

# IAVI Report

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## IIb or not IIb?

### *AIDS Vaccine Trial Sponsors Weigh the Merits of Intermediate-Size Efficacy Trials*

BY EMILY BASS

“To be, or not to be: that is the question,” says young prince Hamlet in the Shakespeare play that bears his name. Over the past year, a punning variation on this question has surfaced in the AIDS vaccine field as trial designers and statisticians have debated the merits and shortcomings of intermediate-size trials—often known as Phase IIb trials—in advancing the search for an AIDS vaccine.

It’s more than a superficial allusion. When the young prince poses his question he is wrestling with the meaning of life. Likewise, the scientific question—IIb or not IIb—reflects soul-searching on the part of a field that faces challenging decisions about how to best invest its limited resources, and how to determine which AIDS vac-

cine candidates should be advanced into large-scale efficacy trials.

In the traditional clinical trials sequence AIDS vaccines move from small Phase I safety studies to Phase II trials, which gather additional information about safety, dosing and immunogenicity in a few hundred people, directly to Phase III efficacy trials that are designed to meet regulatory standards for product licensure.

If there were known correlates of protection for AIDS vaccines then immunogenicity data from Phase II trials could provide some hints about efficacy. But in the absence of such a correlate AIDS vaccine developers have little indication of whether or not a vaccine is likely to be effective

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## VACCINES AT RETROVIRUS 2004

BY RICHARD JEFFERYS

The 11th Conference on Retroviruses and Opportunistic Infections took place from 8-11 February, drawing over 3,500 researchers and activists from around the world into the cavernous halls of San Francisco’s Moscone Convention Center. The main focus of the annual meeting is basic science and HIV treatment, but this year the completion of the AIDSVAX efficacy trials and the controversies surrounding the Phase III Thai prime-boost study ensured that vaccines were a hot topic, both in conference sessions and during the informal hallway tête-à-têtes that are the signature of scientific meetings.

### *Caught in the Act: The Potential of Neutralizing Antibodies Targeting Fusion*

Antibody expert Dennis Burton (Scripps Institute, La Jolla) reviewed the state of scientific knowledge regarding neutralizing antibody (NAb) responses to HIV, focusing on the potential of

strategies to block HIV as it fuses with target cells (viral fusion). Burton began with the role of NAb in protection against viral diseases for which licensed vaccines exist. Resolution of an acute viral infection is thought to be mediated primarily by innate and cell-mediated immune responses, and NAb arise after the symptoms of acute infection are resolved. Once present, NAb typically persist and are available to immediately bind and help block the virus if a secondary exposure occurs (memory B cells can also replenish supplies of NAb if necessary). However, Burton believes it’s unlikely that NAb mediate complete protection—some virus probably leaks through and is dealt with by memory T cells that were also generated during the initial acute infection. Licensed vaccines essentially attempt to mimic the effects of primary infection via the generation of NAb and memory T-cell and B-cell responses.

Burton stressed that these approaches typically

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before they decide whether to embark on a Phase III trial. This is a weighty decision; although the precise size of all trials depends on the local incidence rate, Phase IIIs usually enroll many thousands of people—a massive undertaking that requires lengthy preparation and large investments of financial and human resources.

Enter Phase IIb trials, which are smaller than Phase IIIs but significantly larger than Phase IIs. Their intermediate size gives them statistical power to provide valuable preliminary information about vaccine efficacy, though they are generally not geared towards securing product licensure unless the data show very strong evidence of efficacy. Instead, Phase IIb trials are designed to answer key research questions about one or more vaccine candidates, with the expectation that these data will lead to more trials or help guide basic scientific research in the future.

Phase IIb trials are starting to appeal to many AIDS vaccine trial sponsors. “We don’t know what immunogenicity measures mean or if they correlate with protection,” says HVTN statistician Steve Self (SCHARP, Seattle). “We’re doing the best we can do with Phase I and II trials, but we’re still groping in the dark. Phase IIbs would give us some sense of whether there is any protective effect for these vaccines—and that would be beneficial.”

Although there are no confirmed plans for Phase IIb AIDS vaccine trials at present, IAVI is actively considering Phase IIb trials,

as is the US HIV Vaccine Trials Network (HVTN), which is currently developing plans for a large-scale trial of Merck’s adenovirus candidate. “A Phase IIb test of concept trial is the kind of design that we’re discussing,” says Self. The HIV Prevention Trials Network (HPTN) is also poised to launch an intermediate-size trial of two candidate microbicides before the end of 2004.

The new interest in Phase IIbs marks a shift in thinking for the AIDS vaccine field, which so far has launched only three efficacy trials, all of which were classic Phase III studies geared towards licensure. “We can think of efficacy trials [Phase IIbs and Phase IIIs] as the penultimate step in the process of developing vaccines for licensure and widespread use, or we can think of them as experiments that can provide us with valuable information to be used at early stages in the development process,”

said Susan Buchbinder in her plenary presentation (available online at [www.retroconference.org/2004/pages/webcast.htm](http://www.retroconference.org/2004/pages/webcast.htm)) at the recent Retrovirus conference (see article, page 1) outlining the types of questions that could be answered by Phase III and Phase IIb trials (see Table 1 on page 5).

As Buchbinder and others explain it, the question of IIb or not IIb reaches beyond the pros and cons of adding an intermediate step to the vaccine development process and touches on the way that the field defines its expectations for efficacy trials—to itself, to potential volunteers, and to the world.

#### *What is a Phase IIb trial?*

Phase IIb trials are also known as “proof of concept” trials, intermediate-size or probe efficacy trials, or—to their detractors—underpowered Phase III trials. As these names suggest, this type of trial is often used to gather preliminary information on the efficacy of experimental candidates. “Overall, the major question being asked is: Does it work?” says IAVI Medical Affairs Director Pat Fast.

Although the question is simple, the answers—in some cases—are not. As the number of volunteers is reduced, so is the number of HIV infections that are likely to occur during the course of the trial. And as the number of these infections decreases, so does the precision of the trial’s estimates of vaccine efficacy.

One key difference between a Phase III and a Phase IIb AIDS vaccine trial is the width of the confidence intervals—the range of uncertainty—around the “point estimate” of the vaccine’s efficacy in preventing new HIV infections. This type of efficacy is also known as VEs, or vaccine effect on susceptibility. Broadly speaking, most Phase III trials can estimate VEs within  $\pm 15\%$ . A Phase IIb trial of less than half that size—with less than half the number of HIV infections—has broader confidence intervals of  $\pm 30\%$ .

Given these broad confidence intervals, a Phase IIb trial would only be able to say with certainty whether a vaccine was an outright success or a complete failure. For example, if a Phase IIb yielded a VEs point estimate of 60% ( $\pm 30\%$ ), the lowest possible value for VEs would be at least 30%. Since 30% is widely accepted as a minimum threshold for VEs, this would likely be considered a clear positive answer about efficacy. Likewise, if a Phase IIb trial found that a candidate had a VEs of zero, it would be safe to assume that efficacy was less than 30%—and that the candidate does not warrant further testing in trials.

In most cases a product that showed moderate efficacy in a Phase IIb would be tested again in a confirmatory efficacy trial before seeking licensure; but if the efficacy was very high, a Phase IIb trial could possibly be used as the basis for a licensure application to regulatory authorities.

Point estimates that fell into the middle range—

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—SUSAN BUCHBINDER

over 30% and under 60%—would be harder to interpret. “If you’ve got a point estimate of 40% with similarly wide confidence intervals, then that’s a lot more ambiguous,” says Fast. In this instance, sponsors would have to make judgment calls about whether to continue testing the product, abandon it, or attempt to improve the vaccine design.

In addition to measuring VEs, a Phase IIb AIDS vaccine trial could also be used to detect vaccine-induced reductions in viral load ( $\Delta$ VL), a measurement that will likely be used as a surrogate marker for VE<sub>p</sub>, or vaccine effect on disease progression. VE<sub>p</sub> measurements are particularly important for the current generation of candidates, which primarily induce cellular immune responses and will be evaluated for their ability to improve overall health and prolong life in vaccine recipients who subsequently become infected with HIV.

It could take many years to make a direct estimate of VE<sub>p</sub>. One recent article (*J. Inf. Dis.* 188:179; 2003) by HVTN researchers proposed a definition of VE<sub>p</sub> as the percent reduction in the cumulative risk of progressing to AIDS or death 5 to 7 years after infection. In contrast, viral load set point is established within months of HIV infection in people who do not begin ARVs immediately. Early viral load levels have been linked to long-term disease progression, which is why  $\Delta$ VL could be used as an early indicator of VE<sub>p</sub>.

Phase IIb trials can make more precise measurements of  $\Delta$ VL than they can of VEs. This is because VEs calculations are based on the “binary” variable of HIV status—a person is either HIV infected or uninfected at the end of the trial period. In contrast,  $\Delta$ VL is calculated based on the “continuous” variable of viral load, which can take on a range of values. In statistical analysis, there are generally wider confidence intervals for calculations based on binary variables than those based on continuous variables. “A yes or no [binary] outcome is a lot less statistical information than a number like viral load,” explains Wasima Rida (Statistics Collaborative, Washington, DC).

In her presentation, Buchbinder estimated that 5,000- and 2,000-person Phase IIb trials in a population with a 2% incidence rate could measure vaccine-induced reductions in viral load of 0.75 and 1.0 log<sub>10</sub> respectively at about three months after infection. In contrast, a 12,500 person Phase III trial in the same population would have high statistical power to detect a  $\Delta$ VL effect of 0.5 log<sub>10</sub>.

For now, this added level of precision may not be necessary. Although  $\Delta$ VL is a plausible surrogate marker for VE<sub>p</sub>, there are still many unknowns including how much of a  $\Delta$ VL effect is needed and how long it would have to last to achieve a clinical or public health benefit. Given these uncertainties, it might make sense to do a smaller Phase IIb that can detect a more pronounced viral load effect and provide some information on its clinical benefit before proceeding to a

large-scale trial that will pick up smaller virus load effects which could have less clinical relevance.

#### *Intermediate-size trials in other fields*

In weighing the risks and benefits of intermediate-size trials, many AIDS vaccine researchers turn to other vaccine fields. One example of a best case scenario comes from Merck’s human papillomavirus (HPV) vaccine research project.

In 2002 Merck announced positive results from a trial of a vaccine candidate targeting HPV 16. (There are over 100 strains of HPV, some of which can cause genital warts and cervical cancer.) Since the company ultimately hoped to license a polyvalent vaccine against several strains of HPV, this intermediate-size trial was used as “proof of concept” for the basic vaccine design. The trial followed roughly 1500 volunteers for an average of a year and a half following the final immunization, and monitored them for persistent HPV 16 infection—a surrogate measure of the vaccine’s ability to protect against cervical cancer, which may not develop until many years after initial HPV infection.

The trial showed 100% protection against persistent HPV 16 infection in vaccine recipients. Based on these encouraging data, the company has gone on to launch a full-scale Phase III trial designed to secure licensure for the polyvalent vaccine that targets strains 6, 11, 16 and 18. The trial will include long-term follow up so that researchers can directly assess the vaccine’s efficacy in preventing cervical cancer.

Asked about the worst-case scenario, many scientists utter a single word: Patarroyo. This was the name of the Colombian scientist whose malaria vaccine, SPf66, was tested in a series of trials in Africa and South America. These trials yielded varying, often ambiguous, results—most notably the first African trial that tested the vaccine in nearly 600 Tanzanian children. Although the trial was conducted in a region with high rates of malaria, there were not enough cases of symptomatic malaria (the primary endpoint for the trial) to make statistically precise estimates of the vaccine’s effects. The vaccine was found to be 31% effective in preventing a first episode of clinical malaria but the 95% confidence interval for efficacy ranged from 0% to 52%.

The ambiguity of the results led to a stalemate as to how to proceed. “We agree on the need for further research, but raise the question of whether this should be further field studies or, as we would recommend, more detailed pre-clinical studies,” wrote malaria vaccine researchers Adrian Hill (Oxford University) and Sarah Gilbert (Wellcome Trust, Oxford) in a commentary published at the time. Although a 31% efficacious malaria vaccine might have been beneficial—particularly in areas of high endemicity—the debate over the SPf66 trial data effectively stalled the development of the

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Patarroyo candidate, which has still not been tested in a definitive trial.

Looking ahead, the AIDS vaccine field will likely gain insights from a 3,100-woman multicenter international Phase II/Ib microbicide trial to be launched in 2004 by the HPTN.

HPTN 035 is a 4-arm trial comparing two candidate microbicides, PRO 2000 and Buffergel, with a condom-only and a gel-only arm as controls. The study will collect intensive safety data in 800 women followed for three months each—nesting a more traditional Phase II type study into the expanded design. The trial has been designed so that it could be the basis for a licensure application to the FDA if the estimated VEs for either candidate exceeds 43.6%.

**Why now?**

In the AIDS vaccine field, discussions of Phase IIb dates back to 1994 when the US National Institute of Allergy and Infectious Diseases (NIAID) decided not to proceed with a Phase III trial of two gp120 candidates designed to elicit neutralizing antibodies against HIV. This decision prompted a NIAID-sponsored meeting to consider whether and how smaller, less expensive intermediate-size trials could be used to advance AIDS vaccine research. (A summary of this meeting appeared in *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* 16:195; 1997).

Wasima Rida co-organized the meeting and co-authored the summary report that remains the only peer-reviewed publication on Phase IIbs and AIDS vaccine trials to date. During the 1995 discussion, Rida says, “I really didn’t know which side I fell on. I was afraid that the field could end up with a Patarroyo situation.”

The potential for this type of confusion is a powerful deterrent to some AIDS vaccine researchers. US Vaccine Research Center head Gary Nabel warns that these trials could yield indeterminate results that add to, rather than allay, confusion. “If you have to double the trial size to get statistical significance, then double the trial size,” he says.

Many trial sponsors are now weighing the risk that Nabel identifies against the financial and human risks that come with investing in a Phase III trial. VaxGen’s two Phase III trials cost as much as US\$300 million; the current prime-boost trial in Thailand will cost over US\$100 million. Ed Tramont, head of the Division of AIDS at NIAID, predicts that by 2009 the US government-funded networks will need an additional \$239.9 million over and above projected government spending on large-scale AIDS vaccine trials.

“It truly is a matter of dealing with resources around a substantial amount of scientific uncertainty,” says Self. Much of this uncertainty has to do with the field’s current focus on CTL-eliciting candidates that will be evaluated for their ability to

affect disease progression. Since such a vaccine has never been developed before, vaccine developers will have to learn as they go exactly what types of vaccine effects are acceptable to regulatory authorities—and to communities where vaccines may be used.

“We know from the Food and Drug Administration (FDA) exactly what is useful and licensable for VEs,” says statistician Ira Longini (Emory University, Atlanta). “But as far as reducing viral load, we don’t know what we’re looking for, so the trials need to be more exploratory. We need a variety of trials that are large enough to sort out what makes an efficacious, licensable vaccine.”

Phase III trials are too large and costly to conduct simply to learn more about the characteristics of a candidate. Instead, these trials are geared towards licensure, which means that trial sponsors must set and adhere to well-defined hypotheses and criteria for success. “It’s hard to build a hypothesis for a Phase III trial when you only have a sense of what you may see, or want to see, from a candidate vaccine,” says Emilio Emini, Senior Vice President for Vaccine Development at IAVI.

Emini suggests that the field use Phase IIb trials as a chance to “loosen the statistical criteria”—turning the trial into an investigative research study, rather than a quest for licensure. “In a probe efficacy [Phase IIb] trial, you’re looking for anything at all,” he says. “Given that we’re assessing vaccines with unknown biological effects and with an uncertain magnitude of effect, one could argue that it is the better part of valor to do Phase IIb trials before moving on to Phase IIIs.”

Buchbinder agrees. “Rather than asking, ‘Do we have a vaccine for licensure?’ we need to be asking better questions,” she says, adding that the results of these trials could influence design of future candidates as well as Phase III trial decisions.

Phase IIb trials can also be useful in speeding evaluation of vaccine candidates that have yet to complete the costly, time-consuming steps of “process development” (see article, page 14), since sponsors generally wait to launch Phase III trials until this process development has been completed. “If you don’t have your final candidate—if you still have to do process development for example—then it saves time and money to get an answer from a Phase IIb, even if it is not the final answer,” says Fast.

**New challenges and opportunities**

Although Phase IIb trials could be smaller and cheaper than Phase IIIs, they are not necessarily less work than Phase III trials. If anything, Phase IIb trials could require the field to be more selective in the questions it poses, and more thorough in its education efforts to prepare communities for ambiguous results. “We need to think carefully about what we will do depending on the outcome,” says Rida. “We need to think about what

“If you have to double the trial size to get statistical significance, then double the trial size.”

—GARY NABEL

we will do if we fall into a “gray area”—how do we respond to the community; what do we do next?”

Positive findings would also have to be carefully presented, since a candidate that showed moderate levels of protection in a Phase IIb would still have to be tested in a full-scale efficacy trial to confirm the initial data and gather additional information on vaccine effects in a more diverse population. Trial sponsors would have to work with communities and ethical review boards to explain the need for further research on, rather than licensure of, a vaccine candidate that appeared effective.

Phase IIbs will be closely considered at an April meeting on AIDS vaccine trial endpoints in

Washington, D.C. The NIAID-sponsored event will bring together an international group of regulators, scientists and statisticians from the major AIDS vaccine trials networks to discuss different trial designs and their possible outcomes.

For now, proponents of Phase IIb trials say that the benefit of these trials in terms of giving the field some efficacy data on existing candidates far outweigh the risk that this data will be ambiguous and add to confusion in the field. “For the field to continue to debate in a data-free zone is necessarily bad,” says Self. “I would much rather continue the debate in the zone of ambiguous data—that’s where gutsy scientific decisions can be made.” ♦

**Table 1: Trial Size Depends on Primary Hypothesis<sup>^</sup>**

Trial Objective	VEs ≥ 30%	VEs >0%	VEp ≥ 1 log <sub>10</sub> VL
Type of trial	Phase III	Medium phase IIb	Small phase IIb
No. endpoints	250	100	40
Sample size (2% incidence/yr)	12,500	100	2,000
Power for VL	0.5 log <sub>10</sub> VL	0.75 log <sub>10</sub> VL	1.0 log <sub>10</sub> VL
Power to analyze subgroups <sup>+</sup>	Formal for 1, exploratory for 2	Exploratory for 1	None

VEs = percent reduction in susceptibility to HIV infection

VEp = percent reduction in the cumulative risk of progression to AIDS or death following HIV infection. Reduction in VL set point sometimes used as a surrogate number for VEp.

VL = viral load

\* Endpoints = HIV infections

<sup>+</sup> Subgroup analyses could include comparisons of vaccine effects by gender, race or genetic subtype of the infecting virus

<sup>^</sup> adapted from Susan Buchbinder’s plenary address, HIV Vaccine Efficacy Trials: Lessons Learned and Future Directions, at Conference on Retroviruses and Opportunistic Infections, 2004

work well if natural infection leads to the generation of NABs. But HIV infection rarely induces such responses, and even when present they almost never demonstrate broad activity against a diverse range of primary viral isolates. Therefore Burton thinks that the use of simple mimicry as an HIV vaccine strategy is not likely to produce a protective NAB response.

Burton went on to discuss the reasons why HIV infection generally fails to induce NABs. Chief among them is the poor accessibility of conserved regions of the viral envelope, which are protected from antibody recognition by a highly variable and glycosylated gp120 “glycan shield.” Nevertheless, Burton emphasized that several monoclonal antibodies have been isolated that do possess broad neutralizing activity. An unpublished study by Burton’s colleague James Binley, in collaboration with Chris Petropoulos (Virologic Inc.), tested the activity of a panel of these monoclonal antibodies against close to 100 HIV primary isolates (a mixture of molecular clones and quasispecies) from multiple different clades using Virologic’s luciferase-based neutralization assay. The results indicated an antibody known as 4E10 possesses activity against all viruses tested, although Burton described the potency as “moderate.” The next best antibody in terms of breadth of neutralization was 2F5. Burton pointed out that these two monoclonal antibodies both target gp41, a part of the viral envelope that is submerged beneath the glycan shield and only briefly exposed during the process of fusion between HIV and a CD4<sup>+</sup> T cell. The relative conservation of gp41 epitopes that are exposed during fusion makes them an attractive target for antibody-based vaccine strategies, Burton suggested.

He then emphasized the importance of ongoing research in two key areas. Firstly, it will be important to delineate the levels of NABs required to mediate protection against HIV infection *in vivo*, and understand whether NABs and cell-mediated immune responses can work together to enhance protective efficacy. Secondly, the optimal strategy for designing immunogens to induce NAB responses in humans remains to be discovered. Burton listed the four leading approaches that are currently being pursued (see box).

Burton is particularly excited about the third approach, because the broad neutralizing activity of 4E10 and 2F5 demonstrates that it is feasible; it had been feared that the process of viral fusion might be too brief to allow antibody-mediated inhibition. The brevity does seem, however, to generally prevent the generation of fusion-directed NABs in the setting of natural HIV infection. Thus, the challenge for vaccine designers is to present antigens that elicit 4E10 and 2F5-like antibodies in humans, and Burton feels that if this challenge can be overcome “we might be able to attack a real weakness of the virus because the fusion machinery is really very well conserved.”

### **Consensus env Gene Sequences Encode Functional and Immunogenic Proteins**

One attractive strategy for reducing the genetic divergence between HIV vaccines and circulating viruses is to build vaccines that are based on consensus or ancestral viral sequences (see Bette Korber’s presentation covered in *IAVI Report*, Oct-Nov 2001). A key question related to this approach is whether artificially constructed gene sequences can encode functional and structurally intact viral proteins. Several studies presented at the conference addressed this issue. Feng Gao (Duke University, Durham) has constructed an *env* gene (christened CON6) based on the consensus sequence for the entire group M of HIV. Gao confirmed the structural integrity of the encoded Env protein by demonstrating that it binds monoclonal antibodies known to recognize linear, conformational and glycan-dependent epitopes in a broad array of primary HIV isolates. He then inserted the CON6 gene into DNA and recombinant vaccinia virus vaccines for preliminary immunogenicity studies in mice. Compared to *env* genes from clade B and C isolates, CON6 induced T cell responses (primarily CD4<sup>+</sup>) that showed substantially broader cross-clade recognition of HIV epitopes. However, neutralizing antibody responses were poor.

Denise Kothe (University of Alabama at Birmingham) has taken a similar approach utilizing consensus sequences for clade B and C *env* genes (obtained from Bette Korber) as opposed to the entire group M. Kothe first verified that these genes could mediate infectivity if inserted into an *env*-deleted HIV backbone. These “pseudovirions”

#### **Immunogen Design Approaches to Induce NAB**

- Production of recombinant molecules that mimic the mature gp120 trimer on HIV’s surface.
- Production of envelope molecules that may normally express neutralizing epitopes weakly but engineer them to better express these epitopes (by either subtracting elements from the envelope to try and reveal epitopes, or conversely by adding elements to mask regions that induce ineffective responses and thus appear to interfere with the generation of NABs).
- Generation of stable intermediates of the fusion process to expose conserved epitopes to which antibody could gain access during viral entry into a target cell.
- Production of epitope mimics of the broadly neutralizing monoclonal antibodies determined from structural studies of the antibody antigen complexes.

“We might be able to attack a real weakness of the virus because the fusion machinery is really very well conserved.”

—DENNIS BURTON

were also sensitive to neutralization by monoclonal antibodies that have activity against primary HIV isolates. In ongoing work, Kothe is attempting to improve the ability of the consensus proteins to induce effective antibodies by making modifications that may unmask neutralizing epitopes.

#### **CTL Control of SIVmac239 Challenge after DNA/Sendai Virus Immunization**

Tetsuro Matano (University of Tokyo) presented results from a preclinical study that may offer solace to researchers developing CTL-based AIDS vaccines. Matano and colleagues immunized eight non-Indian macaques with a multi-gene DNA construct (at week 0) followed by a booster using a Sendai virus vector encoding the SIV Gag protein (at week 6). Four macaques received sham vaccinations and served as controls. Three months after the boost all animals were challenged intravenously with SIVmac239.

Surprisingly, 5/8 immunized macaques demonstrated robust control of SIVmac239 viremia, and Matano reported that this has persisted out to a year of follow-up. Three of the eight (and all controls) displayed high viral loads that are more typical of SIVmac239 infection. In an attempt to understand the outcome, Matano compared the CTL responses and viral sequences in the five macaques that controlled viremia to the three that did not. The numbers of Gag-specific CTL (as measured by IFN- $\gamma$  production) were not significantly different between the two groups. However, when the genetic sequence of the SIV *gag* gene was analyzed, it transpired that all five controllers had consistent amino acid changes in their virus, suggestive of CTL escape. In three animals known to be descended from the same parent (and therefore presumed to share a class I MHC haplotype), the escape mutation was pinned down to a lysine to serine change at position 216 of the Gag 207-216 epitope. No such changes were seen in the remaining three immunized macaques.

Searching for an explanation, Matano's group constructed a SIVmac239 variant containing the escape mutation and found that it had reduced *in vitro* replicative capacity compared to wild-type virus. Two unrelated macaques were then challenged with a mixture of the wild-type and mutated SIVmac239 and while both variants could be detected one week after challenge, the escape mutation had disappeared a week later, further confirming its replicative disadvantage. Matano's study echoes recently published work by David Watkins (see Research Briefs, page 18) suggesting that CTL escape can, under some circumstances, inflict a severe fitness cost on the virus. In response to a question from Jeff Lifson, Matano noted that the macaques used in this experiment were from Myanmar, and that SIVmac239 may be slightly less virulent in this subspecies compared to the Indian macaques that are typically used by

American researchers. The shared parentage of several of the animals that controlled viremia also suggests that, while promising, these results need to be confirmed in a larger and more genetically diverse group of macaques.

Another presentation that offered an optimistic perspective on the potential benefits of CTL-based vaccines was a poster by Zoé Coutsinos (Institut Cochin, Paris). Coutsinos looked at the long term outcome after challenge with the highly pathogenic SIVmac251, based on the presence or absence of an SIV-specific CTL response. Out of 17 animals that developed CTL in response to immunization with a lipopeptide construct, 16 are free of signs of simian AIDS after two years of follow up. In contrast, out of seven macaques that did not develop CTL responses after vaccination, only one was asymptomatic after two years, a highly statistically significant difference.

#### **Macaque Low Dose Mucosal Challenge Models**

A longstanding concern regarding the use of macaque models in AIDS vaccine research is that animals are challenged (usually intravenously) with doses of virus far higher than is typical in a real-world (typically mucosal) exposure to HIV. High doses are used to ensure that all unvaccinated control animals become infected, since failure to infect controls often prevents a clean analysis of any vaccine effects in the immunized animals. However, this equates to a per-exposure risk of infection of 100%, whereas the per-exposure risk of HIV infection in humans is thought to be in the range of 0.01% to as high as 10% in male-male transmission (*Nat. Rev. Microbiol.* 1:25; 2003).

Two studies presented at the conference—one theoretical and the other experimental—investigated novel study designs based on repeated, low-dose viral challenges. A poster by Roland Regoes and Silvija Staprans (Emory University, Atlanta) asserted that low-dose macaque challenge experiments involving repeated exposures may be a feasible methodology for assessing candidate vaccines, based on the results of a mathematical model. Their results suggested that a vaccine with 80% protective efficacy could be evaluated using as few as 5-7 animals per group with a statistical power of >95%. In contrast, a single-dose challenge experiment of a comparable vaccine would require as many as 20 animals per group to achieve the same statistical power.

Ron Otten (Centers for Disease Control and Prevention, Atlanta) described the development of a low-dose vaginal challenge model in pig-tailed macaques. To assess feasibility, three groups of two animals were given weekly atraumatic vaginal challenges with either 10, 2 or 0.2 TCID<sub>50</sub> (50% tissue culture infectious doses) of the R5-tropic SHIV162p3 virus. Both animals given the highest dose became systemically infected after three exposures, while those given 2 TCID<sub>50</sub> became

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infected after 4-8 exposures. Neither animal that received the lowest dose became infected after twelve exposures.

Otten then utilized the 10 TCID<sub>50</sub> dose to evaluate the potential protective efficacy of the microbicide candidate, cellulose acetate phthalate (CAP). Three of four CAP recipients remained uninfected after a total of 12 exposures, while three controls and one CAP-treated macaque became infected after the first three challenges. Despite the small numbers of animals used in the experiment, Otten was able to obtain a statistically significant *p* value of 0.015 when the rate of infection was compared between the controls and the CAP group. Otten noted that an attractive feature of this model is that the significance of the *p* value increases as a function of the number of exposures given, suggesting that the approach could also be applied to the evaluation of potential vaccine candidates. A study published after the conference by David Watkins also concludes that low dose mucosal challenges can be used to evaluate vaccine efficacy (*J. Virol.* 78: 3140; 2004).

#### **Disconnect Between Interferon- $\gamma$ Production and Cytotoxicity among HIV-specific CD8<sup>+</sup> T Cells**

The daunting complexity of human immunology presents a severe challenge for researchers attempting to delineate the optimal technologies for measuring vaccine- or infection-induced immune responses. The challenge is well-illustrated by the fact that no definitive correlates of protection against HIV infection have yet emerged, and the immunological mechanisms by which rare HIV-infected long term non-progressors (LTNP) control viremia and maintain health remain relatively obscure. Despite these problems, researchers continue to make incremental progress, and a number of studies addressed some of the outstanding questions in this area.

Mathias Lichterfeld (Partners AIDS Research Center, Boston) presented new data on the measurement of HIV-specific CD8<sup>+</sup> T cell responses in infected individuals. The widely used ELISPOT assay quantitates HIV-specific CD8<sup>+</sup> T cells based on their ability to produce the cytokine interferon (IFN)- $\gamma$ , but this approach may not always capture cells capable of exerting cytotoxic activity. Lichterfeld compared results obtained using ELISPOT with a new flow cytometry-based assay that assesses the cytotoxicity of CD8<sup>+</sup> T cells by looking for the induction of caspase-3 substrates (a marker for apoptosis or cell death) in target cells. One advantage of the caspase-3 assay compared to traditional tests of cytotoxic activity is that it does not require any culturing of the CD8<sup>+</sup> T cells in the laboratory. Lichterfeld found that the IFN- $\gamma$  ELISPOT results correlated poorly with cytotoxic activity. However, a superior correlation emerged when the ELISPOT was modified to cap-

ture CD8<sup>+</sup> T cells producing the cytokine TNF- $\alpha$  in addition to IFN- $\gamma$ . Lichterfeld pointed out that these results are consistent with murine models of CD8<sup>+</sup> T cell exhaustion, which have demonstrated that the capacity for IFN- $\gamma$  production can be maintained long after other important functions—such as cytotoxicity and the production of TNF- $\alpha$ —are lost.

IFN- $\gamma$  ELISPOT assays are also commonly used to quantify HIV-specific CD4<sup>+</sup> T cell responses, but two studies also raised concerns as to whether this approach is capturing a fully functional cell population. Alexandre Harari (Laboratory of AIDS Immunopathogenesis, Lausanne) showed that in controlled cytomegalovirus (CMV) infection, virus-specific CD4<sup>+</sup> T cells comprise a relatively balanced mix of populations secreting the cytokine IL-2 alone, IL-2 plus IFN- $\gamma$ , or IFN- $\gamma$  alone. HIV-specific CD4<sup>+</sup> T cells in LTNPs displayed a similar cytokine profile. In stark contrast, HIV-specific CD4<sup>+</sup> T cells in progressive infection were skewed toward a population only capable of making IFN- $\gamma$ . The quantity of HIV-specific CD4<sup>+</sup> T cells as measured based on IFN- $\gamma$  production alone was not correlated with control of viral load, but there was an inverse correlation between the numbers of IL-2 or IL-2 plus IFN- $\gamma$  producing cells and the level of HIV viremia. A poster from Harriet Robinson's group at Emory University described a similar finding in HIV-infected children.

Stephen De Rosa (Vaccine Research Center, Bethesda) investigated the cytokine profiles of CD8<sup>+</sup> T cell responses to DNA vaccines encoding HIV *gag*, *pol* and *env* in HIV-negative volunteers and compared them to those seen in infected individuals. While vaccination induced T cells that produced a somewhat similar mix of cytokines as those seen by Alexandre Harari in CMV infection and LTNPs (with some cells producing either IL-2 or IFN- $\gamma$  alone and others producing both), the proportion of cells producing the various cytokines differed in infected individuals, with a tendency to skew toward IFN- $\gamma$  production. These data suggest that quantifying HIV-specific T cells based on IFN- $\gamma$  alone may not reveal the full spectrum of immune responses induced by vaccination, and that immunization might induce T cells with broader functional capabilities than those that typically arise in the setting of natural infection (although De Rosa acknowledged that the relationship between these various cell populations and the ability of a vaccine to protect against or control HIV infection remains unknown).

#### **Beyond Help: Direct Effector Functions of HIV-1-Specific CD4<sup>+</sup> T Cells**

The role of CD4<sup>+</sup> T cells in the immune response is generally considered to involve the provision of help to CD8<sup>+</sup> T cell and B cells. In the absence of such help, CD8<sup>+</sup> T cell and B cell



responses generally function poorly or not at all. Occasionally, however, it appears that CD4<sup>+</sup> T cells can exert direct cytotoxic activity themselves. Phillip Norris (Partners AIDS Research Center, Boston) has been investigating this phenomenon in the setting of HIV infection and in a poster documented cytotoxic activity by HIV-specific CD4<sup>+</sup> T cells in four out of ten individuals studied. There has been controversy as to whether such activity may emerge as a result of long term culture in the laboratory, but in one individual the cells could be identified directly *ex vivo*. This particular study participant has maintained a viral load of <50 copies for over 20 years without treatment, and his HIV-specific CD4<sup>+</sup> T cells could mediate >3 log<sub>10</sub> suppression of HIV replication *in vitro*. A poster by John Zaunders also reported the identification of a cytotoxic HIV-specific CD4<sup>+</sup> T cell population in an infected LTNP and identified a similar CMV-specific population in healthy CMV-positive adults. It appears that these cells may represent an underappreciated component of antiviral immunity in humans.

**VaxGen Dénouement: No Efficacy in Racial Subgroups, No Efficacy in Thai Trial**

Dean Follman (National Institutes of Health, Bethesda) addressed the one lingering question regarding VAX 004, the recently completed efficacy trial of VaxGen's recombinant gp120 AIDSVAX vaccine candidate. The question relates to VaxGen's controversial claim that AIDSVAX showed statistically significant protective efficacy among black study participants and a combination of racial subgroups that the company characterized as "Hispanics, Blacks, Asians and Others." Follman led a committee appointed to investigate this claim, made up of representatives from the

CDC, the SCHARP Statistical Center at the University of Washington, and VaxGen.

One of the basic tenets of statistical analyses is that conducting multiple comparisons of different subgroups in a trial greatly increases the risk of finding an erroneously significant result. There are several standard statistical tools that can be applied in order to reduce the risk of error, but when VaxGen first presented their trial results, these tools had not been employed (although the company initially claimed otherwise). After reanalyzing the data, Follman's team found that a significant result could be obtained by chance about 22–24% of the time when data from 15 subgroups were evaluated, leading them to conclude that the unadjusted subgroup data originally presented by VaxGen were spurious.

In the same conference session, Punnee Pitisutithum (Mahidol University, Bangkok) gave the first public presentation of results from the second AIDSVAX efficacy trial, which recruited 2,546 Thai intravenous drug users at high risk for HIV infection. As reported last November in a press release from VaxGen, the vaccine again failed to show efficacy. Pitisutithum fleshed out the details, showing that 106/1161 vaccine recipients became infected vs. 105/1155 that received placebo. Pitisutithum also stated that there were no significant differences in viral load, CD4<sup>+</sup> T cell counts or time to initiation of antiretroviral therapy (ART) between vaccinees and placebo recipients. Taken together, Pitisutithum's and Follman's data sets should finally lay to rest any outstanding questions regarding the efficacy of AIDSVAX. ♦

*Richard Jefferys is Basic Science Project Director at the Treatment Action Group, a New York-based organization advocating for HIV research.*

“Taken together, [these] data sets should finally lay to rest any outstanding questions regarding the efficacy of AIDSVAX.”

# Addressing AIDS in China

## AN INTERVIEW WITH

David Ho, MD, has been at the forefront of HIV research for 20 years and is now one of the most instantly recognizable names in the field. He is the founding Scientific Director and Chief Executive Officer of the Aaron Diamond AIDS Research Center, as well as the Irene Diamond Professor at The Rockefeller University, both in New York City. Ho's research has given an appreciation of the dynamic nature of HIV infection in different reservoirs in infected persons, an understanding that led him and his collaborators to champion combination antiretroviral therapy, including the use of protease inhibitors, an approach that has been invaluable to prolonging the life of infected persons who have access to these drugs. Ho has received numer-

ous awards from the international scientific community and, to give some indication as to the breadth of his influence, was named Time Magazine's Man of the Year in 1996 and awarded the Presidential Medal in 2001. In recent years he has increasingly been involved in HIV/AIDS in China and at the end of last year launched the Chinese AIDS Initiative to promote advocacy, prevention, education, and treatment of HIV/AIDS there. At the same time he began an AIDS vaccine trial in the US to test ADVAX, a DNA vaccine based on subtype C virus developed by his research team. Ho recently spoke to IAVI Report editor Simon Noble about that trial, the Chinese AIDS Initiative and the current awareness of the burgeoning epidemic in China.

## David Ho



In addition to your long and distinguished track record in HIV research, you've recently entered the field of SARS research. Do you think that the SARS epidemic in China and surrounding countries has improved the public health programs in these states, their accountability and transparency?

The SARS outbreak has had a dramatic impact on China because it shook the whole country up in early 2003. I think we all would agree that China did not handle the early part of the epidemic very well. But starting from April, China reversed its course, became much more transparent in handling the outbreak and, I think, to everyone's amazement brought the epidemic under control, even in rural areas, by July. Their effort in the latter part, certainly, was very commendable. I had the occasion of visiting China and Hong Kong multiple times throughout the period and I was impressed. They not only took action for SARS but they also committed lots of funds. In speaking to academic leaders and leaders within the Ministry of Health, it was clear to me that SARS was a wake-up call, and it was also clear to me that it was a great opportunity to have the Chinese government focus on HIV/AIDS, which in reality is a much, much larger problem.

I think now the Chinese leaders realize how important public health and proper health care infrastructure are to their future prosperity, so I have seen nothing but positive changes in terms of the attitude of the Chinese leadership toward AIDS as a consequence of SARS.

Do you think this wake-up call has filtered or will filter through to HIV/AIDS surveillance and

recognition of the problem?

I think at the top level, that is, at the State Council with the Vice Premier and at the level of the Ministry of Health and its leadership, it most definitely has. We've also been doing our advocacy work and it's probably a combination of many events, including SARS, that has improved the political will to do something about HIV/AIDS in China. Certainly, we saw the symbolic gesture that the Chinese Premier made on World AIDS Day by visiting HIV patients.

But even more important than that, Vice Premier Wu Yi spent several days visiting villages with severe AIDS problems in the central province of Henan and this was done quietly, under the radar screen. I just came back from there recently, and it is clear that the government is restructuring their AIDS effort so that it reports to the State Council, elevating the status of this office. So I see a lot of changes but, of course, China is a huge country and a lot of the high-level commitment will take some time to trickle through the system.

Do you think there's a change of attitude towards AIDS in China within the general populace, that it's not seen just as a problem in marginalized communities?

There has been some improvement, although there's a lot more that has to be done. I think the leadership is aware of the situation and will be making the right decisions. But unfortunately, if you speak about the Chinese population in general, I think most do not know anything about HIV/AIDS; most do not care. Most consider it a foreign disease or a disease that affects 'strange' populations. So there's now a disconnect between what the leadership knows and would like to do, and the general population. And I think that will require tremendous educational and outreach effort.

***You mentioned your advocacy work, were you referring to your own Chinese AIDS Initiative?***

Yes, we've been organizing a consortium to help China address its HIV/AIDS problem because we wanted to approach the problem in a very comprehensive manner, with prevention at the center, including testing. Of course, this is prevention outside the vaccine research agenda. But we have also realized that there are multiple other components that will impact on this primary agenda of prevention; that is, we have to deliver care and services and, importantly, antiretroviral (ARV) therapy to help bring people into the prevention effort. The ARV component we have been doing together with the Clinton Foundation.

As for advocacy, it's actually broader than that—it's advocacy, outreach and education and also involves issues related to legal reform and policy changes. We've been working with the schools of journalism, law and public policy at Tsinghua University in Beijing, the leading educational institution in China. We have a series of things in mind including training of the Chinese political leadership and media with courses done at Tsinghua.

We're going to make documentaries and public service announcements and at the end of last year, November 10th, we organized a high-profile conference that involved former President Bill Clinton. It was a very successful event that immediately preceded all these positive changes with the Chinese leadership. AIDS was prominent on the agenda for the Chinese for weeks, so I suspect it all had a pretty positive influence.

***How are AIDS vaccine trials viewed in China, and are they something that your initiative and other Chinese groups are gearing up towards?***

The Chinese government has always emphasized science and technology, so they welcome taking vaccines to China for testing. There are internal efforts underway by various Chinese investigators, particularly in collaboration with European scientists.

We've been speaking to the Ministry of Health and the State Drug Administration for some time now. They know that our two vaccine candidates are derived from a strain from southwest China, the Yunnan province, and that we have taken them through laboratory testing, some animal testing and now one is approved [for investigational use] by the FDA and another one will be submitted soon.

***Which vaccine candidates are those?***

Our first vaccine consists of two DNA vaccines mixed together, which will express five HIV proteins; Gag, Pol, Env, Nef and Tat [called ADVAX]. We've done the *in vitro* evaluation, the immunogenicity studies in animals, and this was taken to the FDA last year and approved [for study in humans]. We launched the clinical trial here in New York late last June, and we've just finished enrolling two dose schedule arms, and we have a third dose coming up, probably in May.

So that vaccine is well on the way. In terms of the regulatory aspect, we've been speaking to the Chinese State Drug Administration and working with a vaccine production facility in China to think about the next step in production there. We need to finish the Phase I here, then we will take this initial product to China.

The second product is a modified vaccinia Ankara (MVA), which contains the same five genes from the same subtype C strain of HIV from southwestern China. For this one, we've done the laboratory evaluation and animal testing, and we have a GMP-grade product that's now in preclinical testing. We're also doing some QA/QC (Quality Assurance/Quality Control) studies and we plan to submit it soon to the FDA. We believe this is one of the best vaccinia vaccines out there—it's stable, it expresses well, it's easy to grow, easy to produce to high titers. So, fortunately, we haven't had the technical problems that have plagued others in MVA development. It's gone well and we're anxious to get it into the clinic. And once again, we'll be testing in New York.

The work on both of these vaccines was made possible by support from IAVI, which has been absolutely vital to our vaccine effort. We had some internal funds that got the project off to a good start but IAVI stepped in fairly early and supported us for the past three years, and that's been great, to be able to take it from the bench to the clinic in that short period of time. But it isn't just financial support. Especially in the past year, IAVI also provided clinical research expertise that complemented our basic scientists here, particularly with respect to development issues and regulatory concerns. If it weren't for that, we'd be struggling. So it's been a very successful partnership and we certainly appreciate that.

***What's your opinion of the current state of Chinese research in general and HIV research in particular? Is there a new funding commitment from the government?***

In general, China has put in a lot of money to support biomedical research, and they have created many new institutes and many positions within the leading universities. They've made a very impressive financial commitment and they're beginning to attract some of the very best people to return.

More than 90% of the people who came to the US [from China] to study, stayed in the US. Now we're seeing the return of some of the very best because living conditions are better, at least in the major urban areas, and they're also making offers to some of the very best that are difficult to refuse. When I visit the various institutes and science parks in Beijing and Shanghai, it becomes very clear what China is trying to do to draw them back.

Now, in the HIV/AIDS area, I don't quite see that yet. Although there is a deeper commitment on the part of the government, I don't think it has trickled through the system to be relevant for AIDS in

China right now. So there isn't a lot of basic HIV research in China, in fact there's almost none. But people are beginning to be more interested in more practical issues in treatment, vaccine development, prevention work and the clinical and epidemiological front.

***How reliant is Chinese science on training in the US and Europe? And how is that now being affected by the recently imposed tougher visa restrictions?***

The short answer is it's impacted a great deal on training in the US and Europe. Since the late '70s, when Deng Xiao Ping and Jimmy Carter opened up the new relationship, there has been a massive outpouring of talent, to the US primarily but to Europe as well. Hundreds of thousands of some of China's very best have been trained.

I think it's really a shame that the post 9/11 changes in visa requirements and immigration laws are keeping out very talented scientists who could come and contribute to—not just learn from, but contribute to—American science. I've had a number of potential post-docs from China, some that I painstakingly selected, only to have them turned away at the visa office in Beijing or some other city in China.

***What is the current state and the potential future use of vaccine manufacturing facilities in China?***

Actually, unlike other developing countries, China has got a tremendous set-up for making vaccines or recombinant proteins. It has at least a dozen such facilities. The one we've been dealing with in Kunming City in Yunnan Province, for example, was built by the Dutch and the GMP conditions would match those in Europe. It makes all of China's polio and hepatitis A vaccine. I know the IAVI experts went there not so long ago and were impressed with this particular site. So China really has a fair amount of capability in that area.

***What about manufacturing facilities for ARVs? Is making their own generic ARVs an important pursuit of the government and public health bodies?***

Yes, there are some pharmaceutical companies involved that are government- owned or government-backed. The Northeast Pharmaceutical Group, for example, has made several generic drugs. And now the private sector is trying to do more. There is a company in Shanghai called Desano [Bio-pharmaceutical Co.] that has made four generic drugs—AZT, ddI, D4T and nevirapine. These are now approved in China and they're fairly cheap. I think some studies remain to be done to demonstrate bioequivalence to the western drugs, but at least part of the therapeutic arsenal is being synthesized in China.

We also have to keep in mind that China has for some years now had an active, not pharmaceutical, but a chemistry effort to make the basic com-

ponents that are the precursors for ARV drugs. We hear so much about Cipla in India or the Brazilian effort, but we have to keep in mind that these places get their basic components from China, they have played a role through that particular function. Now they are trying to expand the process and go on to make the actual drugs. I think it will happen, I'm hearing more and more about other, mostly private companies trying to join the effort.

However, to date China has been respectful of World Trade Organization regulations with respect to intellectual property. So Desano and the Northeast Group have just made generics or drugs that are off patents or not covered by patents, they have not gone on to make 3TC, for example, which is patent-protected in China.

***What do you think are the most important goals for China in terms of its AIDS epidemic in the next five years?***

The most important goal is to make sure the projected numbers of people infected with HIV do not come true. By 2010, the World Health Organization and UNAIDS have suggested that China will have 10-20 million infected, going from approximately one million today. That's a frightening prediction. So the most important goal is to make sure that doesn't happen, and for our own Chinese AIDS Initiative, we want to reduce it by several-fold.

But to achieve that goal, I think the most important thing is to get the information out to the public. You know, here, really, more than anywhere else, knowledge is power and we have to educate the populace about HIV/AIDS and about prevention.

***To switch gears a bit and go on to more scientific issues, what do you consider are currently the most pressing scientific challenges for HIV research?***

I would say development of a protective vaccine is the highest priority in AIDS research. That's mine, and I think many would agree with me.

Aside from a vaccine, I think that developing better drugs is still an important priority. It's clear that combination therapy has been great for American and European patients, but the drugs still need to be improved; there's development of resistance in some patients, and these people need other options. We need drugs that have fewer side effects and are easier to take. So there should be continued incremental development in the therapy side.

The greatest challenge on the therapy side is whether a cure could be obtained or not. There's always a lot of debate on that. I think we all know that it will be exceedingly difficult, given the fact that the virus is now known to be hiding in a subset of CD4<sup>+</sup> T cells. How to purge the virus from that subset will be extremely difficult, but probably not impossible, and so [research] groups have to continue to try. But right now, I can tell you, we

can't think of too many things to do along that front. If we could think of something that was reasonable, we would be jumping on it and pursuing it.

Also, there's still a lot of molecular biology that needs to be defined. Recently, we've learned quite a bit about innate cellular factors that restrict HIV replication, and these things are wonderful, such as the APOBEC3G story or the TRIM5 $\alpha$  story that came out only a few weeks ago. These things are wonderfully revealing about the basic biology of the virus and about how the host fights various pathogens.

But that said, the most important thing is to make sure we develop a vaccine to curtail the further spread of this epidemic. The vaccines that are fairly advanced in development have failed, and now we're looking at vaccines mostly at early stages of development. We could optimistically think that these might offer partial protection, as we're seeing in monkeys with some of the vaccine candidates. Will they be the home run that everyone is looking for? I wouldn't be overly optimistic about that. So I think the next three to five years will be most interesting to see this whole series of DNA or viral vector vaccines advance to efficacy trials.

***Do you think there are any basic immunological questions that should be addressed immediately, that are currently being ignored? We still haven't defined the correlates of protection.***

Well, you answered the question yourself. We are able to protect using, say, live attenuated virus vaccine, but in that system the correlates were never worked out, despite some attempts. But we measure what we know how to measure, and it would seem to some of us who have been in this field for some time that maybe the correlate is in something that we haven't measured well up till now, like innate immune responses, or we haven't been measuring the cellular responses in the proper way with the proper assays.

***The standardization of assays is all focusing on ELISPOTs and interferon (IFN)- $\gamma$ , but it's still not known if that's a good surrogate marker for anything functional in protection. Do you think that's an important issue that needs to be sorted out?***

I think we need to sort all these things out. We tend to gravitate toward things that are quantitative and easy to do. In general there's a rough correlation between those ELISPOTs and, say, protection results in monkeys, so that's somewhat reassuring. But we don't know whether ELISPOT, the way we measure it using IFN- $\gamma$ , is the right parameter. Maybe IFN- $\gamma$  plus another cytokine—IFN- $\gamma$  and Tumor Necrosis Factor (TNF)- $\alpha$ , or IFN- $\gamma$  and interleukin-2 or -12, whatever—would give a better correlation with the killing ability of these CD8<sup>+</sup> T cells. What do we need to actually measure? Maybe it's that killing ability. Until it is well defined, we tend to just do whatever is easy to do. But we are just taking a snapshot when the real situation is more dynamic

and one needs to integrate it over time.

***You mentioned innate immunity, is it being realized now that perhaps we haven't looked closely enough at the innate immune response in the context of HIV infection?***

Yes, from the molecular field we now realize that there are molecules like APOBEC3G that reside inside the cell and have a way of mutating and degrading viral DNA so that the pathogen cannot survive. We've known about IFN- $\alpha$  and IFN- $\gamma$  in other viral systems such as hepatitis infection. They play a very important role, seemingly, in protection.

We've known since 1996 about the  $\beta$  chemokines, and these are probably, in a non-discriminatory way, shutting down HIV that uses particular receptors. And I suspect there are more that could play a very important role. Now, we may not be able to elicit those responses using the old vaccine strategies, because all of those are directed toward acquired immunity. So to induce the proper innate responses would be very difficult, but I have no doubt that the innate immune system is contributing to the control of HIV in every infected person.

***Do you see these new targets, like APOBEC3G and TRIM5 $\alpha$ , as being amenable to manipulation?***

Yes, I think some of these are amenable for development of drugs, small molecule inhibitors. We know about Vif and APOBEC3G; could that be exploited for drug development? We know about TSG 101 and Gag-p6 for budding. So there are new targets for therapeutic development. So I'm hopeful, and I know there are people who are concentrating their drug development on these new targets.

***How's the ADARC trial going with ADVAX? You said earlier you've almost completed enrollment.***

It's going quite well. There are three dose groups, fifteen subjects per group, being done at two sites; here at Rockefeller University and at the University of Rochester in upstate New York. Within each blinded group there are 12 volunteers who receive vaccine and three who receive placebo. The first dose group began late last year. All of them have gotten their third shot already. They're doing fine, no adverse events, that's why we are allowed to move the dose group. Dose two has been enrolled, and that's going well.

In terms of immunogenicity, those samples are just being shipped to IAVI's core lab for testing. A few weeks from now, we may have some read out from the early periods, from dose one.

The concern was whether we would fully enroll, but it's going very well. We got over 400 phone calls from people volunteering to participate in our trial when we only had 49 spots. So it bodes well for not only the third group that we have to enroll, but also the next [MVA] study. We think we could go back to the same pool of volunteers.

“  
...the most important thing is to make sure we develop a vaccine to curtail the further spread of this epidemic.”

—DAVID HO

continued on 19 ►

# Breaking the Bottleneck

## AIDS vaccine researchers and developers address the short supply of manufacturing and process development capacity

BY SHERI FINK

There is a growing consensus within the AIDS vaccine research community that its various members need to work together to confront one of the major challenges to AIDS vaccine development—the acute shortage of vaccine manufacturing and process development capacity. For a candidate vaccine to move from the laboratory into clinical trials, and ultimately into commercial production, a process must be developed for its safe, reliable, well-characterized, cost-effective and large-scale manufacture.

After proposing a global AIDS Vaccine Enterprise (*Science* 300: 2036; 2003), leading vaccine scientists met last summer to begin establishing a strategic plan to accelerate AIDS vaccine development and six working groups were established to examine “needs, gaps, opportunities, and potential initiatives.” Two of these Vaccine Enterprise groups met jointly this February in Bethesda, MD, bringing together twenty world experts on vaccine manufacturing and process development from both the private and public sectors. The two working groups were the manufacturing group chaired by Jerry Sadoff (CEO, Aeras Global TB Vaccine Foundation) and R. Gordon Douglas, Jr. (freelance consultant on vaccines, infectious diseases and global health), and the product development group chaired by Gary Nabel (Director, Vaccine Research Center, U.S. National Institutes of Health) and Emilio Emini, (former Senior Vice President of Vaccine Research, Merck and Co., now with the International AIDS Vaccine Initiative). Their remit is to address the scarcity of AIDS vaccine manufacturing and process development capacity outside of large pharmaceutical corporations, shortfalls that are being blamed for causing delays in clinical trials. Although it will be some time before an effective AIDS vaccine is found, the experts agreed that planning for that day must begin now.

For vaccines, it is often said that the process is the product. Unlike most pharmaceuticals, vaccines are biological products created by manipulating complex systems such as mammalian cell cultures, embryonated chicken eggs or bacterial cultures. The vaccine manufacturing process is therefore subject to the fragility of these biological processes.

A failure to invest in process development may result in a vaccine that is not optimized for stability or yield, which could well affect cost-effectiveness, all critical factors for a product whose greatest demand will come from the

developing nations. This effort requires time, human capital, dedicated manufacturing facilities and, of course, funding. All are in short supply. The difficulty is that the vaccine field, which is already stretching finite resources for basic research and clinical trials, must allocate funds for the production of vaccines that do not yet exist.

There are two related problems. One is a shortage of manufacturing capacity sufficient to provide the quantity of vaccine needed for Phase III clinical trials. The second is a shortage in large-scale manufacturing capacity and space and expertise for process development that will enable scale-up to maximum production once an efficacious vaccine is licensed. As a rough approximation, the process needs to leap from the 200 ml laboratory flask used to produce the

number of years required to bring a vaccine candidate through a Phase III clinical trial. “That means you need to make your major investment at the time you’re starting your Phase III trial,” says Sadoff. “That’s a problem because you don’t know if [your product] is going to work.” The demand (as distinct from the need) for the vaccine is another unknown that will depend on factors such as efficacy, duration of protection, immunization schedule, safety, cost, and stability, the latter two of which are, in turn, influenced by the degree of early investment in process development. Adding to the risk is the question of who will pay for the vaccine in the many low-income countries where it will be needed most.

On the other hand, with more than two dozen vaccine candidates currently in Phase I

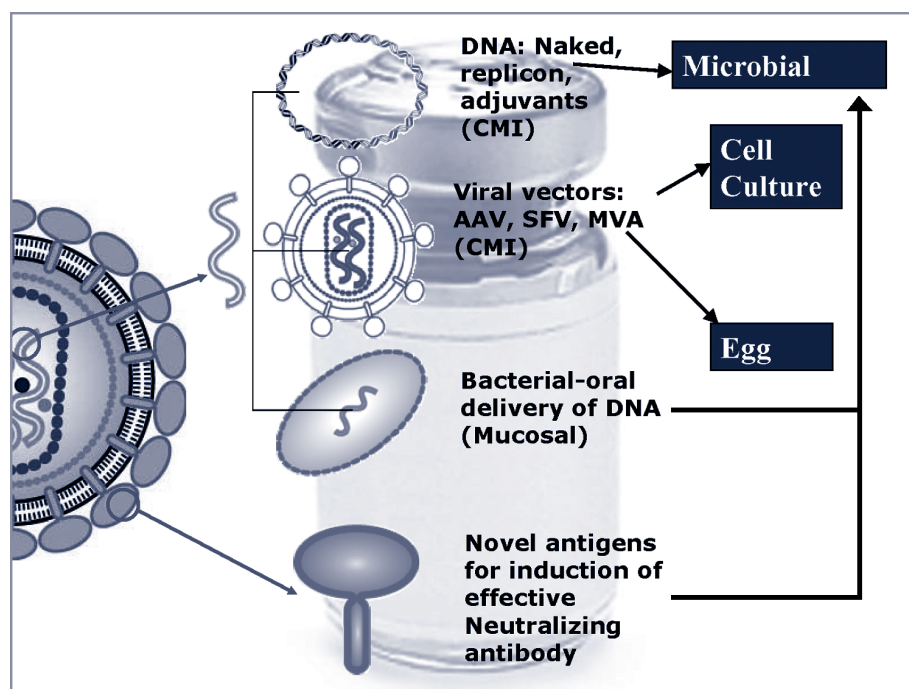


Figure 1. The three types of manufacturing technologies used to produce different AIDS vaccine platforms.

initial vaccine to the several hundred or thousand liter bioreactors needed for clinical trials, and then to the 10,000 to 50,000 liter reactors required to make hundreds of millions of doses of vaccine during full-scale manufacturing of an approved product.

The field has reached a critical moment because of timing. The five or more years required to design, build and license a large-scale manufacturing facility approximates the

or II trials, a failure to make immediate, significant investments in process development and manufacturing capacity will result in delays in bringing a vaccine to market should any of these candidates be found efficacious. When it comes to HIV, those delays will be measured in lives. “Anything you can do to minimize time rather than resources and risk is key. You’re talking about millions of lives” says Sadoff.

### *The state of the field*

Research scientists developing AIDS vaccine candidates outside of major pharmaceutical companies frequently rely on contract manufacturing companies to provide clinical-grade materials for early clinical trials. Because the quantity of vaccine required for a Phase I safety trial is small, little if any manufacturing scale-up is necessary, and the process used by the contract manufacturer is similar to the process used to make the candidate in the original research laboratory.

Even so, scheduling outside contractors for a Phase I trial is often difficult. "One great problem is these contract manufacturers are constantly booked; you have to pay a reservation charge and book your slot [well in advance]," says Eddy Sayeed, Vaccine Production Manager for IAVI. "If one is not able to achieve the timelines, you lose your slot and have to pay a certain amount for not being able to fulfill the requirement."

As much of a problem as these logistical issues pose in early phase clinical trials, vaccine developers face an even tougher question: What to do once they reach Phase III trials and ultimately the need for full-scale production? Outside of the large pharmaceutical companies, there are no pilot, large-scale manufacturing plants for Phase III production capacity of viral vectors, which, along with DNA and protein-based vaccines, are the three major types of current vaccine candidates (see Figure 1). Such plants and the expert workers that go with them are needed not only to manufacture vaccine for trials, but, perhaps even more importantly, to develop an optimized process for large-scale production.

"The process development issue is critical," says Emini. "Process development requires intellectual and physical investment which is housed at the moment almost exclusively within the large biopharmaceutical companies."

Nabel agrees. "If you don't have a manufacturable process and if you don't have vaccine candidates that can really be manufactured to the specifications and the scale that you need, then you don't have a vaccine."

The shortage of process development and manufacturing capacity has led to a bottleneck and, some argue, a slowing of the entire vaccine development pipeline. "People are discouraged from making products if they're worried there isn't a capacity to do trials," said Sadoff.

One AIDS vaccine candidate has progressed through all clinical trial phases, VaxGen's AIDSVAX, a recombinant gp120 protein vaccine. While the mammalian cell culture manufacturing process used to produce recombinant proteins was fairly well characterized, and the process for gp120 had been developed by Genentech and licensed exclusively to the company, VaxGen still had to respond to the potential need for commercial manufacturing capacity. "We were faced with the fact that there was not anywhere, even projected in the future, capacity to produce recombinant proteins even on a moderate scale," says Don Francis, founder of VaxGen.

In February, 2002, during Phase III clinical trials of AIDSVAX, VaxGen and a group of South Korean partners invested US\$113 million in building a large-scale biopharmaceuticals manufacturing facility in Incheon, South Korea, with an initial 50,000 liter bioreactor capacity, expandable to 150,000-200,000 liters. The joint venture also funded the construction of a smaller facility in San Francisco, US, to be used to validate the AIDSVAX manufacturing process. The two facilities were expected to produce 200 million doses of AIDSVAX per year with the completion and licensure of the South Korean facility in 2005 or 2006. Experts outside of VaxGen have complimented the company's approach to developing large-scale manufacturing capacity for its product, which ultimately failed to protect against HIV infection. VaxGen is now recouping some of its investment by putting its US manufacturing capacity to work to produce other recombinant protein products, including anthrax vaccine. However, the unfinished South Korean facility has yet to find a new use, demonstrating that early investment in manufacturing capacity does indeed carry risk. "It's not that the demand is not there," says Francis, who believes the facility will eventually be put to good use once others are convinced that high quality vaccines can be produced in places such as Asia. "It's the first recombinant [protein] manufacturing facility outside the US or Europe."

### *The search for capacity*

AIDS vaccine researchers are working to define the requirements of the field and the capacity that exists worldwide for developing and manufacturing various types of AIDS vaccines, including DNA, viral vector and protein-based vaccines. Early expectations were that major pharmaceutical companies in the industrialized world, which have the expertise and the facilities to undertake process development and large-scale vaccine manufacturing, would take the lead in producing AIDS vaccines. However, it has become clear that these companies do not have a financial interest in tackling the project by themselves. "Companies are staying away from making an HIV vaccine because nobody knows how to do it," says Douglas, "the science isn't there." Even if an efficacious candidate emerges, AIDS vaccine manufacturing may ultimately be complicated by concerns over product liability, intellectual property, and pressure to make early technology transfers into the developing world.

Still, research and development experts from the large pharmaceutical companies of the industrialized nations are active participants in the current discussion about AIDS vaccine production. Companies may end up contributing in very valuable ways, such as offering training in process development and manufacturing, or making their validated cell lines and egg banks available to AIDS vaccine developers.

Emini envisions that large biopharmaceutical companies might have an interest in manufacturing an AIDS vaccine once it has been proven effective. This would still leave the need for early process development unmet. "Process development has to be done up

“  
People are discouraged from making products if they're worried there isn't a capacity to do trials.”

—JERRY SADOFF

“  
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tion worked out—  
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big, you raise the  
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on the vaccine  
...too little, it's  
going to be a  
crisis.”

—R. GORDON DOUGLAS, JR.

front,” he says, “that’s a key gap.”

While large pharmaceutical companies may be willing to manufacture an AIDS vaccine for developed country markets, where profitability or at least return on investment is possible, producing for developing countries is a different question altogether. Yvette Madrid, a freelance vaccine policy consultant, has studied the issue: “For a commercial firm, there’s almost no incentive to build a huge amount of capacity for the developing countries, because that increases their risk; there’s the possibility that the supply won’t be used, or the demand won’t materialize.”

These problems could be mitigated if a donor stepped forward with a large, early, and firm commitment to buy an AIDS vaccine at full cost and provide it affordably in the developing world. While a lack of funding to cover many other needs in AIDS vaccine development may make this possibility unlikely, technology could realistically be transferred to lower-cost manufacturers. “One of the solutions,” says Douglas, “is to have [the vaccine] manufactured in some of the middle-income countries like Brazil or India, where it can be manufactured at lower cost because labor costs are lower.” The manufacturing facility for AIDS VAX in South Korea is a case in point.

#### ***Building a multi-product clinical trials manufacturing facility***

A multi-product clinical materials plant capable of producing 10 or 20 products simultaneously would open the AIDS vaccine development pipeline. If excess clinical materials manufacturing capacity existed, the attitude to testing new vaccine candidates in humans could change, encouraging both a broadening of the search for novel candidates and the testing of finer variations of promising vaccine platforms that differed only in their immunogens.

Various models are currently under consideration. These range from a facility dedicated solely to the production of clinical materials to one that would include full process development capability and the capacity, with additional investment, to be scaled up to commercial 10,000 liter vaccine production. Cost estimates for the different facilities vary, from \$50 million to \$200 million.

One useful model is the \$65 million pilot plant being built in Frederick, Maryland by the Vaccine Research Center of the National Institutes of Health to produce AIDS and biodefense-related vaccines. The plant, a year away from completion, will contain four manufacturing suites of various capacities (two 100 liter, a 400 liter and a 2,000 liter) that will be capable of meeting current Phase III needs. “It’s a nice design,” says Nabel, “it’s flexible and it allows for different scale production.” While this plant will be occupied for the foreseeable future with NIH products, the plans for the facility are being shared openly with the Vaccine Enterprise.

#### ***Manufacturing plans***

The future of full-scale vaccine manufacturing is also being considered. “The object of this exercise is

to identify the end point, a manufacturing facility,” says Don Gerson, Managing Director of Manufacturing at IAVI. “How big is it going to be, what does it have to make, if we’re really going to supply the entire developing world with an AIDS vaccine?” For construction to commence before clinical trials have shown which type of vaccine will be needed, a flexible facility could be built that would allow, with slight modification, for the use of any of the three major types of production processes—egg-based, microbial-based or cell culture-based. “It’s very easy to design for all three possibilities at once,” says Gerson. “If you design just for one, and you go back and say ‘it should have been eggs instead of cell culture,’ you have to start all over again.” Alternatively, Phase IIb clinical trials could be performed prior to construction, providing proof of concept; the merits of Phase IIb trials are currently being debated (see “IIb or not IIb,” page 1).

While the need for a high-efficacy AIDS vaccine in the industrialized world would probably fall under 100 million full immunization courses, the goal of covering estimated world needs for such a vaccine could possibly take peak requirements close to 700 million immunization courses (*Vaccine* 21: 2032; 2003). This could require the work of four to five regional manufacturing facilities, each possibly costing as much as \$300 million to build.

Currently, vaccine manufacturing capacity in general is finite and already causing shortages of licensed vaccines for other diseases. “The only way to make more [vaccine] is to build another plant, and that’s going to take five years,” says Douglas. “If you don’t have the demand situation worked out—not the need, but actual demand—you’re not going to know what size to build for. If you build too big, you raise the cost prohibitively on the vaccine; if you build too little, it’s going to be a crisis. We have to get into these issues now.”

#### ***The future***

“The challenge for the world is to think about how to get to large-scale manufacturing as soon as possible,” says IAVI President Seth Berkley. Across the AIDS vaccine field, the importance of addressing shortages in process development and manufacturing capacity has now been acknowledged. “Researchers in the field recognize the need for coordination, and this is the time to do it,” says Emini. “These are problems that go beyond what any single individual can do, what any single organization can do.” ♦

*Sheri Fink, MD, PhD is a freelance writer whose work has appeared in such publications as the New York Times and Discover Magazine, and the author of War Hospital: A True Story of Surgery and Survival.*



# IAVI Report Online

THE NEWSLETTER ON INTERNATIONAL AIDS VACCINE RESEARCH

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## www.iavireport.org

■ *IAVI Report* is very pleased to announce the launch of its new website. IAVI Report Online is a centralized source of information on all aspects of AIDS vaccine research and associated scientific disciplines—from basic science like molecular virology and immunology to more applied fields such as HIV prevention research.

● Updated daily with highlights from the day's HIV/AIDS news from around the world, plus a round-up of the latest published research relevant to AIDS vaccine development, IAVI Report Online is a one-stop resource for HIV researchers, advocates, policy makers, and anyone else with an interest in the progress towards an effective, preventive AIDS vaccine.

■ IAVI Report Online is home to all of the current and archived articles from the print editions of *IAVI Report* and also *VAX*, a monthly non-technical bulletin available in 5 different language versions—English, French, German, Portuguese and Spanish. IAVI Report Online incorporates a new Early Edition feature that will publish *IAVI Report* articles directly to the web as soon as they are available, ahead of print publication.

● Visitors to the website will be able to subscribe to any of the IAVI Report products in a variety of electronic and print formats, all free of charge.

■ Roberto Fernandez-Larsson, PhD, Web Editor of the IAVI Report Online, is a virologist by training and comes from *AIDScience* website where, as Senior Editor, he developed and headed the *Science* magazine-sponsored AIDS prevention and vaccine research site.

### HIGHLIGHTS OF IAVI REPORT ONLINE

#### Articles:

- Reports: in-depth articles on current topics by *IAVI Report* writers and others.
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- Interviews: important figures in the development of AIDS vaccines address relevant questions.
- Early Edition: *IAVI Report* Early Edition contains articles published online ahead of print publication.
- Primers: AIDS vaccine related questions answered in non-technical format to enable non-scientists to broaden their understanding.
- Five different languages: *VAX* articles are translated from English to French, German, Portuguese, and Spanish.

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**This week's HIV/AIDS Journal Headlines:** Updated weekly, this section contains scientific papers chosen by the *IAVI Report* team as the most significant and relevant to AIDS vaccine research and associated disciplines.

**Hot News section:** This section highlights the most relevant HIV/AIDS news of the week.

**IAVI Database of AIDS Vaccines in Human Trials:** Contains a continually updated, searchable database of past and present AIDS vaccine candidates currently in human testing around the world. The related poster, Ongoing Trials of Preventive HIV Vaccines, is a snapshot of current research activity from the larger database.

**Special Features:** Contains databases, posters, maps, anthologies, and other archived special projects.

#### Other features:

**Calendar of Meetings:** Database on HIV/AIDS-related scientific and other meetings.

**This Week's Researchers:** Searchable database of principal (corresponding) authors of papers that appear in the HIV/AIDS Journal Headlines section.

#### Coming soon:

**Reviewed HIV/AIDS research sites**

**Letters to the Editor**

**French, Spanish, Portuguese and German content pages:** HTML versions of translated content and related links for our non-English speaking readers.

## NEW LIGHT ON HOW HIV IS TRANSMITTED

People chronically infected with HIV usually harbor swarms of slightly different, variant viruses at the same time, usually termed “quasispecies.” Much less is known about the variant or variants that are transmitted from one adult to another or from mother to child.

A group led by Eric Hunter and colleagues at the University of Alabama at Birmingham (UAB) has now studied a group of eight heterosexual discordant couples that transmitted virus in Zambia to determine the evolutionary relationship of donor and recipient viruses (*Science* 303: 2019; 2004), asking the question, what are the characteristics of the virus variants that infect the new host? This information is important to researchers because the characteristics of these variants could be exploited in vaccine design.

They found that the gp120 of transmitted virus variants in newly infected partners were more likely to contain shorter variable

regions (V1 and V4) than the majority of the viruses in donor quasispecies. The shorter variable regions also resulted in the absence of N-linked glycosylation sites, where the sugar components of the molecule attach to gp120. Hunter and colleagues believe that this loss of amino acids and sugars expose the region of the HIV gp120 that binds to the cellular receptor CD4, making it easier for these variants to infect host cells and increasing their infectivity. Therefore, they claim, it is most likely that the viruses that ultimately establish infection through heterosexual contact are more fit for this purpose than other viruses in the quasispecies.

Neutralization studies were conducted with virions pseudotyped with donor and recipient Envs. Recipient pseudotypes, made with Envs from viruses from newly infected patients, were as much as ten times more sensitive to neutralization by plasma from the linked donors than the donor pseudo-

types. Virus variants in patients with long-established infections are usually not sensitive to neutralization by plasma isolated from those patients at the same time.

But the researchers themselves caution that because of the limitations of the sampling used, they “cannot distinguish between a true transmission bottleneck, transmission of the predominantly replicating form in genital tissues, or transmission of multiple forms followed by outgrowth of a particular variant.” The latter possibility is particularly troublesome for vaccine design because it would entail more than one variant being transmitted to the new host.

Jon Cohen, in a commentary appearing in the same issue of *Science*, quotes virologist Douglas Richman (University of California, San Diego) as saying he has evidence contrary to the UAB data in his own matched donor-recipient pairs study, a group of predominantly homosexual men.

## CTL ESCAPE VARIANTS ARE NOT FOREVER

It has been extensively demonstrated that during HIV infection, the cellular immune response exerts selective pressure on the virus and drives a within-patient adaptive evolution. As a result, new virus variants appear that escape recognition by the original CD8<sup>+</sup> cytotoxic T lymphocytes (CTL) response. These escape variants have been associated with a loss of immune recognition and progression to AIDS.

What happens if these escape variants are transmitted to a new host? If these escape mutations, and even new ones derived from them, could propagate in populations this could undermine the efficacy of CTL-based vaccines designed on specific CTL epitopes or even consensus epitopes. Two new papers in *Nature Medicine* shed light on this problem and suggest that this scenario might not take place.

In a study with SIV-infected macaques (*Nat. Med.* 10: 275; 2004), David Watkins (University of Wisconsin, Madison) and colleagues observed that escape variants in a heterogeneous SIV isolate were lost upon passage to new animals. They used cloned

SIV (3x SIV) bearing escape mutations in three immunodominant CTL epitopes to infect macaques, and followed viral evolution after infection. They found that each mutant epitope sequence continued to evolve *in vivo*, often re-establishing the original, CTL-susceptible sequence. They characterized the *in vitro* growth properties of clonal viruses encoding escape mutations in all three epitopes and found that 3x SIV replication lagged behind that of wild-type SIV in the first 96 hours in culture. They construed that escape from CTL responses may exact a cost to viral fitness, the ability of the virus to replicate at normal levels.

In the companion paper in the same issue of the journal (*Nat. Med.* 10: 282; 2004), Philip Goulder (University of Oxford) and colleagues studied a similar scenario in humans infected with HIV from the B- and C-clade epidemics. They studied infected individuals who had human leukocyte antigen (HLA) alleles *HLA-B57* and *HLA-B5801*, which are associated with long-term HIV control and are therefore presumed to exert a strong selective pressure on the virus, and

followed the transmission of virus variants with escape mutations in a Gag epitope. They found that on transmission of escape variants to *HLAB57/5801*-negative individuals, one escape mutant (T242N) consistently reverted soon after transmission to the new host, the most likely explanation being that this escape mutation is associated with a fitness cost to the virus. Conversely, a second escape mutation (G248A) in the same Gag epitope was maintained in the new host. The suggestion that this CTL escape mutation has a negligible cost to viral fitness is supported by a previous study on this specific mutation.

The authors conclude that these two cases are likely to be extreme cases and that there is probably a broad spectrum of CTL escape mutations that have differing degrees of cost to viral fitness.

These two papers demonstrate that intrapatient evolution of HIV driven by CTL escape does not necessarily translate into evolution of HIV at the population level, suggesting that some HIV CTL epitopes will be maintained in human populations.

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International AIDS  
Vaccine Initiative

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.

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## WHY DOES HIV-1 FAIL TO REPLICATE IN SIMIAN CELLS?

HIV-1 fails to replicate in simian cells because of an early block in replication that the virus encounters after it has entered the cell. The block exists in cells derived from several nonhuman primate species, including rhesus macaques, which limits the usefulness of this species as a vaccine or treatment model for human AIDS. Previous coinfection and heterokaryon experiments have suggested that the block in replication is caused by a dominant inhibitory cell factor or activity.

A group led by Joseph Sodroski (Dana-Farber Cancer Institute and Harvard Medical School, Boston) has identified TRIM5 $\alpha$ , a component of cytoplasmic bodies, as the blocking factor (*Nature* 427:848; 2004). Using cells stably expressing TRIM5 $\alpha$  variants, they found that the human TRIM5 $\alpha$  protein was less effective in suppressing HIV-1 and SIVmac infection than was rhesus monkey TRIM5 $\alpha$ . This agrees with previ-

ous findings that suggested that HIV-1 capsids bind the Old World monkey restriction factor more efficiently than do SIVmac capsids. Sodroski and colleagues hypothesize that each virus has evolved in its natural host to achieve an acceptably low level of TRIM5 $\alpha$  interaction, and that vigorous, detrimental capsid disassembly may result when HIV-1 capsids encounter more effective TRIM5 $\alpha$  proteins, like those expressed in simian cells.

Why is it important to understand the early species-specific restrictions to HIV-1 replication? The researchers hope that the elucidation of TRIM5 $\alpha$  protein blocks will suggest approaches to the development of animal models of HIV-1 infection. They also suggest that insight into the HIV-1 uncoating process, a so far poorly-understood aspect of the retroviral life cycle, may reveal intervention targets.

### ◀ INTERVIEW: HO *continued from 13*

*Is that particular to New York City or did you have a similar reaction in Rochester?*

Rochester has a long history of vaccine testing, not just for HIV/AIDS, and they have built up a great program there to attract volunteers. New York, I think, is unique. We have so many people whose lives have been affected by HIV/AIDS, and almost every single one of our volunteers comes to us because of altruism, and not because of the reimbursement fee we give to participants. So we've been particularly moved by the spirit exhibited by our volunteers. It's been phenomenal. ♦

# vaccine BRIEFS

## MAJOR INDUSTRY ENTERS INTO PARTNERSHIP WITH THE MICROBICIDES FIELD

On 29 March 2004, the International Partnership for Microbicides (IPM) and Tibotec Pharmaceuticals, a subsidiary of Johnson & Johnson, announced a collaborative agreement to advance TMC120, a Tibotec product, into trials as an experimental microbicide. This is the first time that a major company has partnered with the microbicides field, which is largely made up of small biotech firms, non-profit groups and academic researchers. Under the agreement, Tibotec has granted the IPM a royalty-free license to develop TMC120 as a microbicide in resource-poor settings.

TMC 120 is an experimental antiretroviral drug in the non-nucleoside reverse transcriptase inhibitor (NNRTI) class. Several other NNRTIs are also being developed as potential microbicides. Many developers in the field believe that the most effective microbicides will combine several compounds with different targets and modes of action. It is hoped that topical formulations of NNRTIs will protect against HIV infection by inactivating virus in semen.

Phase I trials of TMC 120 are currently in progress; should the experimental candidate prove safe, it will move into expanded trials. Under the agreement, Tibotec will bear the costs of current trials and the cost of the compound through Phase II trials; IPM will assume all other development costs. IPM will retain the rights to manufacture and distribute the product in the developing world should it prove effective, a provision designed to ensure that the product will be affordable and readily available to women in the developing world. Tibotec will have the option of marketing the product in developed countries; if exercised, IPM will recover some of the development costs and receive a royalty which can be used to further IPM's mission.

The IPM is a public-private partnership that was founded in 2002 to accelerate microbicide product development. Other activities include support of a center for *in vitro* drug screening and collaborations with pharmaceutical companies and other organizations.

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## NEW STAFF AT THE SOUTH AFRICAN AIDS VACCINE INITIATIVE

In March the South African AIDS Vaccine Initiative (SAAVI) announced the appointments of two new team members: Gatsha Matzithulela, PhD as Deputy Director and Elize Levendal as the new Head of SAAVI Community Preparedness Program, replacing Ashraf Grimwood. Levendal is a nurse and former Executive Director of the Western Cape branch of South Africa's National Progressive Primary Health Care Network; she has also served as the women's sector representative at the South African National AIDS Council. A molecular biologist, Matzithulela also holds an MBA; he has a background in intellectual property and marketing issues in the field of biotechnology. At SAAVI, Matzithulela will have a particular focus on vaccine development.

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## NEW EXECUTIVE DIRECTOR FOR THE AIDS VACCINE ADVOCACY COALITION

Mitchell Warren is the new Executive Director of the AIDS Vaccine Advocacy Coalition, a US-based international non-profit organization that mobilizes support for and awareness of AIDS vaccine research in the US and internationally. Warren was previously the Senior Director for Vaccine Preparedness at IAVI, where he helped direct efforts to increase community and national involvement in AIDS vaccine clinical trials in Africa, Asia and Latin America. Prior to joining IAVI, Warren was the Vice President for International Affairs at the Female Health Company where he worked with governments, donor agencies and non-governmental organizations to expand women's HIV prevention options and to design and implement delivery programs for the female condom. In his new role, Warren will continue to focus on global advocacy and says that AVAC will "find increasing ways to have communities around the world be meaningfully engaged in the vaccine development process."

## UNIVERSITY OF MASSACHUSETTS MEDICAL SCHOOL BEGINS CLINICAL TRIAL OF MULTICLADE AIDS VACCINE

In April 2004, scientists at the University of Massachusetts Medical School (UMMS) began recruiting 36 healthy, HIV uninfected volunteers for a Phase I human trial of a new preventive AIDS vaccine formulation.

The vaccine strategy was developed by Shan Lu, PhD, associate professor of medicine and the head of the HIV vaccine effort at UMMS, in collaboration with Advanced BioScience Laboratories (ABL). The ABL-UMMS team was funded by a HIV Vaccine Design and Development Team (HVDDT) contract from the National Institute of Allergy and Infectious Diseases. This vaccine formulation was recently approved as an investigational new drug (IND) by the US Food and Drug Administration. The strategy uses a polyvalent DNA prime followed by a protein boost; both candidates are based on five different strains of HIV originally isolated from infected patients in five locations around the world. The researchers hope that the polyvalent prime-boost combination will induce both neutralizing antibody and cellular responses against HIV. It is not yet known whether the global diversity of HIV clades will require AIDS vaccines to be matched to locally circulating strains, or whether it will be possible to develop a single, universal vaccine against all HIV subtypes. Lu and his collaborators hope that future trials of their multiclade vaccine will help address this question. The DNA component of the vaccine contains one HIV *gag* gene (clade C) plus 5 HIV *env* genes (one clade A, two clade B, one clade C, and one clade E). The DNA inoculations are boosted with 5 different recombinant gp120 proteins (same isolates as the DNA component). The protein boost is administered in combination with QS21 adjuvant.