



IAVI Report

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Immunity's yin and yang

A successful vaccine must first avoid being eliminated by pre-existing immunity before it can promote a protective immune response

By Philip Cohen

In the hunt for better vaccines, researchers are engineering viruses and bacteria into harmless vectors, delivery vehicles for genes from pathogens, to safely stimulate protective immunity. But as researchers attempt to domesticate these microbes to deliver genes for vaccines, possible immune reactions against those vectors become important obstacles to overcome.

Consider, for example, human adenovirus serotype 5 (Ad5), a naturally-occurring virus that has been engineered so that it carries HIV genes, creating an AIDS vaccine vector. Results from a preliminary human clinical trial of such a vaccine by Merck were very encouraging; the vaccine elicited the strongest cellular immune responses yet seen for an AIDS vaccine.

But since the natural form of Ad5 regularly infects humans, causing a severe version of the common cold, experts were worried that many people may already have "pre-existing immunity"—antigen-specific antibodies and immune cells—able to attack the Ad5 vaccine vector. And that worry was confirmed. In clinical trials of an early version of the vaccine containing a gene for the HIV Gag protein, the response to the vaccine was blunted in people even with moderate levels (a titer over 1:200) of pre-existing anti-Ad5 antibodies. In the US and Europe about one-third of the population has pre-existing immunity able to significantly reduce vaccine efficacy—and in developing countries that could be as high as 80% of the population. The specter of pre-existing immunity cast a shadow over this otherwise promising trial.

That's why last fall's announcement by Merck was as welcome as it was unexpected. It turned out that a higher dose of a newer version of the vaccine carrying three HIV genes elicited detectable immunity in a broader spectrum of volunteers. "We were seeing immune responses in 60-70% of people, including those with high levels of pre-existing immunity," says Robin Isaacs, executive director of HIV vaccine clinical research at Merck. "We don't really know why, but it suggests that Ad5 vaccination could be useful for more people."

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Industrial strength research

Collaborative efforts in AIDS vaccine research are adopting some characteristics common in the biopharmaceutical industry

By Catherine Zandonella

When President John F. Kennedy vowed to put a man on the moon by the end of the 1960s, he mobilized the nation's top scientists and engineers to the task. The Manhattan Project in the 1940s and the Human Genome Project in the 1990s—each of these projects succeeded on the back of huge sums of money, the best scientific

expertise, and an unprecedented level of collaboration among scientists.

Despite significant gains in basic knowledge, twenty years of research have yet to yield an effective preventive vaccine against HIV. Like landing a man on the moon, the search for an AIDS vaccine requires money, minds, and collaboration. The field is mov-

ing towards a broad consensus as to what are currently the most important scientific questions. And with funding now becoming more available and more interest concentrated on the task, global health leaders are focusing on the need for cooperation and collaboration among research groups to work more effectively. The new collabora-

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The Merck Ad5 story may be a small triumph in the larger ongoing battle against pre-existing immunity that vaccine designers face. But researchers have been busy investigating many other strategies to help vectors escape immune destruction so that they can do their job.

The problem of pre-existing immunity isn't unique to Ad5 or even to vaccine design. GlaxoSmithKline, for example, recently announced plans to develop an HIV/AIDS vaccine based on an existing measles vaccine, against which many people have childhood immunity. The same issue has arisen for various vectors for vaccines and gene therapy based on poliovirus or BCG (a bacterial strain used as a vaccine against tuberculosis) and adeno-associated virus (AAV).

But while Ad5 is not unique in presenting the challenge of pre-existing immunity, it provides an illuminating example. It is a popular vector—it's now being explored as a vaccine vector for HIV, Ebola virus, anthrax, and the SARS coronavirus, as well as many applications in gene therapy—and many strategies to overcome pre-existing immunity to this vector are being developed.

The attraction of Ad5 lies partly in its ability to infect many types of cells, both actively dividing and non-dividing. It is easy to make the virus defective in replication, and it doesn't integrate into chromosomes, addressing two clinical safety issues. Genes incorporated into the virus are expressed at high levels, an important advantage for presentation of vaccine antigens and therapeutic proteins. The virus is also easy to grow in large scale tissue culture. For vaccinologists, its best feature is that it is a natural born stimulator of immunity—a single administration induces strong antibody and CD8⁺ T cell responses. And it can also induce immunity after being delivered under the skin, into muscle, into the blood, orally, or intranasally.

Some important details of how pre-existing immunity interferes with subsequent use of Ad5 have also been worked out in animal models. Inoculation of the virus elicits strong neutralizing antibodies against viral capsid components, the most potent of which are against the adenovirus hexon protein. Antibodies block the free virus, stopping its entry into cells and the expression of its genes, and therefore subsequent antigen presentation. Any viral particles that manage to pass the antibody gauntlet can still be destroyed by Ad5-specific CD8⁺ cytotoxic T

lymphocytes (CTLs). As a result of this double hurdle, few viral particles can sneak through, generally not enough to kick start a strong immune response against vaccine components or to boost previous vaccinations with the same vector.

Isaacs says the problems with pre-existing immunity to Ad5 were known when the Merck team began to build their HIV vaccine vector. It was no great surprise when these researchers found that in people with antibody levels over 1:200, they saw a poor cellular immune response, barely above background, even at the highest dose of 1×10^{11} infectious particles. "Increasing the vaccine dose seemed to overcome, at least partially, the impact of pre-existing immunity to Ad5," says Isaacs. "But the response rates were still much worse than in those subjects with low, less than [1:200], Ad5 antibody titers."

With those results in hand, Merck began the Phase I safety trial of the next version of the vaccine, a trivalent mixture of vectors containing genes for three different HIV proteins (Gag, Pol and Nef). The 118 volunteers included people with a broad range of anti-Ad5 antibodies, up past 1:4000. But when the company started to recruit the 1500 volunteers for the larger Phase IIb trial of this vaccine, they decided to exclude people with pre-existing immunity above 1:200 since their previous work suggested the vaccine would have little or no effect in this population.

That's when Merck got a pleasant surprise. Analysis of data from the Phase I trial of the trivalent vaccine showed that it behaved quite differently from the version carrying a single HIV gene. They saw that 60-70% of people had a detectable cellular response to HIV proteins based on IFN- γ ELISPOT assays, including people with levels of antibody 10 to 20-fold higher than the 1:200 cut off. Based on that new data, Merck, in collaboration with the HIV Vaccine Trials Network (HVTN) and the NIH Division of AIDS, decided to double the size of the Phase IIb trial, with the second 1500 drawing from individuals with a large range of pre-existing anti-Ad5 specific antibodies over 1:200. And at the recent 13th Conference on Retroviruses and Opportunistic Infections (CROD), Larry Corey, Principal Investigator of the HVTN, announced that they will partner with Merck on a second Phase IIb trial of the trivalent vaccine in South Africa that will include volunteers with anti-Ad5 antibodies.

So why was the second version of the vaccine more immunogenic? There are two obvious possibilities, according to Isaacs. There could be some immunological synergy when the three HIV proteins are presented together, rather than one at a time. The dose of vector may also have been crucial. The monovalent dose was 1×10^{10} particles while the trivalent dose was 3 times higher, containing 1×10^{10} particles of each component. That extra dose of vector may have been just sufficient to allow more viral particles to bypass antibodies at the site of injection, invade cells and express their HIV genetic cargo. "My guess is that both factors count, your gene inserts and the dose," says Gary Nabel, director of the Vaccine Research Center (VRC) at the National Institutes of Allergy and Infectious Diseases. In the VRC's clinical trials of Ad5 vectors, Nabel says his team has also noticed that certain immunogens, such as the HIV Env protein, are particularly good at getting around pre-existing immunity compared to others like Gag. "Even with antibodies around, a good immunogen gives more punch for the same amount of protein," he says.

Whether similar strategies could help overcome pre-existing immunity for other Ad5 vaccines or vectors isn't clear. But the HIV community is eager to see if larger trials confirm the result and show these vaccines to be effective at preventing infection. Meanwhile Merck, the VRC, and other research groups are exploring other ways to tackle pre-existing immunity.

One approach taken by Nabel and Dan Baruch at Beth Israel Deaconess Medical Center in Boston and their colleagues is to attempt to lower the threshold of Ad5 vector particles needed for immunogenicity in the face of pre-existing immunity. This team investigated sensitizing the immune system of mice to HIV proteins by injecting them with plasmid DNA vaccines, either alone or in conjunction with adjuvants such as the cytokines GM-CSF and MIP-1 α . In naive mice primed with a DNA vaccine, a dose of 10^6 particles of Ad5 carrying the gp120 gene was enough to boost cellular immunity so that 25% of CD8⁺ T cells were antigen-specific. In mice that were pre-immunized with Ad5, a similar cellular response required a dose of 10^9 particles. If Ad5-exposed mice first received a prime of a cytokine-adjuvanted DNA vaccine, a subsequent dose of

10^7 Ad5 particles gave the same cellular response, suggesting that pre-existing immunity was partly overcome.

These researchers suggested that the DNA priming elicited the production of antigen-specific memory T cells that then readily responded to smaller doses of the same antigen presented in the Ad5 vector (*J. Virol.* **77**, 8729, 2003). This strategy is now being tested in human trials by the VRC group. In data presented at meetings from trials VRC009 and VRC010, Nabel has reported that the CD8⁺ T-cell response from the DNA prime/Ad5 boost can be 10- to 100-fold higher than for the DNA or Ad alone. In Merck trials, however, DNA/Ad5 was found to be no better than Ad5 alone, suggesting that the composition of the vector and vaccination protocol may be crucial for this effect.

It also turns out that Ad5 vector delivered mucosally sometimes performs far better in the context of pre-existing immunity than when delivered systemically. A report from Hildegund Ertl, James Wilson, and their colleagues at the Wistar Institute and University of Pennsylvania analyzed the effects of route of vaccine administration on the development of antibodies against rabies glycoprotein when its gene was delivered in adenovirus vectors. Mice exposed to Ad5 (but not the rabies glycoprotein gene) were subsequently given Ad5 vector containing the glycoprotein gene either orally or as an intramuscular injection. As expected, the development of antigen-specific rabies protein antibodies elicited by the injected vector was highly blunted by pre-existing Ad5 immunity. But the response of the animals to the same rabies protein vector delivered orally was unaffected by previous Ad5 exposure of the animals (*J. Virol.* **77**, 10780, 2003). Later experiments showed the ability of orally-delivered vector to overcome pre-existing immunity was dose-dependent: rabies protein antibody induction was reduced by about half at a dose of 2×10^5 particles, but not affected in animals given a dose of 2×10^6 vector particles. The reason why ingestion of the virus overcomes pre-existing immunity isn't clear, but the authors suggest that the vast number of cells in the gut that carry the coxsackie Ad receptor (CAR), used by Ad5 to invade cells, may make neutralization by antibodies difficult.

But this trick doesn't appear to work for every mucosal route or every antigen. Nabel's group assessed the ability of intranasally-



**My guess
is that both
factors count,
your gene
inserts and
the dose**



Gary Nabel



Another strategy that has emerged is to use genetic engineering to alter Ad5 vectors to escape pre-existing immunity by giving the virus a new immunological face



delivered Ad5 to overcome pre-existing immunity. The nasal route is of interest to researchers working on sexually-transmitted diseases because application at this site can result in strong secretion of protective antibodies in the genital mucosa. However these researchers found that mice previously exposed to Ad5 had reduced antigen-specific antibody titers when later given an intranasal dose of an Ad5 vector carrying genes for HIV Gag, Pol, and Env proteins compared to animals with no previous Ad5 immunity given the same vector. They also found that intranasal delivery resulted in infection of the nervous system through the olfactory bulb, suggesting this method may raise safety concerns about potential neurotoxicity (*J. Virol.* **77**, 10078, 2003).

Another strategy to help Ad5 vectors evade immunity is to use chemical tricks to hide the virus. Suresh Mittal's team at Purdue University has encapsulated Ad5 inside alginate microparticles as a way to shield vector proteins from neutralizing antibodies and immune cells. The particles are small enough (5 to 10 micrometers) to be taken up by antigen presenting cells such as macrophages and dendritic cells. In their mouse study, they looked for gene expression of a bacterial β -galactosidase gene carried in the vector in the trachea and lungs after inoculating either naïve animals or those that had received one or two previous injections of Ad5. For the unencapsulated vector, gene expression dropped dramatically with one or two previous Ad5 immunizations, to about one-third and one-eighth of that in naïve animals, respectively. In contrast, encapsulation of the virus preserved at least 75% of the expression level in animals with no prior exposure to Ad5. The absolute level of gene expression in naïve animals was initially 50% lower for the encapsulated vector, however, which could lower the efficacy of an encapsulated vaccine (*Gene Ther.* **9**, 1722, 2002).

Chemistry has also been used to alter the Ad5 vector surface by linking various activated forms of polyethylene glycol (PEG) molecules. Wilson's team found that these modifications did not affect the ability of "pegylated" virus to invade cells, but it did reduce the effect of antibodies and immune cells raised against the unmodified virus. Interestingly, the addition of PEG to the surface doesn't make the virus invisible to antibody recognition. If a vector treated with the same version of a pegylated virus is adminis-

tered again, the second inoculation is blunted by pre-existing immunity. But PEG appears to disguise the virus by hiding epitopes recognized on the native virus and creating new ones, suggesting that by shifting PEG chemistries, the same vector could be used multiple times in the same animal or person (*Hum. Gene Ther.* **13**, 1887, 2002).

Another strategy that has emerged is to use genetic engineering to alter Ad5 vectors to escape pre-existing immunity by giving the virus a new immunological face. The most successful approach to date has been to incorporate the major structural component of the Ad5 viral capsid, the hexon protein, from different serotypes of adenovirus. But researchers at Merck have encountered at least two problems with this approach. First, presumably due to structural constraints of the viral capsid, the majority of these chimeric viruses can't replicate. And even though this approach allows the virus to overcome anti-Ad5 antibodies, CTLs reactive to other Ad5 proteins blunt the response (*Hum. Gene Ther.* **13**, 311, 2002). At CROI, Barouch presented promising data on a chimeric Ad5 vector in which only the seven short hexon hypervariable regions of Ad5 were exchanged from human adenovirus serotype 48 (see *CROI covers advancements from start to finish*, this issue). This chimeric vector replicated well in complementing cell lines and effectively evaded anti-Ad5 immunity in both mice and rhesus monkeys.

Researchers have also begun developing new vectors based on one of the other 50 or so known human serotypes of adenovirus. Merck researchers are working on a vector based on adenovirus serotype 6 (Ad6). According to data presented at the AIDS Vaccine 2005 conference, pre-existing immunity against Ad6 is significantly lower than for Ad5: 40% of people in Europe and 35% in US had a titer above 1:200 for neutralizing antibodies against Ad5, while only about 7% and 17% of the same populations have neutralizing antibodies against Ad6. Merck has not yet published data on the immunogenicity of Ad6 vectors.

Another human adenovirus, serotype 35 (Ad35), has also emerged as a leading candidate for a vaccine vector. Prevalence of pre-existing antibodies against Ad35 is lower than Ad5 in every population yet examined. For Ad35, neutralizing antibodies were present in less than 10% of populations in Europe, US or Asia and in approximately 20-30% of African populations, while prevalence of Ad5 anti-

bodies in the same four populations are 50, 30, 40, and 90% respectively (*J. Virol.* **77**, 8263, 2003; *AIDS* **18**, 1213, 2004). And a group at the University of Pittsburgh School of Medicine has reported that even when Ad35 antibodies are present, they are usually at low titers (*Clin. Diagn. Immunol.* **11**, 351, 2004). Barouch, working with the Dutch company Crucell, has shown in mice that Ad5 pre-existing immunity does not significantly suppress antigen-specific cellular responses in mice to the SIV Gag gene carried in an Ad35 vector (*J. Immunol.* **172**, 6290, 2004). An HIV/AIDS vaccine based on the Ad35 vector is being developed in a collaboration between IAVI and the Crucell group.

So far, though, these rarer adenovirus serotypes don't live up to Ad5 in every regard. "The rare human Ad serotypes have proven less immunogenic than Ad5 in both mice and monkey studies to date," says Barouch. "Whether this is also true in humans is an empiric question." Barouch's team has also experimented with engineering Ad35 to be more immunogenic by replacing a section of the capsid fiber protein, the "knob," with that of Ad5. This chimeric virus uses the CAR receptor to invade cells instead of CD46, which is the natural receptor for Ad35. This small change results in enhanced immunogenicity of Ad35 in mice and monkeys. But this improvement also had its costs, as it rendered the virus much less stable (*J. Virol.* **79**, 14161, 2005).

The search for other immunologically-distinct alternatives to adenovirus has sent researchers screening viruses isolated from other species. The most closely related of these are the nine serotypes of chimpanzee adenoviruses. Serotypes 6 and 7 (Pan 6 and Pan 7; the vectors are referred to as AdC6 and AdC7) are being actively developed as HIV/AIDS vaccine vectors based on work from the Ertl and Wilson labs. They found that humans rarely have neutralizing antibodies to these viruses and vectors based on them are not affected by high levels of pre-existing immunity to Ad5 or for the other chimpanzee adenovirus in mice (*J. Virol.* **75**, 11603, 2001). These vectors can also induce high levels of antigen-specific cellular immunity even against the backdrop pre-existing immunity to Ad5 (*J. Immunol.* **170**, 1416, 2003).

One of the potential benefits of having a number of vectors from different adenovirus serotypes available is that they can be used in

prime boost protocols. For a chimpanzee vector carrying a truncated gene for the HIV Gag protein, these researchers found that priming mice with AdC6 followed by a boost from AdC68 (based on chimpanzee adenovirus serotype 68) resulted in antigen-specific CD8⁺ T cells expanding to a frequency of 40% of total (*J. Immunol.* **171**, 6774, 2003). The order of vector usage seems to be important for both the size and character of the immune response. A prime-boost-boost immunization of four Chinese rhesus macaques with AdC7, AdC6, and Ad5 Gag-vectors achieved a sequential improvement of Gag-specific antibodies in four Chinese macaques, while their use in the opposite order was far less effective. However, the Ad5, AdC6, and AdC7 order appeared superior for the induction of Gag-specific CD8⁺ T cell responses (*J. Virol.* **78**, 7392, 2004). "The downside of these viruses is that you are using something new and the experience in humans is much less, which sets the regulatory hurdles higher," says Ertl. IAVI has started a collaboration with GlaxoSmithKline to produce HIV/AIDS vaccine chimp adenovectors that were licensed from the University of Pennsylvania.

Beyond chimpanzees there is a menagerie of adenovirus serotypes from other species: 10 from cattle, 2 from dogs, 7 from sheep, 5 from pigs, and at least 4 from birds. Some of these are already being investigated for use in veterinary vaccines against, for example, bovine herpesvirus, canine distemper virus, and classical swine fever virus. The ovine adenovirus serotype 7 (OAd7) has also been tested in mice as a vector for a hepatitis C virus vaccine and found to be effective at eliciting IFN- γ secreting T-lymphocytes even in animals with antibodies against Ad5 (*Vaccine* **22**, 2717, 2004).

One irony about overcoming pre-existing immunity is that if one of these new vectors is actually incorporated into an effective vaccine, it could quickly become a victim of its own success. "If you vaccinate everyone in a population with an HIV adenovirus vaccine, you can't turn around and use the same vector against malaria," points out Ertl. That's why the battle against pre-existing immunity isn't just the search for one perfect vector for each vaccine application, but the hunt for a whole toolkit of related approaches that overcome pre-existing immunity time after time. "Once we have something that works," says Nabel, "there's no telling how many ways we'll want to use it in the future." 



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Hildegund Ertl

tive efforts come in the shape of consortia or virtual institutes: they aren't bricks and mortar establishments but rather collectives of independent research groups keen to share ideas and resources.

So a new research model is emerging, one which emphasizes coordination of efforts, rapid sharing of positive and negative results, structured decision making, accountability, and consideration of long-term goals. If this model sounds familiar, it is because it has much in common with the industrial model of research used by biopharmaceutical companies. But precisely what constitutes this industrial template of research, and which particular characteristics should be adopted, is currently a subject of discussion.

Curiosity-driven v. goal-oriented

Generally speaking, industry is very protective of its research and closely guards its intellectual property and expertise. But within a given corporation research has long followed this collaborative model with a single product-oriented goal in mind, where a project is driven through teams with different expertise until completion.

Conversely, academic scientific research has traditionally been a pretty solitary endeavor, heavily reliant on the principal investigator's specific expertise and interests. Researchers at universities and institutes tend to work in small groups, sharing their results at conferences and through publication in scientific journals. This academic model meant that a research group would work on a specific aspect of a scientific endeavor, publish their results, and then another group would take on a related question, adding incrementally to the pool of accumulated knowledge.

While the investigator-initiated, curiosity-driven model has thrown up serendipitous findings in just about every scientific discipline, a mission as vast as developing an AIDS vaccine requires coordination on the level of the great scientific endeavors of the past. "Focused development programs similar to those within industry are needed so that people can make informed decisions about what is working and what is not," says John Shiver of Merck.

Over the last few years, support for an industry-like research environment for AIDS vaccines has gained momentum. In 2002 IAVI teamed with a number of academic and government research groups to establish one of the first such research programs, the Neutralizing Antibody Consortium. A collaborative effort involving investigators from across the US, the consortium members share standardized methods and common reagents. Even more importantly, they share their ideas and discoveries freely and plan some of their experiments collaboratively.

In June 2003 a group of twenty-four scientists, including Nobel laureates Harold Varmus and David Baltimore, proposed the creation of a Global HIV Vaccine Enterprise, an "alliance of independent entities" that brings together many of the key players in AIDS vaccine research and

calls for the coordination of efforts to improve vaccine discovery. The Enterprise has consulted widely and published early last year its scientific strategic plan (www.hivvaccineenterprise.org/plan/index.html) that outlines the key unanswered questions in AIDS vaccine research and has helped build consensus across the whole field.

Last year the US National Institutes of Health (NIH) awarded funds to start the Center for HIV/AIDS Vaccine Immunology (CHAVI), a consortium of largely academic research groups with leadership at Duke University, North Carolina (see *A new virtual Center*, *LAVI Report* 9, 4, 2005). Additionally, the Bill & Melinda Gates Foundation will grant up to \$360 million over the next five years for the creation of research centers that function as a network of collaborating institutions. Part of the goal is to get people working across disciplines, says José Esparza, who is coordinating the effort. "We would like to bring together complementary expertise that otherwise would not be available."

The most important aspect of an industry approach... is in the decision-making process, management, and oversight of a project

Taking stock of industry

The ability to bring together that expertise is just one of the strengths of the industrial approach. Perhaps the most important aspect of an industry approach, and where it differs most from the investigator-led academic model, is in the decision-making process, management, and oversight of a project. Instead of relying on the typically academic decision-making by consensus and committee, industry cultivates accountability by adopting an organizational structure that gives authority to individuals. As the late, eminent vaccinologist Maurice Hilleman put it, "there must be a point where authority prevails and the buck stops."

Vaccine candidates are stewarded from research to development by an experienced project manager or team. This management structure ensures that researchers are communicating and milestones are being met, which are important throughout the project. "The project manager provides the discipline to march the product through all the phases of its development," says Gary Nabel, director of the Vaccine Research Center (VRC) at the NIH.

For early stage research the project manager can help the investigator understand factors outside the researcher's area of expertise, such as whether a project has the potential to pass regulatory hurdles. The project manager can advise on the potential for adverse immunological events and other toxicity issues and the ease of scaling up the manufacturing process so that only workable technologies move from the discovery phase into further development, says Nick Jackson, a former vaccine researcher at GlaxoSmithKline Pharmaceuticals and now a project manager for IAVI's collaboration with GSK. "The bottom line is that [project management] adds structure and accountability, while striving to maintain flexibility so that it doesn't stifle the creativity of the researcher," says Jackson.

A project manager is an asset to any research lab, says David Ho, sci-

entific director of the Aaron Diamond AIDS Research Center. “We have come to appreciate how important that is to keep things on track. There are many management stages that are not common in research labs. Even at the formative stage the investigator needs to think about many things in parallel and the timelines involved,” he says. “A project manager helps the group follow the timeline they set for themselves, and it draws your attention to the things that need to be done at a certain time.”

In academia, the job of project management usually falls to the principle investigator, and becomes one more responsibility on top of supervising graduate students, writing and submitting papers, and applying for grants. “It would serve any lab to have [project managers] around to see that work is done efficiently, but in AIDS vaccine research they are indispensable,” says Ho.

Publish or perish

A shift to a more industry-like way of doing research could be tough for some investigators who are accustomed to the relative freedom found in academia. Abandoning investigator-led research completely is not the solution. Curiosity-driven research has provided numerous innovations. Penicillin, for example, was discovered when Alexander Fleming left some moldy culture plates in a drawer. But he would never have realized what he was seeing if not for the incremental work of microbiologists before him.

Yet the academic system has some shortcomings that make it less than ideal for AIDS vaccine development. Of course collaboration in academia does go on, but these relationships are often fluid and informal and may be terminated for reasons that have nothing to do with the research. Formalizing the collaboration with intellectual property agreements can encourage researchers to be more invested.

The lack of oversight inherent in academic freedom means that major questions can go unanswered, simply because nobody sought out that knowledge or someone was refused a grant to study it. Some problems might simply be too difficult or too resource-expensive for a small research group to tackle alone. For example, studying mucosal immunity is prohibitively expensive due to the difficulty in collecting samples from the gut.

Publication of research studies in scientific journals is the lifeblood of academic science. A strong publication record can make a scientist’s reputation and is a strong motivator for funding agencies to renew grants. This emphasis on publishing often means that research groups closely guard their own research results to ensure they aren’t scooped, and this becomes a strong disincentive to collaborate with other research teams. Also, in the rush to publish proof-of-concept studies researchers may not give sufficient thought to how well the technology will translate to the clinic.

The publish-or-perish mantra may also keep researchers from

admitting that a project is not working out as hoped. Few researchers publish negative results, either because they fear it may reflect badly on them or because journals refuse to publish them. Yet publishing negative results keeps other researchers from following blind alleys. “Negative data can be quite useful in this field in terms of rejecting and moving on to the next concept,” says Dennis Burton, an immunologist at The Scripps Research Institute in La Jolla, California.

The research grant structure can provide a perverse incentive to continue with mediocre projects, says Burton. If a researcher finds that his or her research isn’t returning the hoped-for results, or won’t translate well to the clinic, he or she is not likely to immediately call up the funding agency and tell them so. Instead, the researcher might keep that work going through the end of the grant while starting up new projects. “People keep the ball in the air long enough to get the grant renewed,” says Burton. “But what you really want to do is reject failed concepts as

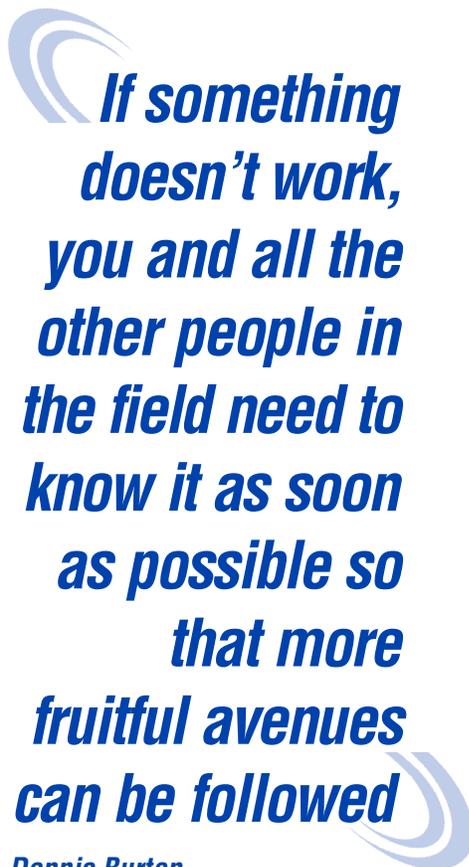
soon as possible. If something doesn’t work, you and all the other people in the field need to know it as soon as possible so that more fruitful avenues can be followed.”

Making it happen

The new industrial-style collaborations are meant to sidestep these pitfalls. CHAVI contains some aspects of the industrial model but retains the committee ethos commonplace in academia. Their strategic plan includes both a discovery phase and a product development phase, each of which has a team leader, and the teams are organized according to the unanswered questions posed by the Enterprise’s scientific strategic plan. An internal committee evaluates whether the discovery phase is producing viable approaches. Meanwhile, a product development evaluation committee looks at what comes out of the discovery teams and decides if a project is ready to be subjected to timeline management pursuant to moving forward with clinical trials. “We hope that by organizing in this manner we can have both a discovery phase where serendipity is clearly needed and a product development phase where we rapidly evaluate and optimize the vaccine candidate,” says Barton Haynes, director of CHAVI.

CHAVI is essentially a not-for-profit company in an academic setting. It has a chief scientific officer and chief medical officer, much like a corporation. The challenge is to give the scientist enough freedom, while at the same time keeping the science focused, says Haynes. “It is a grand experiment and the key is the interdisciplinary approach using components of the corporate model to focus the firepower to solve a very hard problem.”

The approach will put the focus on successful projects and allow the jettisoning of vaccine candidates before moving to full-scale clinical trials. For candidates that are ready for further testing, vaccine developers are increasingly using the Phase IIb trial, an expanded Phase II trial that incorporates efficacy as an endpoint. Merck is taking this approach with a Phase IIb trial of its adenovirus-based vac-



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cine candidate. The goal of these trials is to get answers sooner on whether a vaccine has the potential to work.

IAVI has been active in maintaining that research must adhere to project guidelines in a timely fashion, as shown in its support for the development of a vaccine using a modified vaccinia Ankara (MVA) vector. In 1993 the NIH had identified MVA as a promising vector for delivering antibody-inducing HIV genes. Over the next five years additional research supported that finding, and at the end of 1998 IAVI made the decision with its Oxford University and University of Nairobi partners to conduct Phase I/II trials to evaluate a DNA/MVA combination vaccine in Africa and elsewhere. "In the course of four years we did trials in five different countries," says Seth Berkley, president and CEO of IAVI. These trials were done in parallel to evaluate the candidate as quickly as possible, trading off time against money.

At the end of the trials, the data showed that the DNA/MVA vaccine did not induce an adequate level of immunogenicity. With so much time and effort invested in a strategy, some institutions might have been reluctant to drop it, but IAVI made a decision to end the project—although IAVI still funds other MVA research. "Products that don't pass the bar are terminated quickly without waste of resources so that funds are used efficiently," says Berkley.

The experiments themselves will always take time, but the idea is to accelerate the other aspects of research and development so that laboratory experiments, such as waiting to see if animals develop an immune response, become the most time-consuming thing. "The challenge would be to have no delays except for the time it takes for experiments to finish," says Berkley.

Orderly exploration could help quickly eliminate dead ends. "There's this finite immunological space," says Nabel, "and testing a vaccine candidate/strategy means that, if it doesn't work, you can close off that space and move on."

Secure funding

Some of the drawbacks in traditional academic research, like the publish-or-perish dilemma, stem from researchers having to constantly compete for grant funding. Contingent upon shifting business priorities, industrial researchers usually know their funding is secure as long as the project is continuing to make progress towards its milestones. One option being discussed is to fund quality academic researchers for longer periods of time and make grant renewal less firmly tied to publishable data. "A degree of independence from current funding mechanisms could foster innovation," says Burton.

Secure funding might have another desired effect: luring experienced researchers into the field of AIDS vaccine research. Especially needed are experts in basic immunology, says Bruce Walker, director of Harvard Medical School's Center for AIDS Research. "In the HIV immunology field," says Walker, "there are very few of the mouse

immunologists, those that have helped to define how the immune system works, who have made the transition to HIV research."

It is not easy to attract people who are successful in other areas to change focus and work on HIV, says Walker, and new, secure funding could help, especially if it came with few strings attached and with an attitude that says, "We expect you to not worry about funding and publications, just get the job done." More formal funding arrangements could ensure that groups work in tandem for prolonged periods.

As for providing incentives for new researchers to enter the vaccine field, some young researchers may be concerned that working in large collaborative groups hurts their chances for publication and therefore promotion. Haynes says that he is already talking to the deans at the universities participating in CHAVI about basing promotion and tenure

less on publications and more on recognizing productivity and group contributions. An AIDS vaccine effort will differ from industry practices in significant ways. Rather than keep results close to the vest, AIDS vaccine researchers will share them widely. Intellectual property rights will be protected through patents to enable sharing of ideas, so that biopharmaceutical companies will have incentive to take vaccine candidates into development and manufacturing.

Hard science

A research model alone won't lead to a vaccine. "No matter how good your industrial model is, you have to have the basic strategy first," says Ho. "The harmonization of practices and assays are good for the field, but we should not be misleading ourselves that those are the true obstacles—the obstacle is a scientific one." Mitchell Warren, executive director of the AIDS Vaccine Advocacy Coalition, agrees. "We shouldn't forget that no matter what research model is used, much basic research remains to be done," he says. "I would hate to see people stuck debating about what is the correct model."

Harmonizing research practices and assays will help ensure that the search for the vaccine is efficient and takes as little time as possible. Yet in applying the industrial model

to AIDS vaccine research, we must take care, says Esparza. "In 1997, President Clinton promised an AIDS vaccine in 10 years, and he compared it to Kennedy's initiative to put a man to the moon. But there is a critical difference. Putting a man on the moon was an engineering problem, whereas an AIDS vaccine is a research problem. We know where the moon is, but we don't know where we will find an AIDS vaccine."

The risk, says Esparza, is that we will invest in the wrong vaccine candidate, before we've had a chance to explore all the options. "Right now the goal is not to build a spaceship to the moon," says Esparza. "It is to develop many probes that we will send to different regions of space." 

Catherine Zandonella, MPH, is a freelance writer whose work has appeared in Nature and New Scientist.



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Bruce Walker

CROI covers advancements from start to finish

Highlights of recent HIV meeting run gamut from basic science to HIV prevention and vaccine research

By Kristen Jill Kresge

The 13th Conference on Retroviruses and Opportunistic Infections (CROI), which took place from 5-8 February, often struck a historic chord as many plenary and keynote speakers acknowledged the passage of important landmarks in the battle against the AIDS pandemic. The Annual Bernard Fields Memorial Lecture given by Bette Korber of Los Alamos National Laboratories during the opening ceremony helped set this tone. Korber began by explaining the genetic diversity of HIV, gazing all the way back to the 1930s when HIV first entered humans. This historical theme continued with other researchers who stopped to review and reflect on the progress of the field spanning relatively shorter time frames, including the 25 years since the first AIDS cases were reported in the US and almost a decade since the advent of highly active antiretroviral therapy (HAART).

But the almost 4000 researchers convened in Denver took away more than a history lesson. Presentations at the meeting also covered the most recent advancements in understanding the immunological events that occur early in HIV infection, using new strategies to prevent transmission of the virus, and vaccine vector development and studies that may help to elucidate the immune responses required to prevent infection. Mario Stevenson of the University of Massachusetts, a member of the conference committee, reminded the audience that these developments in both treatment and prevention remain an imperative since despite 25 years of research and innovation, not a single HIV-infected individual has been able to successfully clear the infection and an effective vaccine hasn't yet been found.

Beyond a gut feeling

A highlight from the basic science track at the conference was the expanding body of knowledge on the immunological events that occur in early SIV/HIV infection in animal models and humans. Following on publications from Mario Roederer of the Vaccine Research Center (VRC) and Ashley Haase at the University of Minnesota last year (*Nature* **434**, 1148, 2005; *Nature* **434**, 1093, 2005), presentations at CROI contributed further evidence for the critical depletion of memory CD4⁺ T cells in mucosal tissues during acute SIV infection in several species of primates and added some new theories on the complicated dynamics of depletion and subsequent restoration of these cells during HAART.

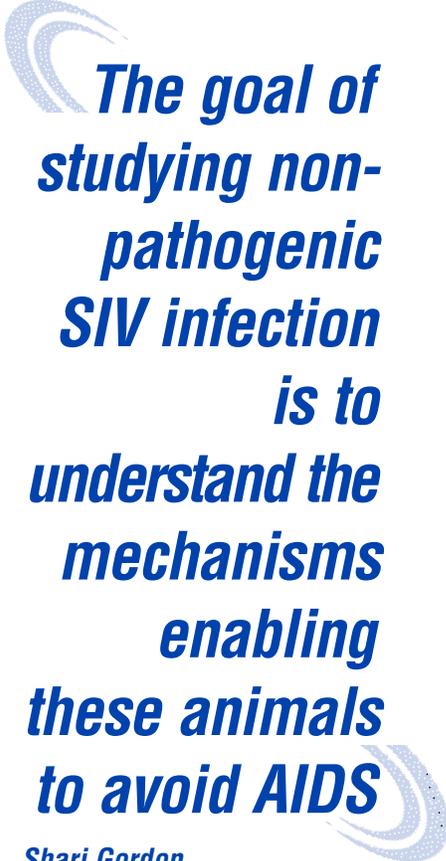
The original work by Roederer and Haase showed that a decisive and rapid depletion of this subset of immune cells in the gut-associated lymphoid tissue (GALT) occurred within days of SIV infection in rhesus macaques (see Research Briefs and *Research at the Extremes*, *LAVI Report* **9**, 2, 2005). This massive and very early immunological destruction in the GALT, and other mucosal tissues where large numbers of CD4⁺ T cells are found, probably plays a key role in the eventual immunodeficiency of these SIV-infected animals. Protecting these cells during acute infection has therefore become of increasing interest to both clinicians and vaccine scientists.

Two presentations at CROI focused on the events of acute infection in two other primate models, African green monkeys and sooty mangabeys, which unlike macaques are natural hosts for SIV and don't progress to AIDS after infection. This makes them an interesting model for studying any possible connection between early immunological events and disease progression.

Ivona Pandrea from the Tulane National Primate Research Center in Louisiana looked at the loss of CD4⁺ T cells in GALT during SIV infection in African green monkeys (Abstract 37; www.retroconference.org/2006). Even prior to infection, these monkeys have fewer CD4⁺ T cells expressing the CCR5 receptor, which were identified by Ron Veazey, also of the Tulane National Primate Research Center, as the target cells for SIV infection in the macaque model. Pandrea compared the CD4⁺ T cell depletion in six African green monkeys and four rhesus macaques infected with SIV_{agm} (African green monkey SIV).

What Pandrea observed was that both African green monkeys and macaques had very similar responses to infection in peripheral blood and in the GALT during chronic infection. Starting at day 28, Pandrea reported a dramatic depletion of these cells in both species and after 60 days, there were no measurable CD4⁺ T cells in the GALT of the African green monkeys. These cells recovered slightly after two years, but still remained lower than pre-infection levels. However the extensive damage that occurred in the GALT of African green monkeys did not have any pathogenic consequences and, despite a sustained high level of viral replication, none of these monkeys showed signs of disease progression.

But other groups have postulated that immune activation is also



The goal of studying non-pathogenic SIV infection is to understand the mechanisms enabling these animals to avoid AIDS

Shari Gordon



**Another area
of interest
among
researchers
is the ability
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undo the
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immune cells
during acute
infection**



necessary for progression to AIDS and disease-related morbidity and could play a role in preventing the restoration of the immune system (*Nat. Immunol.* 7, 235, 2006). To address this Shari Gordon, who works in the laboratory of Guido Silvestri at the Emory Vaccine Center in Atlanta and her collaborators looked at the depletion of CD4⁺ T cells as well as the level of immune activation in the mucosal tissue of sooty mangabeys, another species that does not progress to AIDS when infected with SIV (Abstract 36).

Previous studies from Silvestri's group have shown SIV-infected sooty mangabeys maintain low levels of immune activation, as measured by T cell proliferation, despite high viral replication (*J. Virol.* 79, 4043, 2005). Gordon's presentation at CROI centered on an experiment comparing the depletion of CD4⁺ T cells in the peripheral blood, lymph nodes, and mucosal-associated lymphoid tissue (MALT) of 18 SIVsm-infected sooty mangabeys and 10 control animals. Samples of mucosal tissues were obtained by rectal biopsy and broncho-alveolar lavage.

Gordon observed that the SIV-infected animals had a significant depletion of CD4⁺ T cells in both the rectal and lung samples as compared to the uninfected controls, although the level of damage varied between animals. This reduction in CD4⁺ T cells in the MALT occurred despite overall low levels of immune activation, including activated or proliferating T and effector cells, measured in the mucosal tissues. Even though the CD4⁺ T cells were significantly depleted, in the absence of immune activation, these animals still did not progress to AIDS. Gordon and her colleagues are now looking for a possible correlation between the level of cell depletion and the degree of immune activation in sooty mangabeys.

"Ultimately, the goal of studying non-pathogenic SIV infection is to understand the mechanisms enabling these animals to avoid AIDS despite levels of viral replication that would be highly pathogenic in HIV-infected humans," says Gordon.

Another area of interest among researchers is the ability of both monkeys and humans to undo the damage wrought on these critical immune cells during acute infection. Several studies at CROI looked at how the introduction and time on HAART influences the restoration of the CD4⁺ T cells during chronic infection.

Gordon's study evaluated the repopulation

of CD4⁺ T cells in the rectal mucosa of 6 sooty mangabeys after initiation of therapy with 2 antiretrovirals (ARVs), tenofovir and FTC. Half of the animals placed on therapy showed an increase in the percentage of these cells at this mucosal surface, suggesting that the immune mechanisms designed to reverse this damage are still active in non-pathogenic SIV infection.

Several groups also presented on the effect of HAART in human studies. Jason Baker from Haase's laboratory looked at both the pre-treatment levels and the increase in CD4⁺ T cells in the lamina propria of 8 people with acute HIV infection, 15 who were pre-symptomatic, and 7 who had AIDS (Abstract 41). In 12 of these individuals that were followed for 6 months, he observed only a 10% increase in the CD4⁺ T cells in the gut after starting HAART. These same individuals had an average 32% increase in CD4⁺ T cells measured in peripheral blood samples.

Another study looked at five individuals on HAART for at least three years and found that even after this length of time there was not a significant replenishment of CD4⁺ T cells in the gut. This second study, presented by Jason Brechley from the VRC, also found in HIV-infected individuals on HAART, 10 times more HIV-infected CD4⁺ T cells in the gut than in peripheral blood (Abstract 38).

This finding suggests to Brechley that the local concentration of ARVs in the gut may be much lower than in blood, allowing viral replication at mucosal surfaces to continue at high levels. This residual viral replication along with the continued depletion of the CD4⁺ T cells at mucosal surfaces can hinder immune reconstitution. Brechley proposed that the varying concentration of ARVs could be due to the high number of cells in the gut that express P-glycoprotein, a toxin pump found on both lymphocytes and epithelial cells known to expel protease inhibitors.

"This is a possible explanation for why the CD4⁺ T cells aren't restored," he says. Brechley is now looking at the level of viral replication in the GI tract in more HIV-infected individuals on HAART and is also comparing ARV concentrations to further determine if this is what impairs reconstitution of the CD4⁺ T cells.

There was also a study at CROI from Satya Dandekar at the University of California Davis on HIV-infected individuals who are able to restore CD4⁺ T cells in the gut (Abstract 39).

Dandekar looked at 50 HIV-infected individuals that initiated HAART either during early infection (defined as within 4-6 weeks after exposure) or during chronic infection when CD4⁺ T cell counts dip below 400 cells/ml of blood. The 30 individuals who started HAART during early infection had significantly greater increases in CD4⁺ T cells during the first 2 years of therapy as compared to those who didn't begin HAART until later. One person was actually able to restore cells to pre-infection levels.

"Studies suggest that treatment during acute infection is better because it may attenuate the degree of depletion that would have otherwise occurred," says Brenchley. "It would probably work best if you could start as early as possible. This could be why post-exposure prophylaxis works so well."

And since the recovery in the gut differs significantly from that in the blood, Dandekar suggests HIV-infected individuals should be monitored by mucosal responses to obtain a better measure of how well HAART is working.

In another presentation, Binhua Ling of the Tulane National Primate Research Center reported on the restoration of CD4⁺ T cells in a colony of Chinese rhesus macaques, a subspecies of macaque that is genetically different from the Indian macaques most commonly used in these studies (Abstract 40). Ling began using this sub-species because of a lack of available Indian macaques but soon noticed that it took many of these animals 2-3 times longer to develop AIDS. Ling followed 10 SIVmac239-infected Chinese macaques, 4 of which were classified as long-term non-progressors (LTNPs) during the acute and chronic stages of infection. She found little difference between the two groups during the acute phase but after about six months, during chronic infection, the LTNPs had a greater ability to restore and maintain CD4⁺ T cells in the GALT.

Further experiments by Ling showed that depletion of the CD8⁺ T cells in two of these LTNP animals prevented a repopulation of the CD4⁺ T cells. Preston Marx, a virologist at Tulane who worked on this study suggests that immune control, mediated by the CD8⁺ T cells in the mucosal tissues, is required to suppress the virus for CD4⁺ T cells to be replenished. He and others are now looking to see if there is a specific MHC allele associated with suppression of such a highly pathogenic virus in these LTNP Chinese macaques.

Marx believes this model will be useful in understanding what allows some HIV-infected individuals to have markedly slower disease progression. "No one knows what the immunopathology is for someone who will be a long-term non-progressor," he says. "We hope that by looking more closely at these animals we can learn something that helps understand this process in humans."

Pep for PrEP

Using ARVs to prevent HIV transmission, a concept known as pre-exposure prophylaxis (PrEP), has attracted much attention over the past year. Much of this had to do with the closure of two trials testing the efficacy of the ARV tenofovir in blocking HIV transmission. The premise for these trials is simple; people at high risk of HIV infection swallow a single pill of tenofovir each day to see if it lowers their rate of acquiring HIV when accompanying other risk-reduction behaviors. The biological plausibility of this approach to HIV prevention was demonstrated with tenofovir in monkeys several years ago by the drug's manufacturer Gilead Sciences, but clinical trials testing PrEP only recently began in several countries. Although trials are still ongoing in the US, Thailand, Botswana, Peru, and Ghana, the trials in Cambodia and Cameroon were stopped last year after activist pressure.

At CROI, those following PrEP research received some promising news. Researchers from the US Centers for Disease Control and Prevention (CDC) showed that a combination of two ARVs, an idea now being called combo-PrEP, may be even better at blocking HIV transmission in rhesus macaques (Abstract 32LB). Walid Heneine of the CDC presented data on the efficacy of tenofovir and FTC, two non-nucleoside reverse transcriptase inhibitors, in preventing rectal SHIV (a hybrid of SIV and HIV) transmission in six macaques. The animals received a subcutaneous injection of 22 mg/kg of tenofovir and an oral dose of 20 mg/kg of FTC for 9 days prior to viral challenge.

The SHIV challenge virus was administered repeatedly at a dose equivalent to 3.8×10^5 virus particles using the repeated rectal challenge model. All 9 control animals were infected after at least 4 exposures, while all treated animals remained free of infection after a total of 24 rectal inoculations. Three months after stopping tenofovir/FTC treatment, all these animals still remain uninfected.



It is likely in my mind that antiretroviral therapy will serve as part of our overall HIV prevention strategy



Myron Cohen

A presentation at last year's CROI showed that tenofovir was able to delay but not entirely prevent infection in macaques when challenged with SHIV using this same model. So the results from combo-PrEP were particularly exciting. A possible explanation for the notably improved response to the tenofovir/FTC combination is the exposure of the drug at mucosal surfaces. Myron Cohen of the University of North Carolina presented data from his laboratory showing that the concentration of FTC is 600 times higher in the human female genital tract than in blood, making it a promising choice for PrEP. In order to determine how much this additional drug contributes to the efficacy of the combo-PrEP regimen, Heneine and colleagues also tested the efficacy of FTC alone in six macaques. They found that after 10 viral challenges 4 of the monkeys remained uninfected.

These impressive results suggest that this combination could be highly effective in preventing sexual transmission of HIV. Both researchers and prevention activists were enthusiastic about this study and some groups have already called for clinical trials with this regimen. Gilead has already licensed the co-formulated version of these two ARVs in a single pill known as Truvada and this makes it even more appealing for human studies. Although efficacy in this repeated rectal challenge model does not guarantee similar results in clinical trials, "it seems it may more closely approximate what is happening in humans," says Susan Buchbinder of the San Francisco Department of Public Health, who applauded the research.

"It is likely in my mind that antiretroviral therapy will serve as part of our overall HIV prevention strategy," said Cohen in his presentation on the future of HIV prevention.

Blocking superinfection

Ever since the first reports of HIV superinfection, including the announcement by Bruce Walker of Harvard Medical School at the 2002 International AIDS Conference in Barcelona, vaccine researchers have been on notice. The idea that the antibodies produced in response to HIV infection are not capable of neutralizing a virus that differs only slightly was great cause for concern. But based on research presented at CROI there is still hope that such protection does occur after a certain length of infection.

Evidence from both macaque studies and documented cases in humans indicate that infection with a second virus is most likely to occur within a window period of primary infection. There aren't any reported cases of superinfection in cohorts of chronically-infected patients, according to Robert Grant of the Gladstone Institute in California. Grant presented data on the neutralizing antibody responses to various viruses in a cohort of HIV-infected individuals and their HIV-infected partners (Abstract 92). All partners in this

study had genetically distinguishable viruses at baseline, were not taking ARV therapy, and had established their partnership after becoming infected. The partners engaged in an average of 200 unprotected episodes of intercourse during this study.

Of the 9 individuals that were recently infected there were 5 cases of superinfection, while among the 12 individuals that were HIV infected for more than 2 years, there was no evidence of superinfection.

Grant found that when he measured the neutralizing antibody responses in the superinfected individuals, they had higher titers to their partner's virus than to autologous virus but that overall the responses were weak and narrow. In the individuals that were exposed but remained infected with only a single virus, the neutral-

izing antibody responses were cross-protective to autologous virus, virus from their partner, as well as to 10 epidemiologically unrelated viruses from other individuals. This finding led Grant to conclude that neutralization of the partner's virus might be blocking systemic superinfection in chronically HIV-infected individuals. Grant and his colleagues are now analyzing genital mucosal sites in these individuals to see if the antibodies created at these surfaces are blocking infection or if a superinfection is localized within the genital compartment.

The results of this study were good news to researchers but emphasized again the importance of finding a vaccine that can induce broadly neutralizing antibodies.

Ways around pre-existing immunity

Lawrence Corey of the HIV Vaccine Trials Network was given the task of summarizing the status of AIDS vaccine research at this year's CROI. He highlighted in his discussion the eight viral vectors that are currently being evaluated in clinical trials. Of these the one that is furthest along in clinical testing and perhaps holds the greatest promise, is the adenovirus serotype 5 vector (Ad5). But a major obstacle for this vector is the prevalence of pre-existing immunity (PEI; see *Immunity's yin and yang*, this issue).

"Apart from the problem with pre-existing immunity, Ad5 appears to be an excellent vaccine vector," said Dan Barouch of Beth Israel Deaconess Medical Center in Boston at CROI. One strategy for getting around issues with pre-existing immunity is to use a naturally-circulating adenovirus that is not as prevalent worldwide, such as Ad35. However in preclinical studies this vector is much less immunogenic than Ad5, according to Barouch.

To circumvent problems with PEI and maintain the immunogenicity of the Ad5 vector, Barouch and his colleagues have been tinkering with the hexon-capsid protein of Ad5, which is the primary target for neutralizing antibodies against the virus (Abstract 179LB). This group conducted experiments where they replaced the



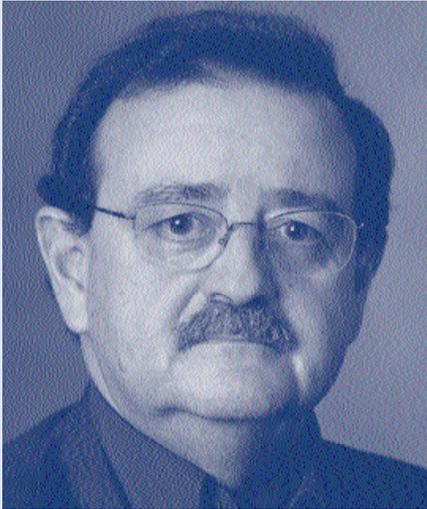
Dan Barouch

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An Interview with

José Esparza



HIV vaccine developments

José Esparza MD, PhD has been a leader in the international HIV arena for almost two decades and for much of that time has been a consistent champion for AIDS vaccines, particularly for developing countries. He currently wears two hats, one as Senior Advisor on HIV Vaccines at the Bill & Melinda Gates Foundation, as well as head of the interim secretariat for the Global HIV Vaccine Enterprise.

Esparza began his research career at the Venezuelan Institute of Scientific Research in Caracas, Venezuela, where he rose from graduate student to full professor. During that time he completed his PhD at Baylor College of Medicine, Houston, Texas, and managed a two-year spell as a visiting professor at Duke University, North Carolina. His research interests have encompassed epidemiology to molecular biology in human virology, including herpes simplex viruses, Venezuelan equine encephalitis virus, and rotaviruses.

In 1986 he joined the World Health Organization in Geneva, and soon became a leader in that organization's HIV/AIDS programs, with an emphasis on vaccines. Esparza then moved in 1996 to the Joint United Nations Programme on HIV/AIDS (UNAIDS) to lead their HIV vaccine programs. In 2004 he joined the Bill & Melinda Gates Foundation and has been an important figure in establishing the nascent Vaccine Enterprise. IAVI Report Editor

Simon Noble recently spoke to Esparza about the Gates Foundation, the Vaccine Enterprise, and recent developments in the field of AIDS vaccines.

The Gates Foundation is now a leading global health organization. Consequently it now has a very wide scope of activities—in comparison to other diseases, what emphasis does the Foundation place on HIV?

The Gates Foundation is a family foundation and our mission—which reflects the vision of Bill and Melinda Gates—is to help address fundamental inequities in the world, something that Bill has referred to as the random geography of birth: A child born in the US or Europe has a totally different perspective in relation to access to health, education, housing, the future in general, when compared to a child who is born in, say, the middle of Africa. They have identified that two of the major factors of this inequity has to do with lack of access to education and healthcare. Because of that, global health—the emphasis is to develop interventions that can be used in developing countries—takes about 60% of our funding at this time.

When AIDS appeared 25 years ago, it not only created major inequities but also helped to identify existing major inequities, including inequities to do with access to education that helped prevent many people in the North from becoming infected but that wasn't widely available in the South. And that inequity between the haves and the have-nots was then increased when antiretroviral drugs were developed.

I hope that once an HIV vaccine is developed it will not contribute to further increasing the gap between the North and the South. If the vaccine that is developed is not appropriate for developing countries because of the cost, because of the regimen for administration, because of the sub-type specificity, or for some reason it's not available in developing countries, then that would be the ultimate tragedy.

About \$1.1 billion of the \$6 billion in global health grants to date have gone towards HIV. This proportion may change as we find where the strategic opportunity arises, so we can move there and make a difference.

How much emphasis is placed on HIV prevention versus treatment?

Today, in 2006, we don't see a dichotomy between prevention and treatment. Both are important components of our response to AIDS, they have to go hand in hand. But ten years ago, there was confrontation between prevention and treatment. When I was at the WHO I remember making the point that an organization that focuses on prevention can't disregard those who are infected.

We're very encouraged by the recent progress on treatment, but there's still a long way to go on prevention. We are developing a broad prevention portfolio, including

microbicides, vaccines, pre-exposure prophylaxis, behavioral components like the Avahan initiative in India to empower disenfranchised populations, and others. We work with other partners to ensure that the response to the epidemic is not skewed in any direction but remains comprehensive, rational, and durable.

There have been major efforts in the last three years, like PEPFAR and the '3 by 5' program, to increase access to therapy. That's wonderful, and it has facilitated the work of organizations like IAVI that focus on prevention.

You used to work on rotavirus, it must be exciting to finally see an effective vaccine come to market. Does this example have lessons for AIDS vaccine development?

Yes, I started working with rotavirus soon after it was discovered, in 1974. The ultimate goal was the development of a vaccine, and I am thrilled that now we have one, or maybe even two. I often use rotavirus as a paradigm for HIV vaccines. The development of the vaccine took over 30 years of hard work, with multiple efficacy trials conducted in industrialized and developing countries. A key strategic decision was when the end point to measure vaccine efficacy was changed from prevention of infection to prevention of disease, which is a current discussion in the HIV vaccine community, although there are important differences.

In rotavirus, there is not natural immunity after infection, children who are infected with rotavirus can be re-infected and re-infected. Conventional wisdom will tell you that if there is no post-infection immunity, forget about a vaccine. The eureka moment came when Al Kapikian at the NIH and others realized that the primary infection was the more pathogenic, and when a child was re-infected the disease was generally very mild. That actually led to a change in the paradigm: A vaccine may not be able to prevent infection, but let's measure instead the severity of disease. And voila—there was a clear difference.

There are other parallels with HIV vaccines. There are many rotavirus types—although the problem of rapid mutation does not apply—and one vaccine is based on one type, and the other vaccine on four types. How much cross-protection between types exists? With correlates of protection, the rotavirus vaccine protection seems to be better than the level of circulating and mucosal antibodies would predict. So the immune correlates of protection are not very clear. Also, the rotavirus vaccine was developed without a good animal model.

The other lesson for HIV vaccines of course is the need to conduct multiple, multiple Phase III trials. To some extent, it was an empirically-developed vaccine. It wasn't this approach that scientists

tend to have, that you do a trial or an experiment to prove that your hypothesis was correct. It was actually a very exploratory, empirical approach to vaccines that they learned by doing. Overall, the rotavirus vaccine gives us some good lessons for HIV, including the need to maintain a balance between rational development and empirical testing. There is no substitute for clinical trials.

Do you think that within the AIDS vaccine field people are seeing that prevention of infection, sterilizing immunity, is such an elevated goal that perhaps we have to lower the bar and go for prevention of disease? Do you think that's being tacitly agreed upon?

I don't think so, or at least for me it's not clear. I think that the *intermediate* goal of a vaccine may be prevention of disease rather than infection. But we don't have the information to conclude that this should be the ultimate goal of a vaccine. Experience is that people who become infected will, sooner or later, progress to disease. And accepting this intermediate goal too early could actually prevent the research that is needed to see if we can develop a vaccine that confers sterilizing immunity. There are many, many opinions in this field and I think we need more than opinions; we need facts. Science is not what you believe but what you know.

I think that debate is still ongoing. I would caution against premature agreement in the scientific community that the goal should be a vaccine that just prevents disease. This is work in progress; we don't yet know the limitations.

An effective rotavirus vaccine was introduced into the US in 1999 but was subsequently associated with intussusception (a folding of the intestine) in fewer than 1:10,000 children given the vaccine, leading to its withdrawal. In industrialized countries rotavirus is a fairly innocuous infection but in developing countries almost half a million children die each year from dehydration

caused by rotaviral diarrhea. Do you think this has any relevance for AIDS vaccines, that perhaps we have to look at the risk-benefit analysis of a vaccine in the context of a particular country?

I'm very familiar with the intussusception story, and we lost an opportunity there. The strategy for introducing most vaccines, still in use today, is introduction first in industrialized countries to try to recoup some of the development costs and then move it to developing countries. The conversation about rotavirus vaccine took place after the intussusception issue was identified in the US, when the vaccine was not actually in use in developing countries.

There is a very, very heavy political component to this. It's very difficult for decision-makers in developing countries to justify using a vaccine that was found not to be safe enough for the US. I've seen all the calculations of how many lives will be saved, because intus-



I would caution against premature agreement in the scientific community that the goal should be a vaccine that just prevents disease



susception, as you say, is a very rare phenomenon, but from the very beginning I knew that it would be too much to ask for decision-makers in developing countries to accept a vaccine that had been found unsafe.

Now, had the rotavirus vaccine been simultaneously tested and introduced into industrialized and developing countries, I think the discussion would have been different. Developing countries may have different risk-benefit decisions than industrialized countries. A risk-benefit analysis takes into account burden of disease, the cost of the product, its ease of use, and many other factors that typically differ from country to country. But the value of life is the same anywhere in the world, and if the perception is that you are proposing a substandard vaccine in developing countries, that will not fly.

So that's a lesson to learn for HIV vaccines: Simultaneous testing and simultaneous introduction of the vaccine in developing countries, and allowing developing countries to make their own risk-benefit analysis. A major priority for the Foundation is helping to ensure that health products that could save lives reach the people who need them the most as quickly as possible.

Given those provisos, do you think there is a global perception now that HIV/AIDS within developing countries has become such a crisis that perhaps these conversations can now be broached again?

Yes, I think so, and the reason is that there is far greater research literacy in developing countries today than ten or 15 years ago. Then it was very difficult because basically you went to developing countries to ask them 'Please trust me. Trust the NIH. Trust the WHO.' It's very difficult to have a rational and pragmatic discussion when you're asking people to trust you. Today, I think that thanks to the work of many people, IAVI included, vaccine literacy is much higher.

What do you think sets apart the Enterprise's way of doing business? What does it offer above and beyond the collaborative efforts already underway?

I have been observing the field of HIV vaccines for many years, and I saw a major strategic shift after the results from the recent Phase III trials. On one hand, the research community realized that developing an HIV vaccine was one of the major scientific challenges we were confronting and that a much more intense and rational effort was needed to complement the more or less empirical approach taken up till then. The other shift was what I call from the "solitary hunting approach" to "pack hunting," as prehistoric man did when he shifted from hunting small animals to mammoths. If we want to succeed on this hunt, we need to reorganize ourselves in a more purposeful and targeted way, as that proposed by the Enterprise. Collaborative efforts should expand beyond exchanging reagents and talking in meetings, to a more structured and account-

able way—perhaps we should look at how collaboration is structured in industry.

But I want to clarify that the Enterprise does not propose to replace the creativity of individual investigators, which we consider essential. The Enterprise proposes to supplement that creativity with systems and structures that provide the critical mass, a clear blueprint for research that may lead to discovery and product development, access to information and resources, a supportive political and community environment, and the financial resources to achieve success.

I recently had a conversation with Rino Rappuoli, who for me is the quintessential, practical vaccinologist, and he thinks the industrial model is very much an engineering approach.

If you want to put a man on the moon, you know the laws of physics that will govern how a rocket will fly from the earth to the moon, so it's an engineering problem, an industrial problem. The problem we have with HIV vaccines is that we are to some extent in a pre-industrial stage, because we don't have a solution, and we have to explore many avenues, we have to build many small space probes rather than one big spaceship. However, if the industrial model means a more targeted effort with clear milestones, with clear go-no go decisions, in which the different partners are accountable for their contribution, then I like the industrial model.

A number of people believe there is a strategic gap in the field of HIV vaccines. We can divide the vaccine development process, from an idea to injection into the arms of people, through four phases. The first is the discovery phase, creation of new knowledge, and the NIH is the driving force. Most NIH-funded scientists stop at that level, publication is basically their goal. The second is the translational or, as I like to call it, maturation phase. The third phase is the manufacturing phase, the typical industrial approach, the engineering

phase. And the fourth phase is the delivery of the vaccine, we have a vaccine, how do we make it available to people around the world?

My feeling is that we have a strategic gap in the second phase, and that is one of the areas where the Enterprise can make a major contribution and help mature ideas so that they become interesting leads for industry to follow, and then work together with industry as real partners, because we need industry for their process development and manufacturing expertise.

What are your initial impressions of how CHAVI is developing?

I think that the success of CHAVI is related to the success of the Enterprise: We need to improve the way scientists interact with each other and CHAVI represents a new model for increased collaboration. CHAVI and the NIH decided to first identify the core leaders



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and then asked those leaders to develop the network. The foundation is taking a similar approach, although we first identified the members of the network, and now we are working with them to establish a collaborative group.

What I see from CHAVI is that they are making a very serious effort to do two things. One is to bring new partners to the field. Now, of course many of the leaders are veterans of the HIV vaccine effort, because you have to go to those people who have the knowledge that we need to tap. The trick is getting the same players to play a different game, a more cooperative game. But CHAVI is also making an effort to bring in new players, and in their list of collaborators you see more people from developing countries, more strategic alliances being created with people who are actually newcomers to the field.

The second is to bring new money to the field, and the NIH has pledged up to US\$350 million for CHAVI. That is additive money, and hopefully it'll create a culture of more collaborative work than the typical NIH R01 grants. We are eager to see CHAVI's progress: I'm very optimistic that CHAVI is a critical contribution to the Enterprise.

It seems that the Enterprise's strategic scientific plan has been quite a step forward, and that at least the broad scientific questions, have been agreed upon.

I'm going to say something you may not be expecting: While the plan is important, in fact what's more important is the process that led to the development of the plan. That's a process of bringing people together and challenging them to identify the key questions and potential ways to address those questions, and I think that discussion between 150 scientists from around the world was very helpful.

The plan was agreed upon by everybody. Why? Because it basically represents what we would call the current paradigm. I wrote an article (*International Microbiology* 8, 93, 2005) on the Enterprise where I address the issue of group think versus individual thinking, and I refer to one of my favorite philosophers, Thomas Kuhn, who said that in the scientific community knowledge moves not by gradual increment but by scientific revolutions.

Thinking within the scientific community is defined by the current paradigm because the individual thinking that the scientific community values so much is constrained by preexisting data and available tools, but also by the ability of getting grants or getting published,

by the peer review system in general. If you are a very innovative, creative person, maybe you will not get the grants and your papers will not be published. So the scientific plan that we have today is mostly a current paradigm plan, but still have to explore harder how to bring in new paradigms, real innovative ideas.

Kuhn proposed that when the current paradigm doesn't provide a solution to a scientific problem, then the scientific community jumps to a new paradigm. Now, when you try to jump to a new paradigm, you're trying to jump with ideas that are not supported by data, because they are new. Most of the time those new ideas outside of the paradigm are wrong. So jumping to a new paradigm will require taking risks to a level that most organizations cannot accept.

Although the solution for an HIV vaccine may come out from the current paradigm we need to be constantly looking at innovative research, how to bring really new science, not only theoretical knowledge but also even instrumentation and bioengineering tools.

What do you see as the next big step in the Vaccine Enterprise?

A year ago the Gates Foundation issued a Request for Proposals, inviting the scientific community to submit innovative ideas and approaches to accelerate HIV vaccine development focusing on vaccine discovery, both antibody and T-cell inducing vaccines, and laboratory standardization. We're planning to make an announcement within the next few months. We're structuring these projects as an interactive and collaborative network that shares information, reagents, and ideas, not only among themselves but with other key partners of the Enterprise, including CHAVI, IAVI, and others.

Our major priority is expanding the research agenda to implement the scientific priorities identified in the Enterprise plan. Also, the Enterprise is almost ready to appoint its first Chief Executive and to establish its permanent secretariat. In the short term, our other priorities are to update the current scientific plan and establish approaches to monitor its implementation by the Enterprise partners, start the implementation of activities in the areas of clinical trials capacity, have a reality check on our approaches to working with industry in areas of process development and future manufacturing, fine-tune our investment menu—a list of financial needs to implement the priorities identified in the scientific plan—and use it to raise the necessary funds for the Enterprise partners and for the secretariat. 

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hypervariable regions of the hexon protein with genes from the corresponding region in the Ad48 virus and compared the responses to the unadulterated Ad5 vector.

In mice with high levels of PEI to Ad5 the cellular immune responses, as measured by tetramer binding assays, to a dose-limiting immunization with the traditional Ad5 vector expressing SIV Gag were blunted significantly. But when the chimeric vector was tested, the mice with high PEI had responses similar to those seen in mice without any PEI. Even when the chimeric vector was administered as both a prime and boost in mice with high PEI, both immunizations were highly immunogenic.

Barouch in partnership with researchers at Crucell, a biotechnology

company in the Netherlands, then administered a 10^{11} viral particle dose of both the hexon-chimeric vector and the Ad5 vector expressing SIV Gag in rhesus macaques with and without anti-Ad5 immunity. The Gag-specific cellular immune responses (quantified by IFN- γ ELISPOT assays) elicited by the traditional Ad5 vector were suppressed by more than 4-fold in animals with high PEI but remained unchanged in those who received the hexon-chimeric vector.

These manipulations of the Ad5 vector look like a promising route for avoiding problems with PEI in populations where it is prevalent, and Barouch suggests that this approach is ready to move into clinical trials soon. He did, however, also allude to potential large-scale manufacturing issues with the chimeric vector. 

Research Briefs

Vaccine helps chimp cells fight HCV

About 3% of people are chronically infected with hepatitis C virus (HCV), putting them at elevated risk of liver disease and cancer. There is currently no vaccine to protect against HCV infection and developing one has been challenging, partly because antibodies that neutralize one HCV strain don't work on the highly diverse range of viruses found in infected individuals.

Studies of spontaneous viral clearance in humans and chimpanzees suggest that cellular responses can also control HCV infection. Now a new report finds that an adenovirus vaccine that elicits a robust HCV-specific cellular response in chimps reduces viral replication, protects against liver damage, may improve viral clearance—and can work against a virus with significant genetic differences from the strain used to construct the vaccine (*Nat. Med.* **12**, 190, 2006). This is encouraging news for adenovirus-based AIDS vaccines attempting to elicit protective cellular responses.

The prime-boost-boost vaccination regimen used three vaccine components sequentially: injections of human adenovirus serotypes 6 and 24 (Ad6 and Ad24) and electroporated plasmid DNA. The five vaccinated chimpanzees received doses of the vectors containing a seg-

ment of the HCV genome and five control animals received the same vaccination schedule but with vectors containing the HIV-1 *gag* gene.

In vaccinated animals, researchers saw an increase in the number of IFN- γ secreting HCV-specific CD4⁺ and CD8⁺ T cells after just the Ad6 prime. After the Ad24 boost, there was an additional increase on CD8⁺ T cells, but not CD4⁺. The DNA boost resulted in up to a 4-fold increase in the frequency IFN- γ secreting CD8⁺ T cells and a 2 to 10-fold boost in CD4⁺ T cell responses. None of the control animals developed cell-mediated immunity to HCV in response to vaccination with the *gag* gene vectors.

All the animals were then challenged with H77, an HCV strain that is 13% different at the protein level from the virus used to create the vaccine. In the vaccinated animals, the average peak of viral blood titers was 100 times lower than in the control group. All control animals developed acute hepatitis as judged by increases in the blood levels of two liver enzymes, while no such increase was found in the vaccinated animals. It was not possible, however, to demonstrate a statistically significant improvement in viral control given the high rate of spontaneous clearance: 4 of 5 vaccinated chimpanzees cleared the virus (one developed a relatively weak T cell response), as well as 3 of 5 controls. —PC

Poliovirus quasispecies cooperate to cloud the brain

No RNA viral genome, whether it is HIV or poliovirus, is really alone during a natural infection. A hallmark of RNA viruses is a high rate of replication errors which rapidly generate a mutational cloud of related genomes known as a quasispecies.

Because of their rapid mutation rate, RNA viruses can evolve rapidly to, for instance, develop resistance to antiviral drugs. But laboratory experiments and mathematical models suggest that the actual error rate must be balanced between two extremes. If too many mutations develop too quickly the virus population is driven to extinction. Too slow a rate of evolution leaves the virus less able to generate beneficial mutations to deal with adverse conditions.

And according to a new report from researchers in California and Pennsylvania, a quasispecies is not simply the sum of the abilities of its individual members. Instead they find evidence in a mouse model of poliovirus infection that the viruses of a quasispecies population cooperate to invade the central nervous system (CNS), suggesting that evolution of such viruses can take place at the population level, rather than the individual level (*Nature* **439**, 344, 2006).

The researchers began by identifying an unusually accurate mutant viral polymerase, G64S, which generates six times fewer mutations than wild type, while still replicating the virus at the same rate. During replication this mutant poliovirus generates a less diverse quasispecies. To test the consequences of increased accuracy, mice were given intramuscular injections of wild-type and

mutant viruses, a mode of delivery known to lead to rapid CNS infection. Animals injected with G64S developed paralysis later and only at much higher viral doses than those receiving the wild type. The dose at which 50% of the animals died was 300 times higher for G64S. When the mutant was delivered intravenously, it was unable to establish infection in spinal cord and brain.

The researchers showed that the limited ability of the mutant to invade the CNS was related to the lack of quasispecies diversity by treating stocks of the G64S with chemical mutagens, bringing its frequency of mutation to wild type levels. Even though this population still contained the same accurate polymerase, its ability to invade the CNS was improved and the lethal dose dropped to the same level as wild-type virus. However, samples of the formerly chemically-mutated G64S isolated from brain tissue were not able to invade the CNS again when re-inoculated intravenously, suggesting the ability to invade the CNS was the result of diversity and not the result of the selection of particular neurotropic viruses.

As a final test of the theory that quasispecies diversity *per se* determined pathologic behavior such as CNS invasion, the researchers used a version of the G64S virus marked with a *SacI* restriction site as a genetic barcode. As expected, this virus was not able to invade and replicate in the brain when inoculated alone intravenously. But when it was co-inoculated with wild type or chemically-mutated G64S it was found in brain. The researchers conclude that a diverse population of viruses in a quasispecies can evolve as a group to accomplish certain tasks cooperatively such as gut colonization, immunological evasion, or penetration of the blood-brain barrier. —PC

Research Briefs

Revealing HIV core values

The formation of a proteinaceous bullet-like core that encases HIV's RNA genome is an important step in the maturation of the virus after it buds from an infected cell. A British and German team of researchers has now determined the three-dimensional structure of the core in mature virions, providing a new model for a core assembly mechanism (*Structure* **14**, 15, 2006).

As HIV buds from a cell, it forms a particle with an outer viral membrane and inner proteinaceous core of capsid proteins. Typical of retroviruses, the membrane and core can vary considerably in size and shape, although the membranes are generally spherical and most cores are conical with rounded edges. In a crucial step in the viral replication cycle, upon infection the core disassembles. For reasons that are unclear, this uncoating of the genome must be precisely timed. At least one host factor with antiviral activity, TRIM5 α , may work by corrupting the uncoating process (see *Making a monkey out of HIV*, *IAVI Report* **9**, 3, 2005). One key question the heterogeneity of the particles raises is how capsid core proteins manage to create a well-defined structure that can accommodate a wide variation in size.

To derive core structures, the researchers used cryo-electron microscopy, which allowed them to view individual particles at high res-

olution in nearly physiological conditions. Electron densities of 75 particles were recorded at 2 degree tilt increments through a range of 132 degrees and the resulting data assembled by computer into a 3-D image.

As expected, the virions were roughly spherical with diameters ranging from 106 to 183 nanometers (nm).

The paper focuses on 40 of these virions that contained a single, complete core of conical morphology. Others contained incomplete, indistinct cores or, in the case of four large virions, two cores. One advantage of cryo-electron microscopy is that it allows examination of the membrane and core simultaneously. The diameter of the broad end of the core was found to strongly correlate with virion diameter and mirrored the curve of the membrane about 12 nm away. In contrast, the average diameter of the narrow end and the angle of the cone did not correlate with virion size.

The researchers say the data argue for formation of the cone starting at the narrow end, with the diameter and core angle being determined by intrinsic properties of the capsid proteins and perhaps other molecules, including the viral genome. The core then continues its growth until constrained by the viral membrane. Ultimately, a better understanding of core dynamics could lead to the design of drugs that disrupt this important viral structure. —PC

Immune cell chimera identified

The link between innate and adaptive immunity has been further strengthened. In two reports researchers describe the hybrid features of a new distinct immunological cell type in mice, the interferon-producing killer dendritic cell (IKDC).

Like natural killer (NK) cells, activated IKDCs can destroy cells lacking self MHC molecules. Like plasmacytoid DCs (PDCs), they can secrete relatively large amounts of interferon (IFN)- α . And typical of conventional DCs (cDCs), they can process and present antigens to T cells, altogether providing another bridge between innate and adaptive immune responses.

In the first study, researchers studied the morphological, phenotypic, and developmental characteristics of the new cell population (*Nat. Med.* **12**, 207, 2006). The IKDCs displayed a set of surface markers that overlap those seen on NK cells, PDCs, and cDCs, but with some clear differences that set them apart. Gene expression profiles were also reminiscent of the other cell types, with all IKDCs expressing the NK-activating receptor NKG2D. Under the transmission electron microscope IKDCs were morphologically distinct from cDCs, PDCs, and NK cells. IKDCs were sensitive to the TLR9 ligand, unmethylated CpG oligodeoxynucleotide, and in response developed the dendrites and veils typical of DCs.

Functional characterization showed that spleen- (but not lymph node-) derived IKDCs could lyse target cells through either NKG2D- or Ly49b-dependent pathways, but only after activation with CpG. In

a recombinant *Listeria monocytogenes in vivo* infection model, the researchers looked for direct evidence of antigen-presenting cell (APC) activity, and found that lymph node (but not spleen) IKDCs could present antigen to naïve T cells. The authors contend this is consistent with a model where IKDCs lose their cytolytic NK activity as they migrate to draining lymph nodes and develop APC activity there. They propose that IKDCs extend the DC family and constitute a cell type with dual innate effector functions and antigen-presenting capacity.

In the second study, researchers investigated the role of these cells in tumor immunosurveillance. In a mouse melanoma model the research team showed that IKDCs rather than NK cells recognized and lysed tumor cells in a TRAIL-dependent manner. TRAIL is an apoptosis-inducing ligand and its expression is induced by IFN- γ ; this study shows that TRAIL expression on IKDCs was induced by activated IKDCs' own IFN- γ expression.

This second, more functional study also perhaps suggests why such a hybrid cell that combines the function of two other cell types might be beneficial; perhaps sometimes it's better to have all these functions coordinated by a single cell.

Researchers will now be fervently searching for a human equivalent to get an idea of the medical relevance of this new cell type. Future studies will investigate just how common and important this cell type is and its role in specific aspects of different infections. Researchers will also have to ask to what extent previously described activities are actually due to IKDCs rather than NK or other cell types. —SN

Pharmexa-Epimmune initiates Phase I AIDS vaccine trial

Pharmexa-Epimmune, a US subsidiary of a Danish vaccine and immunotherapy company, recently initiated a Phase I AIDS vaccine trial in partnership with the HIV Vaccine Trials Network (HVTN) to evaluate the safety and immunogenicity of two candidate vaccines given either alone or in combination. This trial, HVTN 064, will enroll 120 HIV-uninfected volunteers at 4 sites in the US (Baltimore, Rochester, and 2 sites in San Francisco) and in Lima and Iquitos, Peru.

The first candidate, known as EP HIV-1090, is a DNA plasmid vaccine comprised of antigens from Gag, Pol, Nef, and Vpr that code for proteins conserved among several HIV clades (A, B, C, D, E, and G). This candidate was tested in a previous Phase I trial with the HVTN in 42 volunteers. The second vaccine candidate, EP-1043, is a recombinant pro-

tein vaccine comprised of T helper cell epitopes from HIV clade B Env, Gag, Pol, and Vpu administered with an Alum adjuvant. This vaccine candidate is intended to interact with CD4⁺ T cells and cause their proliferation.

In the first part of this trial, 24 volunteers will be randomized to receive placebo or 4 injections of either a low or high dose of EP-1043. After analyzing the safety data, investigators will then evaluate the preferred dose in the second part of HVTN 064, where volunteers will be randomized to one of three groups: EP-1043 or placebo, EP-1090 or placebo, and both candidates versus placebo.

All volunteers will be monitored over 11 site visits during the course of a year. This trial is being sponsored by the US National Institutes of Allergy and Infectious Diseases within the National Institutes of Health and both vaccines are being manufactured by Pharmexa-Epimmune.

Phase I AIDS vaccine trial opens in India

India started the country's second Phase I AIDS vaccine trial in January in Chennai to determine the safety and immunogenicity of a modified vaccinia Ankara (MVA) vaccine candidate at varying doses. The vaccine candidate, TBC-M4, uses an attenuated and non-infectious vaccinia virus vector to deliver *env*, *gag*, *tat-rev*, and *nef-RT* HIV genes from clade C, the most commonly circulating in India.

The DNA coding sequences for the included genes were isolated from recently HIV-infected individuals in India by the National AIDS Research Institute in Pune (NARI). Half the volunteers enrolled in IAVI D001 will receive the low dose of TBC-M4 and safety data from this group will be evaluated by an independent advisory board before inoculating the second group with the higher dose. All volunteers will receive three injections of the candidate vaccine.

This double blind, placebo-controlled trial will enroll and follow 32 volunteers at the vaccine

trial centre at the Tuberculosis Research Centre over 2 years. This newly-established center includes a clinical facility as well as an HIV immunology and virology laboratory where all trial samples will be analyzed. All clinical and laboratory staff completed training on study operations and data management, as well as on gender issues related to AIDS vaccine clinical research.

IAVI is sponsoring the trial in partnership with the Indian Council of Medical Research and the National AIDS Control Organization of India. Therion Biologics, a biotechnology company in Cambridge, Massachusetts, collaborated with IAVI on the development of TBC-M4 and is manufacturing the vaccine for use in the trial. Several other Phase I AIDS vaccine clinical trials are currently ongoing in the US and Brazil that use MVA as a vector.

India is also conducting another Phase I AIDS vaccine trial that began last year at NARI with tgAAC09, an adeno-associated virus vector vaccine manufactured by Targeted Genetics in the US. This trial is also sponsored by IAVI.

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IAVI is a scientific organization founded in 1996 whose mission is to ensure the development of safe, effective, accessible, preventive HIV vaccines for use throughout the world. IAVI focuses on four key areas: Accelerating scientific progress; education and advocacy; ensuring vaccine access and creating a more supportive environment for industrial involvement in HIV vaccine development.

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 23 countries, IAVI and its network of collaborators research and develop vaccine candidates. IAVI's financial and in-kind supporters include the Bill & Melinda Gates Foundation, the New York Community Trust, the Rockefeller Foundation, the Starr Foundation; the Governments of the Basque Country, Canada, Denmark, the European Union, Ireland, the Netherlands, Norway, Sweden, the United Kingdom, and the United States; multilateral organizations such as the World Bank; corporate donors including BD (Becton, Dickinson & Co.), Continental Airlines, DHL and Pfizer; leading AIDS charities such as Broadway Cares/Equity Fights AIDS, Crusaid, Deutsche AIDS Stiftung, and the Until There's A Cure Foundation; and other private donors such as the Haas Charitable Trusts. For more information, see www.iavi.org.

Kenya begins enrollment for Phase I vaccine trial

A Phase I AIDS vaccine trial sponsored by IAVI in collaboration with the Vaccine Research Center (VRC) at the National Institute of Allergies and Infectious Diseases (NIAID) began enrolling volunteers this January in Kenya. The trial, IAVI V001, initially started in Rwanda and was expected to enroll a total of 64 volunteers in these countries. However after early success in recruiting volunteers the target number for both countries will be increased, pending regulatory approval by the local Institutional Review Boards in

Kigali and Nairobi.

The trial in Kenya is being conducted in partnership with the Kenya AIDS Vaccine Initiative (KAVI) at the University of Nairobi. The trial staff are promoting initiatives to recruit women for this trial, including holding community seminars within homes or offices targeting only women.

This is one of many ongoing trials testing the safety and immunogenicity induced by a “prime-boost” vaccination regimen with a DNA plasmid vaccine and an adenovirus serotype 5 (Ad5) vector that was developed at the VRC (see Vaccine Briefs, *IAVI Report* 9, 5, 2005).

Trial shows HSV-2 suppression can reduce HIV shedding

Almost a dozen clinical trials are now ongoing to see if drugs to suppress herpes simplex virus-2 (HSV-2) can reduce the risk of HIV transmission and infection. These studies were initiated because of mounting evidence that a causal relationship exists between HSV-2 and HIV infection (see *HIV prevention in a pill?*, *IAVI Report* 9, 4, 2005). Researchers have hypothesized that HSV-2 infection could increase shedding of HIV in the genital tract and therefore increase both transmission and acquisition of HIV, but a relationship between these infections has not been firmly established in a randomized, controlled, clinical trial until now. At the 13th Conference on Retroviruses and Opportunistic Infections (CROI) held in February, researchers from the London School of Hygiene and Tropical Medicine (LSHTM) in the UK in collaboration with the Centre Muraz in Bob-Dioulasso, Burkina Faso presented data from the first “proof of concept” trial to show that this is in fact what happens.

This study enrolled 140 women infected with both HIV and HSV-2 in Burkina Faso and randomized them between the placebo and treatment arm, where they received a 1000 mg dose of the anti-herpes drug valacyclovir once a day for 3 months. The women were followed for a total of 9 months, 3 months prior to and for 3 months following treatment. Over 12 visits, researchers measured the levels of HIV and HSV-2 shedding in the genital tract by cervicovaginal lavage enriched by cervical swabbing, as well as the plasma viral load of HIV for each volunteer.

At baseline, the average CD4⁺ T cell count was 519 cells/mm³ in the treatment group and 482 cells/mm³ in the placebo arm. Any women that met the World Health Organization’s criteria for starting antiretroviral therapy were excluded from this trial and offered treatment in study ANRS 1295b, where researchers are studying the

effects of combining valacyclovir and antiretrovirals (ARVs) on HIV transmission.

Throughout the course of the study, 93% of the visits were completed and Nicolas Nagot from LSHTM, who presented the results of this study at CROI, reported that the average compliance to the medication was 97%. Researchers observed that women that received valacyclovir had significantly less HIV shedding than those that received placebo. The mean reduction in the treatment group was 0.26 log copies/ml, while genital shedding actually increased in the placebo arm by 0.09 log copies/ml. Nagot also reported that HIV shedding in women on acyclovir wasn’t as persistent, with 33% of women shedding at fewer than half the visits as compared to 14% of women who were in the placebo group.

Valacyclovir also significantly reduced the level of HSV-2 shedding with only 19% of women shedding HSV-2 at least once in the treatment group compared to 54% in women who received placebo.

As a secondary endpoint researchers looked at the difference in plasma viral load between the two groups. They found that women taking valacyclovir also had a greater reduction in HIV plasma viral load than controls. The average plasma HIV load drop was 0.5 log copies/ml, while the controls had an average viral load increase of 0.1 log copies/ml. The amount of HIV RNA detected in the genital tract was also significantly lower in the women on valacyclovir and future studies will address whether this is due to the overall reduction in systemic viral load or due more directly to the action of valacyclovir on genital HIV shedding.

This is the first study to verify the causal relationship between HSV-2 infection and HIV shedding, according to Nagot. And although this study does not show a direct link between HSV-2 suppression and HIV transmission, results from ongoing trials will help establish a possible role for HSV-2 suppressive therapy in HIV prevention.