conserved regions of HIV, which are taken from four major HIV clades. Hanke and colleagues injected this insert intra-

muscularly into rhesus macaques in different forms. Three DNA primes were followed by a boost of the insert in a human Ad5 vector and then by another boost of the insert in a modified vaccinia virus Ankara (MVA) vector. This "DDDAM" heterologous prime-boost regimen was then followed by two injections of the same conserved sequences in the form of 46 separate peptides of 25-28 amino acids length. To avoid immunodominance, the peptides were injected at six separate sites.

Data on the immune responses to this "DDDAMSS" regimen in three rhesus macaques showed that the injection of the peptide pools further increased the magnitude of the cellular immune response induced by the "DDDAM" regimen by 30%, and also made the T-cell responses broader. Hanke said that while the immune responses induced by the DDDAMSS regimen are not as broad as the ones recently reported for mosaic vaccines, the responses induced by the regimen are of a higher magnitude because they are focused on the conserved epitopes. "[The DDDAMSS regimen focuses] all the might of the T-cell immune responses on the fewer, but invariant epitopes," Hanke said. Early clinical trials will start later this year with a modified "DDDCM" regimen that uses chimpanzee instead of human adenovirus, he added.

Further insights into transmission

In the last few years, researchers have learned that in most heterosexual transmissions just one transmitted virus variant is responsible for productive infection in the recipient, suggesting that there is a genetic bottleneck which limits the degree of variation as the virus is transmitted (see *HIV Transmission: The Genetic Bottleneck, IAVI Report,* Nov.-Dec. 2008). However, it is still unclear where this bottleneck is located, and which factors determine which virus ultimately gets transmitted.



At the meeting, Cynthia Derdeyn, an associate professor of pathology and laboratory medicine, and Eric Hunter, a professor of pathology and laboratory medicine at Emory University, presented data from their labs that suggest that the genetic bottleneck is not in the donor. Debrah Boeras, a postdoctoral fellow in Hunter's lab, used single genome amplification to analyze the viruses present shortly after transmission from vaginal swabs from six female donors and semen from three male donors. A comparison to the sequences of the transmitted viruses showed that while the donor fluids contained many different virus variants, it was not the most common variant that ended up getting transmitted. "What's new about our data is the idea that in the genital compartment of the donor partner you have a major dominant variant circulating, but that doesn't seem to be the one that's transmitted," Derdeyn said. "It suggests that it's not stochastic, it's not simply that the most frequent variant is the one that's transmitted. It's something else about the variants that get transmitted. The bottleneck is not in the genital compartment of the donor."

In addition, Derdeyn and Hunter found no difference between viruses taken from the blood of donors and recipients around the time of transmission in terms of which cell types they preferentially infect. They all depended on high levels of CD4 and CCR5 receptor expression to infect target cells, and all infected $CD4^+$ T cells well, but not macrophages. "It seems like macrophages are probably not the initial target cells," Derdeyn said.

The observation that there are many different viruses in the genital fluids of donors is consistent with evidence presented by Suzanne English, a graduate student at the University of Oxford, which suggests that different viruses get transmitted to different recipients even when the donor and route of transmission are the same. English and colleagues analyzed HIV sequences from two MSM that were both rectally HIV infected from the same donor in the same night, just minutes apart from each other. The researchers used sequence analysis to confirm that the transmitted founder viruses in the two recipients came from the same donor, but they found that the sequences were too different to have come from the same transmitted virus. Instead, the two were so different that they are estimated to have been evolving prior to transmission for at least several years. One thing is for sure, the researchers were lucky they had the opportunity to study a case like this, English said. "It's very, very difficult to find two patients who were infected by a single donor by the same route in the same night who were then both sampled on the same day 63 days later." ■

INVESTING IN *Surprise*

Efficacy trials may be costly, but some researchers argue that they are the best way to advance AIDS vaccine research and development

By Regina McEnery

It was two years ago that the AIDS vaccine field, stung by the disappointing results of the STEP trial that showed Merck's adenovirus 5 (Ad5)-based vaccine candidate (MRKAd5) had no effect, called for basic discovery research to become a higher priority (see *Balancing AIDS Vaccine Research, IAVI Report*, Mar.-Apr. 2008). The shift from clinical development to basic research was endorsed by the National Institute of Allergy and Infectious Diseases (NIAID), the leading funder of AIDS vaccine research, which also partly funded the STEP trial.

Yet despite a broad scientific consensus that the best and possibly only way to develop an AIDS vaccine is to try and solve some of the key biological questions that have hindered progress, the recently completed RV144 trial spurred many researchers to emphasize the unique and important value of clinical research. The RV144 trial, which tested Sanofi Pasteur's canarypox vector-based candidate ALVAC-HIV (vCP1521) and AIDSVAX B/E (the genetically engineered version of HIV gp120 originally developed by VAXGEN), resulted in the first evidence of vaccine-induced protection against HIV (see *Raft of Results Energizes Researchers*, *IAVI Report*, Sep.-Oct. 2009).

Difficult to execute, sometimes controversial, and, until RV144, lacking any hint of efficacy, the handful of AIDS vaccine efficacy trials conducted over the years have raised new questions and caused some researchers to reconsider what is required for vaccine-induced protection against HIV. "Truth is, the only way we are learning what actually works and what doesn't is from efficacy trials," says Larry Corey, a University of Washington AIDS researcher who heads the HIV Vaccine Trials Network (HVTN) created by NIAID. "We have to continue doing them."

This sentiment is shared by researchers who aren't principally involved in conducting clinical trials. Norman Letvin, a professor of medicine at Beth Israel Deaconess Medical Center whose research involves nonhuman primate studies of HIV vaccine candidates, echoed the importance of clinical research in a recent commentary in *Science* magazine (*Science* **326**, 1196, 2009). "The results of the Thai trial underscore the extraordinary importance of also performing focused human clinical trials of vaccine strategies," Letvin wrote. "Just as the recent failure … in the STEP trial could not have clearly been predicted based on the preclinical experiments that had been carried out, the findings in the Thai

trial were not expected based on preclinical studies and human immunogenicity data."

The results of the 3,000-person STEP trial illustrate how difficult it is to faithfully recapitulate HIV infection in animal models. The vaccine candidate had been successful in lowering acute viral load in macaques challenged with a hybrid simian/human immunodeficiency virus (SHIV) strain (SHIV-89.6P), but a similar effect was not observed in humans (see *Getting It Right Early*, *IAVI Report*, Sep.-Dec. 2007). The results of the RV144 trial also took researchers by surprise because the immunogenicity of the two vaccine candidates in earlier clinical trials was one of the main points a cadre of leading scientists used to argue against the launch of this large trial.

"We have learned to expect the unexpected in our efforts to generate an effective HIV vaccine," wrote Letvin.

Economies of scale

Clinical discoveries, although incredibly useful, don't come cheap. It is estimated to cost about US$500 million to develop a new vaccine. Since the turn of the century, the pace of vaccine research and development has quickened—there are now more than 80 vaccine candidates in the pipeline, and about 30 of them target diseases for which there are no vaccines currently available, according to the third edition of the State of the World's Vaccines and Immunization (www.who.int/immunization/sowvi/en). By the end of 2008, the total number of vaccines on the market reached 120, making this decade the most productive ever in the history of vaccine development.

Large-scale efficacy trials are one piece of the extensive preclinical and clinical testing that is required to bring a vaccine to market. Because of the thousands of volunteers that need to be identified, screened, recruited, and tracked over the course of these long-term trials, later-stage trials also tend to be one of the most expensive links in the vaccine development chain. About 90% of the $130 million that the company VAXGEN invested in AIDSVAX—a gp120 protein vaccine candidate that was originally developed by the biotechnology company Genentech—was spent on two separate, but simultaneously conducted, Phase III efficacy trials, according to Don Francis, founder of VAXGEN, who is now director of Global Solutions for Infectious Diseases, a San Francisco-based non-profit organization that holds the intellectual property rights to AIDSVAX. These studies enrolled close to 7,500 men who have sex with men (MSM) and injection drug users from North America, Thailand, and The Netherlands. The RV144 trial, which enrolled about 16,000 participants and lasted six years, cost $105 million, less than its projected cost of $119 million.

Vaccine efficacy trials can be notoriously large and expensive for other diseases as well. Rotavirus, a common cause of diarrheal disease, which kills 500,000 children annually in the developing world, is a prime example of how much it can take to get a vaccine to market. Two huge Phase III trials, which each enrolled at least 60,000 infants from Europe, the US, and Latin America, were conducted to test the efficacy of Merck's Rotateq and GlaxoSmithKline's Rotarix vaccine candidates. The trials were so large because they had to rule out a very minor safety concern with an earlier rotavirus vaccine that was pulled from the market. These trials were estimated to cost between $263 million and $394 million respectively (*Vaccine* **27**, 6627, 2009). Both vaccines were ultimately approved by the US Food and Drug Administration.

Aeras Global TB Vaccine Foundation, which currently has four tuberculosis vaccine candidates in clinical trials in Africa and two other candidates expected to enter clinical testing next year, estimates it will cost about $120 million to conduct a Phase III licensure trial of a single candidate. And the Health Policy Division of the George Institute for International Health in London, which studies product development issues surrounding neglected diseases in poor countries, estimated in 2006 it would cost between $85 million and $95 million for a Phase III malaria vaccine trial of 15,000 individuals. There is currently no vaccine against the insect-born disease, which is endemic in more than 100 countries and claimed about a million lives in 2006. A Phase III trial of malaria vaccine candidate RTS,S/AS01 was recently launched in Africa by GlaxoSmithKline Biologicals.

Other biomedical interventions against HIV can also be costly to evaluate in clinical trials. A Phase IIb microbicide trial of 3,099 women in Africa and the US known as HPTN 035 that found modest, though not statistically significant, benefit in reducing HIV transmission cost $90 million, while a Phase III trial of 9,385 women, known as MDP 301, which tested the same microbicide candidate in Africa and determined it was not effective cost $64 million. HPTN 035 had a 30-month follow-up period for volunteers,

[TRIAL CO$T]

A well-designed and executed clinical trial can provide important information that may lead to the design and development of improved HIV vaccine candidates. But clinical trials, particularly large-scale efficacy trials, don't come cheap. Below are some of the major factors that influence trial cost.

HIV Incidence

The lower the incidence of HIV in a target population or region, the more volunteers that must be screened and recruited for researchers to determine if the vaccine is effective in preventing or controlling HIV.

Recruitment and Retention

If a high-risk group is transient or difficult to reach, trial organizers often have to devote more money to recruit and retain them in a trial. To encourage retention, clinical trials may also provide payment to volunteers, either in the form of reimbursements for traveling expenses or as a fixed-rate payment for each study visit.

Inclusion and Exclusion Criteria

Exclusion criteria can drive up the cost of a trial. For example, after a post-trial analysis determined that pre-existing immunity to the adenovirus serotype-5 (Ad5) may have been an HIV risk factor for vaccinated, uncircumcised men who have sex with men (MSM) enrolled in the STEP trial, a subsequent trial using another Ad5 vector restricted enrollment to only circumcised MSM with no pre-existing immunity to Ad5. This affects the number of volunteers that must be recruited and screened.

compared to a 12-month period for MDP 301, which contributed to its higher cost. HPTN 035 also cost more because it tested two different microbicide candidates, involved clinical trial sites on two continents, and involved more specimen collection.

But not all HIV prevention trials are this large or expensive. Three recent Phase III trials in Uganda, Kenya, and South Africa that evaluated the impact of adult male circumcision on reduction of HIV infection enrolled more than 11,000 men and cost less than $30 million, says Robert Bailey, a University of Illinois epidemiologist who led the Kenya trial. All three trials were stopped early after infection rates were found to be significantly lower among heterosexual men 18-24 months after undergoing the surgical procedure compared to the uncircumcised group. "For less than $30 million we have an intervention that is at least 60% effective," notes Bailey.

Of course, a one-time surgical procedure is less expensive than the cost of administering six shots and collecting multiple cell samples, as was the case in RV144. "The repeated analyses of immune responses would add to the cost of a trial," acknowledges Bailey.

Determinants of cost

Not surprisingly, the single biggest factor that drives the cost of a vaccine trial is the number of enrollees. The more people that need to be recruited and screened, and the more volunteers that need to be tested, evaluated, and monitored over several years, the more it costs to run the trial. Jerald Sadoff, formerly chief executive of Aeras and now chief medical officer at the Dutch biopharmaceutical company Crucell NV, says the average total study cost per subject is about $7,700 for a Phase II or Phase IIb test-of-concept trial of a tuberculosis (TB) vaccine candidate, an estimate he based on an analysis of three TB vaccine studies. Based on this estimate, it would cost about $12 million to conduct a trial enrolling 2,200 individuals. Sadoff believes the calculations are probably about the same for an AIDS vaccine trial of similar size.

But there are numerous factors that can affect the cost of a prevention trial, notes Peggy Johnston, NIAID's director of the Vaccine & Prevention Research program (see *Trial Co$t*, page 12). These range from the number of trial sites involved, the salaries paid to employees at the clinical research centers, the population that is being targeted, the exclusion and inclusion criteria, the frequency and complexity of specimen collection, as well sample shipping and storage.

"For instance, the MSM populations in US cities where there are vaccine clinical trial sites are fairly educated populations," says Johnston. "They tend to be tuned in and have access to information so recruiters can reach them more easily. In contrast, high-risk black women tend to be more geographically spread out, especially in the southern part of the US. There seem to be no consolidated locations where recruiters can readily identify the hundreds if not thousands of volunteers that might be needed to meet enrollment criteria. This then would require additional sites and/or more staff to meet enrollment goals."

Glenda Gray, director of the Perinatal HIV Research Unit at the University of the Witwatersrand in Soweto, South Africa, has worked on a number of different HIV prevention trials involving different populations. Serodiscordant couples are, by far, the most difficult and most labor-intensive to recruit and retain, according to Gray. "First you have to get the couples into your center and then the couples have to commit to staying together for the duration of the trial," she says. "There is huge attrition along the way. I guess they yield important results, but they are really hard to study and find."

Reimbursement of trial participants is also a factor that affects the cost of a trial. South Africa, for instance, has a policy requiring that trial participants be paid a flat rate for every trial visit. Advocates of the practice believe this encourages trial participation, but critics have argued that payments should be linked to actual time spent and expenses incurred per visit to prevent over-compensating, or under-compensating, participants (*SAMJ* **98**, 926, 2008). Jennifer Koen, a project researcher with the HIV/AIDS Vaccine Ethics Group at the University of KwaZulu-Natal, says that some kind of payment is typical in clinical trials, although they are usually framed as reimbursements for expenses and not a flat payment per trial visit as is the case in South Africa. "It seems that the setting of a standard payment for all site visits may not be the case in many developing countries," says Koen.

Some researchers contend that international clinical trial networks such as the HVTN, which involve large groups of investigators conducting trials across multiple sites and countries, can also make trials more expensive. "Vaccine trials that are part of a larger network require a lot more

Laboratory Specimens

Blood, cell, and tissue samples collected from volunteers are essential, but the volume and types of samples collected can both add to trial cost. Mucosal samples, for instance, are more complicated and time consuming to collect, and fewer laboratories are equipped to analyze them.

Equipment and Storage

Most specimens need to be stored and preserved properly in freezers, sometimes for years, for future analysis. When storage in an off-site laboratory is required, shipment of the samples under temperature-controlled conditions is necessary.

Manufacture of Vaccines

Manufacturing, testing, and supply of clinical-grade vaccines using well-defined processes in an appropriate Good Manufacturing Practices facility is critical for the conduct of efficacy trials. Cost can vary depending on complexity of the manufacturing process.

coordination," says Bailey. "There's more bureaucracy, protocols need to be standardized, and there are multiple independent review boards. You are dealing with different communities and different leaders in the communities. I feel that these networks are often much more cumbersome and expensive than necessary."

Corey disagrees. "The HVTN is every bit as efficient if not more efficient," he says. "And aside from simple small Phase I trials, almost every HIV efficacy trial or larger trial is conducted at multiple sites." Corey also thinks networks like the HVTN offer other advantages, including consistency. "The most important thing is that the data that comes out of a trial be interpretable within the context of the field. A network has a common lab, common structure, and brings some semblance of order from one trial to the next," he says.

Sample collection

The collection and storage of laboratory samples is another one of the biggest expenses in conducting clinical trials, but is an area where researchers have some flexibility in how much they spend. RV144 trial investigators, for instance, were conservative in how many blood and cell samples they collected, partly to cut costs. They also limited the types of samples collected—no mucosal samples were collected in this trial. Francis says because RV144 was conducted in a low-risk population, scientists would have needed to collect mucosal samples from a large population in order to allow accurate comparisons of HIV-infected and uninfected volunteers. For that reason, the research team never seriously considered collecting mucosal samples, although this data may have proven instructive. "Considering the resistance from many sectors to undertake this study when it was proposed, we should all be grateful to those wise few who successfully pushed this study to fruition," says Francis.

Myron Cohen, director of the Institute for Global Health and Infectious Diseases at the University of North Carolina, says the amount of sampling is crucial. "The decision is often made that it is prohibitive to collect biological samples," says Cohen. "If it's semen from a man or vaginal secretions, that's a big deal. And if you collect the samples, then you have to spin them down and store them properly. It all adds to the cost."

For instance, about half of the 500,000 blood samples collected during the course of VAXGEN's two Phase III trials are being stored in 34 sub-zero freezers powered by generators the size of a large bedroom. Francis secured a grant from the Bill & Melinda Gates Foundation just to help pay for the preservation of the samples.

But if you ratchet down the sampling, says Cohen, you end up with the scientific equivalent of a flight data recorder minus any data. You won't learn much about what happened, he said.

An economical study

Not surprisingly, some researchers and AIDS advocates have questioned the cost of efficacy trials, saying the money would be better spent on basic research. Not long after RV144 was launched in the fall of 2003, 22 top AIDS researchers questioned whether the trial should be conducted because the vaccines being used in the prime-boost regimen had performed poorly in previous trials (*Science* **303,** 316, 2004).

So when the surprising findings were released by US and Thai investigators in September 2009, the US Army, which funded about a quarter of the study, made a point of complimenting the research team for coming in under budget at $105 million. "The Thais did a remarkable job on this. I think the word is heroic," said Eric Schoomaker, the Surgeon General of the US Army. "They did a remarkable job of acquiring volunteers and conducting the trial almost flawlessly and did not spend as much money as we estimated it would take."

Jerome Kim, deputy director of science at the US Military HIV Research Program, says the trial benefited from fruitful partnerships with the Thai Ministry of Public Health, which provided the use of its facilities, including laboratory space to process the specimens and clinic sites to screen and recruit volunteers, all free of charge. US Army and US National Institutes of Health (NIH) officials who worked on the trial were paid through their respective institutions rather than from RV144 funds, which also lowered the overall trial costs. Additionally, Sanofi Pasteur donated the ALVAC candidate. "In addition to providing a surprising conclusion, funding for the trial was leveraged by the different collaborators and this decreased the overall cost of RV144," says Kim.

Still, not everyone agrees that the resources on RV144 were well-spent, even given the positive results. "The Thai trial used two vaccines, neither of which showed adequate pre-trial immunity, and claimed to show a modest (and questionable) efficacy," says Ronald Gray, a professor in population and family planning at Johns Hopkins University.

But others, including Glenda Gray, believe these large-scale efficacy trials are incredibly important. "The only time we have learned about the effectiveness of these vaccines has been when they have been tested in large-scale human trials," she says. "We are going to learn about safety and a little bit about immunogenicity in smaller trials but the big studies are the ones where we can begin to understand the biology of transmission. To scale back on those would be a tragedy."

The role of partnerships

Pharmaceutical companies have been a primary driver in late-stage clinical development of candidates, including those tested in the RV144 trial. But industry's role in AIDS vaccine research and development has been waning. According to the HIV Vaccines and Microbicides Resource Tracking Group, investments in AIDS vaccine research and development declined from $961 million in 2007 to $868 million in 2008—a 10% drop that was blamed largely on a 61% decline in commercial investments from the pharmaceutical and biotechnology sectors (see *Vaccine Briefs*, *IAVI Report*, July-Aug. 2009). About $170 million was spent in 2008 on clinical research of vaccines by all public and private funders (see box at right).

By far the largest funder of AIDS vaccine efficacy trials—and HIV prevention trials in general—is the NIH, which will spend about $3 billion on AIDS research in 2010 (see *Despite Recession, New Funding Stimulates Scientific Research*, *IAVI Report*, May-June 2009). NIAID funds most of the NIH-related AIDS research, from bench science to efficacy trials. NIAID split the cost of the STEP trial with Merck, which developed MRKAd5, paid 80% of the cost of RV144, and will be funding the entire cost of a recently launched Phase II trial known as HVTN 505 that is testing the safety and efficacy of a DNA/Ad5 prime-boost regimen developed by the Vaccine Research Center at NIAID (see *Vaccine Briefs*, *IAVI Report*, July-Aug. 2009).

HVTN 505, which is likely to cost about $45 million, is a scaled-back version of the Partnership for AIDS Vaccine Evaluation (PAVE) study, which initially planned to test the vaccine regimen in 8,500 people around the world at a projected cost of $140 million (*Science* **321**, 472, 2008). The PAVE protocol team revised the original study following the results of the STEP trial, which showed an increased risk of infection in uncircumcised men with pre-existing antibody immunity to Ad5.

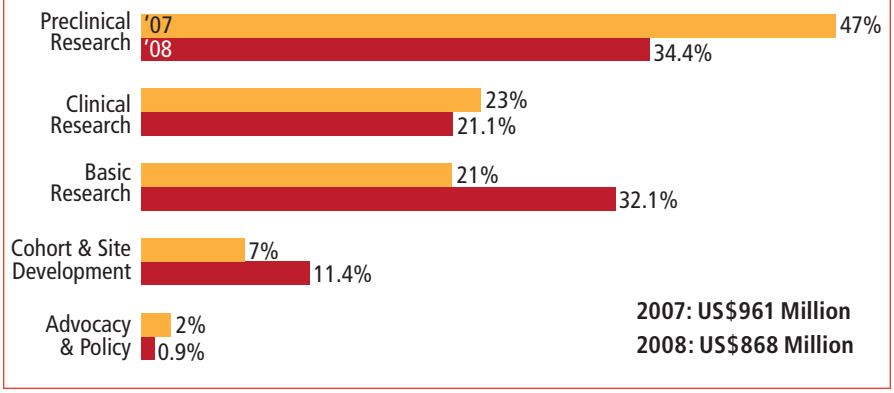
While VAXGEN sponsored two Phase III AIDS vaccine efficacy trials, some pharmaceutical companies have been reluctant to invest in AIDS vaccine research. Sanjay Gurunathan, associate vice president for clinical development at Sanofi Pasteur, the vaccine division of the sanofi-aventis Group, says there are many hurdles when it comes to vaccine development, and with an HIV vaccine, both the investment and scientific risks are large, making it that much more difficult.

James Tartaglia, vice president of research and development at Sanofi Pasteur, says the swirl of criticism surrounding the start up of RV144 was a "little nerve-wracking," but Sanofi believed all along that the vaccine should be tested for efficacy and never considered backing out of the trial even when others suggested it should not go forward. "We weathered it," he says simply. "After that, we just wanted to make sure we executed the trial according to international standards so at the end of the day we would have a result we could rely on, whether it was a plus, minus, or in between."

Tartaglia and Gurunathan say AIDS vaccine research needs these productive partnerships between pharmaceutical companies and the public sector to move forward. "I don't think one single entity can actually shoulder the burden of developing an AIDS vaccine," says Gurunathan. "It has to be a collaborative effort and there have to be partnerships involved in order to be successful in the near future." ■

Total Investment in HIV Vaccine Research

Money spent on pre-clinical and clinical research has declined in recent years, while dollars for basic research have increased, reflecting the shift in resources toward solving some of the key scientific problems impeding the development of an improved pipeline of AIDS vaccine candidates. The HIV Vaccines and Microbicides Resource Tracking Working Group, which collected the figures shown below, will be releasing their 2009 numbers for total HIV vaccine investment later this year.



Research BRIEFS

New Insights on Antibody Inhibition of Cell-associated HIV Spread

HIV CAN SPREAD EITHER as free particles, or from infected cell to uninfected cell. One important mode of cell-to-cell spread occurs through virological synapses (VS), contact zones that form transiently between uninfected and infected cells and enable HIV to pass through. Previous studies found that cell-bound spread is more efficient, although estimates vary as to how much. Also, some previous studies have concluded that HIV transmitted through VS might be harder for drugs or antibodies to reach than free HIV particles, which would be a concern for HIV vaccine and drug design.

A research group led by Quentin Sattentau, a professor of immunology at the University of Oxford, and colleagues has revisited these issues (*J. Virol.* **84**, 3516, 2010). The researchers found that *in vitro*, cell-associated HIV spread between CD4+ T cells via VS is about ten times more efficient than cell-free spread.

They also found that neutralizing antibodies such as b12, 2F5, and 2G12, as well as other HIV entry inhibitors, can inhibit VS-mediated HIV spread between CD4+ T cells and entry of free HIV particles into such cells equally well. They determined this by mixing uninfected CD4+ T cells with CD4+ T cells infected with R5-tropic HIV, or with free particles of R5-tropic HIV in the presence of the entry inhibitors. Researchers then measured the synthesis of new HIV DNA to indicate infection of the target cells. This is "encouraging for vaccine and drug design," the authors conclude.

Sattentau and colleagues also found that some inhibitors such as the b12 antibody could destabilize the VS when added after VS formation. Even when added after VS formation, b12 colocalized with HIV and host cell proteins at the VS and could inhibit HIV infection of the target cells as measured by viral HIV DNA synthesis.

"We are pretty sure that b12 is getting into the synapse," Sattentau says. "In effect it's neutralizing virus within the synapse." Consistent with this, electron tomograms of VS between uninfected and infected CD4+ T cells showed sufficient space for large molecules such as antibodies to gain access (see image, below).

"What people were worried about was that if the virus could efficiently spread cell-to-cell across junctions that are really sealed, then antibodies would be useless and perhaps so would some of the other inhibitors," Sattentau says. "We don't think it is like that. We think that these synapses are actually rather porous open structures and antibodies can get in."

This doesn't mean that VS-mediated HIV spread between cell types other than CD4+ T cells is also easy to inhibit. "This is a T cell-T cell synapse," Sattentau says, "and you can't necessarily say the same thing about other synapses. We work also on macrophage-T cell synapses, and they look much tighter."

The observations of Sattentau and colleagues differ from those of other studies, such as a 2007 study which showed that antibodies such as 2F5 did not block HIV transfer from T cell to T cell through VS (*J. Virol.* **81**, 12582, 2007).

This might be due to different experimental approaches such as the assays used to assess infection, according to Benjamin Chen, an associate professor of medicine at the Mount Sinai School of Medicine, who led the 2007 study. In the 2010 study, Sattentau and colleagues measured inhibition of HIV DNA synthesis as an indicator of target cell infection, whereas Chen and colleagues in the 2007 study measured inhibition of the transfer of HIV Gag proteins from the infected to the uninfected cells, which doesn't always result in an infection of the target cell. Because 2F5 doesn't block Gag transfer, but does block fusion of the virus with the target cell membrane, the two approaches could yield different results.

In the most recent study, researchers used cultured cells that have been infected with HIV for about a week, Chen says. His concern is that virus that has been repeatedly propagated in chronically infected cells is more likely to carry deletions in regulatory genes such as *nef* or *vpu*. This may make the virus more likely to behave in a manner similar to free virus. In addition, Chen says it will be important to test different sera and different viruses with Envelopes that more closely resemble those circulating *in vivo*. Last year, Chen and colleagues found that serum isolated from HIV-infected people can neutralize cell-free HIV infection better than cell-associated infection of CD4+ T cells (*Science* **323**, 1743, 2009). —*Andreas von Bubnoff*

CMV Superinfection No Longer Shrouded in Mystery

Researchers have elucidated an important aspect of the mechanism that enables cytomegalovirus (CMV) to overcome pre-existing immune responses and therefore superinfect rhesus macaques already infected with the virus (*Science* **328**, 102, 2010). The research team, led by Louis Picker, a professor of pathology at Oregon Health & Science University (OHSU), and Klaus Früh, a professor of molecular microbiology and immunology at OHSU, found that to superinfect, rhesus CMV needs genes that prevent major histocompatibility complex (MHC) class I of infected host cells from presenting CMV proteins to $CD8^+$ T cells. "The virus prevents the infected cell from putting a big sign up that says, I am infected, kill me!" Picker says of this immune evasion strategy.

Last year, Picker led a study that showed that rhesus macaques that were already CMV infected could be superinfected with a CMV vector expressing SIV genes (*Nat. Med.* **15**, 293, 2009; see *Research Briefs, IAVI Report,* Mar.-Apr. 2009). The mechanism that enabled this superinfection was not known, although in *in vitro* experiments, Früh had shown that rhesus CMV expresses genes called US2, 3, 6, and 11 that can downregulate MHC class I presentation. The *in vivo* significance of such genes was unclear, because infecting naive mice with CMV lacking genes with a similar function didn't have any effect (*J. Immunol.* **172**, 6944, 2004).

Then Früh and Picker joined forces and infected rhesus macaques by subcutaneous injection with rhesus CMV with and without these genes. They found that wildtype CMV can superinfect rhesus macaques at much lower, more physiologic doses than previously shown. They also showed that CMV lacking the US2, 3, 6, and 11 genes could infect rhesus macaques that were never infected with CMV. In contrast, CMV lacking these genes could not superinfect rhesus macaques with preexisting immunity to CMV, unless their $CD8^+$ T cells were depleted.

This suggests that CMV superinfection, but not initial CMV infection, requires the US-gene-mediated downregulation of MHC class I presentation of CMV proteins on the surface of infected cells, which normally activates CMV-specific $CD8^+$ T cells. The study also found that this requirement for MHC class I downregulation disappears later, presumably because CMV moves to places where it is hidden from $CD8^+$ T cells.

"This is the first [study] to show the importance of MHC class I immune evasion genes for the ability of CMV to superinfect," says Ann Hill, a professor of molecular microbiology and immunology at OHSU who led the 2004 study of CMV lacking such genes in naive mice. "This provides a really appealing answer to the puzzle of what these genes do for the virus."

Although the findings are in rhesus macaques, human CMV has very similar genes, suggesting that it uses the same immune evasion mechanism. This is good news for the development of CMV as a vector for candidate HIV vaccines because it suggests that widespread preexisting immunity to CMV would not hamper the use of CMV as a vector for HIV vaccine candidates in humans. Last year, Picker and colleagues showed that a CMV vector expressing SIV genes could protect rhesus macaques from systemic infection after low-dose rectal challenge with SIVmac239 (*Nat. Med.* **15**, 293, 2009; see *Raft of Results Energizes Researchers, IAVI Report,* Sep.-Oct. 2009). They found that CMV, a replicating vector, induced effector memory T cells, which Picker suggests are better at protecting from challenge virus in mucosal tissues than the central memory T cells induced by non-replicating vectors.

Picker says the new study shows that CMV vectors for HIV vaccine candidates need to have the US genes in order to elicit immune responses in people with preexisting CMV immunity. However, CMV still needs to be attenuated to keep it from causing problems in people with compromised immune systems. "We are working to make the virus safer," Früh says. "We already have put the first attenuated viruses into monkeys and the results so far look good."

The new findings on CMV superinfection also suggest that it may be difficult to develop a CMV vaccine that prevents infection in individuals with a compromised immune system, including fetuses whose mothers haven't yet been exposed to CMV. CMV infection of fetuses is the main cause of non-genetic birth defects such as deafness. The new study suggests CMV would likely be able to overcome any immune response induced by such a vaccine, in the same way it can overcome previous CMV immune responses when it superinfects. "The natural infection doesn't even prevent superinfection, so having a vaccine to prevent infection is not going to work," Picker says.

However, while a CMV vaccine probably won't be able to prevent CMV infection, it should be able to protect against disease by keeping the virus in check, Picker says. "You could have a vaccine [where] the mothers would still get infected, but if they got infected during pregnancy, they wouldn't transmit to their fetus," Picker says. "So it's still possible to make a useful CMV vaccine. It's just not possible to make a sterilizing CMV vaccine."

While the study shows that the US2, 3, 6, and 11 genes are required for CMV superinfection, it is still not clear what biological advantage CMV gains from being able to superinfect its hosts. "Why would the virus want to do that?" asks Früh. One possibility is that CMV needs to be able to overcome immune responses to other immunogens that happen to cross-react with CMV even to establish initial infection. Another possibility is that superinfection enables different viral species to get into people, says Picker. "You have something for evolution to operate on." —*Andreas von Bubnoff*

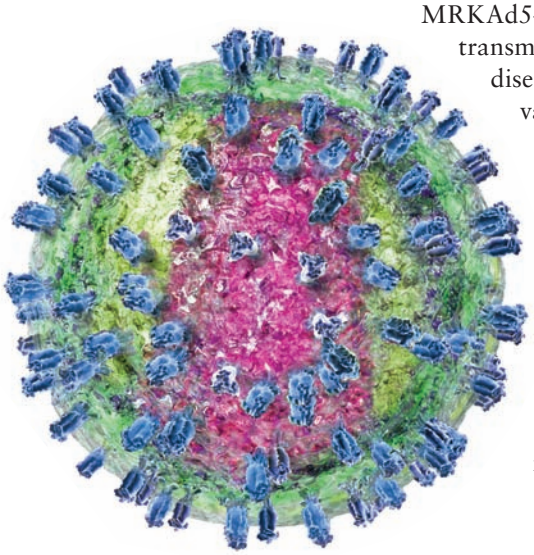
Vaccine BRIEFS

Investigators Tap Social Networking to Pique Interest in Vaccine Trial

With the pace of enrollment slower than investigators would like, a number of sites recruiting volunteers for HVTN 505, a Phase II AIDS vaccine trial conducted by the HIV Vaccine Trials Network (HVTN), have turned to social media and even online classified sites such as craigslist to try and draw volunteers to the study.

Along with traditional outlets like billboards, newspaper and radio advertisements, printed handouts, and social events, clinics are now tapping social media sites with relish to generate interest in a trial that, since its launch last summer, has recruited only 200 of the 1,350 volunteers needed to meet protocol. About 3,000 individuals have inquired about the trial and about 600 were eventually screened, but two-thirds failed to meet the eligibility criteria or decided against joining. The US-based trial is seeking to enroll HIV-uninfected men who have sex with men (MSM) or transgendered women who have sex with men at 15 sites in 12 cities. The trial is testing the safety and efficacy of a DNA/Ad5 prime-boost regimen developed at the Vaccine Research Center (VRC) at the National Institute of Allergy and Infectious Diseases (NIAID; see *Vaccine Briefs*, *IAVI Report*, July-Aug. 2009).

Investigators described the pace of enrollment as slower than usual and blame this on a number of factors. Cheryl Stumbo, a communications specialist at HVTN's headquarters in Seattle, says that some trial sites are still confronting a skeptical public that is unsure whether the vaccine will actually work after another Ad5-based candidate developed by Merck—known as MRKAd5—failed to prevent transmission of HIV or slow disease progression in vaccinated volunteers enrolled in the Phase IIb test-of-concept STEP trial.



Participation in HVTN 505 is also limited to circumcised MSM, or transgendered women who have sex with men, with no pre-existing Ad5 immunity because the results of the STEP trial suggested that male volunteers who received the vaccine had a higher risk of acquiring HIV if they were uncircumcised and had pre-existing antibodies against the Ad5 vector as compared to placebo recipients with the same characteristics.

Stumbo also says the recession has "put some people in bad moods," which may be delaying them from doing something altruistic, like joining a vaccine trial.

It's not clear how much of an impact the use of social media will ultimately have on helping HVTN 505 reach its enrollment goal. Peggy Johnston, director of the Vaccine Research Program in NIAID's Division of AIDS, says while no "immutable deadline" has been set for HVTN 505, the goal is to complete the trial within four and a half years after the first volunteer was enrolled. Johnston says vaccine shelf life is not usually a factor in influencing pre-determined trial futility. "Unless trial length is predicted to be so long that the total cost becomes fiscally indefensible, or if results from other trials become available and make HVTN 505 irrelevant, NIAID remains committed to the completion of HVTN 505," says Johnston.

Using the Internet to draw attention to AIDS vaccine trials is not new, of course. HVTN established a separate website for the STEP trial, which was launched in 2004, and a companion study launched in South Africa in 2007 known as Phambili. What is relatively new is the ways in which trial sites are utilizing newer forms of social media, such as Facebook.

Although it does not recruit volunteers for the individual trial sites, HVTN's headquarters has been posting information about HVTN 505 on its Facebook page and posting advertisements on the Facebook pages of men who live in the same geographic region as a trial site and whose demographics seem to fit the eligibility guidelines for enrollment.

Trial sites are also using social media to reach men in their regions. For instance, the San Francisco site recently aired a video of nine volunteers who had participated in previous vaccine trials. The volunteers talked about what it was like to participate in the trial and addressed some of the misconceptions regarding AIDS vaccine candidates.

Jennifer Sarche, director of community programs for the HIV Research Section of the San Francisco Department of Public Health, says viewers can easily share the video with

friends on Facebook or MySpace. So far, the website (www.SFisReady.org) with the video and information about AIDS vaccine trials happening in San Francisco, has had about 2,000 visits since its January 20 launch. San Francisco has about 35 volunteers enrolled in HVTN 505, says Sarche.

Sarche views the use of social media strategies as part of a larger goal of community education. "We believe the Internet is a place for people to learn more about vaccines at their own pace," she says. "Then, if a person has seen that their friend is one of our sites' fans, and has watched our videos, and read more about it, they'll be a person who is more likely to stop and talk when they see one of our recruiters on the street. And, fully half of our enrolled participants have come from that active street outreach."

The Fenway Institute (formerly Fenway Community Health) in Boston has started using craigslist to recruit volunteers. After obtaining approval from the trial's Institutional Review Board, Fenway posted a listing seeking volunteers for HVTN 505 on two separate craigslist pages, including one that seeks volunteers for clinical trials.

In addition, a Fenway recruiter combs craigslist's personal ads looking for men who appear to fit the profile that the trial is seeking. Then they contact the individuals and ask if they would consider participating in the study. Coco Alinsug, Fenway Institute's recruitment coordinator, says the site has screened about 90 MSM for the HVTN 505 trial, and enrolled about 17 of them, most of whom were found through their online efforts. —*Regina McEnery*

[UPCOMING CONFERENCES]

DATELINE 2010: GLOBAL HEALTH GOALS & METRICS *June 14-18, 2010*, Washington, D.C. Global Health Council's 37th Annual International Conference

XVIII INTERNATIONAL AIDS CONFERENCE *July 18-23, 2010*, Vienna, Austria. Rights Here, Right Now emphasizes the central importance of protecting and promoting human rights as a prerequisite to a successful response to HIV

14TH INTERNATIONAL CONGRESS OF IMMUNOLOGY *August 22-27, 2010*, Kobe, Japan. An innovative and invigorating international congress reflecting the direction of immunology in the 21st century

CDC Creates New Center for Global Health

A NEW CENTER CREATED BY THE US CENTERS for Disease Control and Prevention (CDC) is aiming to consolidate and extend the agency's work in global health, including HIV/AIDS, with two of its major goals centered on health security and extending people's lives.

The Center for Global Health will include about 1,000 workers, largely drawn from departments and programs that already exist within the CDC, and will be led by Kevin DeCock, the former director of the World Health Organization's Department of HIV/AIDS. DeCock most recently served as the director of CDC Kenya.

While the CDC largely focuses on and funds domestic health programs, its international scope is substantial. "There is no way all of the CDC's international work can fall under this new center," says DeCock. "In fact, an important function of the new center will be to do everything it can to facilitate and support the global work that lies outside the center."

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>
> *– Kevin DeCock*

The new center fits into US President Barack Obama's plan to invest US$63 billion over six years in a new Global Health Initiative that has been included in the proposed 2011 spending package now undergoing legislative review. The largest slice of the initiative—about $50 billion—includes the President's Emergency Program for AIDS Relief (PEPFAR), established during the Bush Administration and reauthorized in 2008.

DeCock says a more global approach was inevitable. "The global discourse has moved away from these large disease interventions to highlight neglected areas such as lags in maternal/child health or broader themes of strengthening health systems." He added that for a Global Health Initiative to work there will need to be measurable outcomes. "That was one of the strengths of PEPFAR, particularly with regard to treatment."

DeCock said it is also important not to lose sight of the "unfinished business" of HIV/AIDS. "Collectively, we should be proud of what we have achieved, but treatment coverage is still less than 50% of those who need it. For the other 50%, nothing has changed. The emergency is not over. We must not lose sight of that."

He says long-term funding for global HIV/AIDS programs will need to include innovative financing mechanisms, a broader array of government donors, and greater involvement among countries who now receive support from PEPFAR or the Global Fund to Fight AIDS, Tuberculosis and Malaria. —*Regina McEnery*

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