IAVI REPORT
The Newsletter on International AIDS Vaccine Research Vol.5 No.4 September-November 2000

Gallo Meeting Features New Protection Data from Primates

Enhanced protection via cytokines and the latest on tat-containing vaccines were hot topics at this year's conference

by Richard Jefferys

Every year the Institute for Human Virology (IHV) hosts a six-day science marathon that, in deference to IHV's well-known director, many people refer to simply as "The Gallo Meeting." The 2000 event packed in nearly 300 presentations on diverse topics, including about 30 relating to HIV vaccines.

Enhancing Vaccine Effects with IL-2

Among the conference highlights was Norman Letvin's presentation on a vaccine approach (in collaboration with Merck Research Laboratories) that utilizes naked DNA containing the *env* and *gag* genes combined with low doses of the cytokine IL-2. (The IL-2 used in these experiments was joined to part of a human antibody (Ig), resulting in an IL-2/Ig "fusion protein" that remains in circulation longer than native IL-2.)

In animal studies, 12 rhesus monkeys received the DNA vaccine at weeks 0, 4, 8 and 40. Four of them

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IAVI Launches Two Vaccine Trials in Oxford

by David Gold

Efforts to advance two IAVI-sponsored candidate HIV vaccines received a major boost with the announcement that clinical trials of one vaccine have begun, and a second one has received regulatory approval to start.

On 21 September, Wayne Koff, IAVI's Vice President for Research and Development, announced that an HIV vaccine using a modified vaccinia Ankara (MVA) vector was approved for Phase I testing by the Medicines Control Agency in the United Kingdom. The vaccine is based on subtype A strains of HIV, the most common strain in Kenya and in many other parts of Africa. It encodes a consensus clade A *gag* sequence along with epitopes shared between subtypes A and B, and "mini-genes" encoding these epitopes as 20-mer peptides. The MVA vaccine was designed by Oxford University's Thomas Hanke and manufactured by Impfstoffwerke Dessau-Tornau (IDT), a German pharmaceutical company. It is the second component of a prime-boost vaccination strategy.

A few weeks earlier, the first component – an HIV DNA vaccine – entered Phase I testing in Oxford, in a trial that will involve a total of 18 volunteers. Oxford researcher Andrew McMichael told the *IAVI Report* that, as of 30 October, about 50% of the needed volunteers had been immunized. The researchers will randomize participants to either the DNA or the MVA vaccines. They then plan to seek approval for testing both vaccines in combination.

Like the MVA vaccine, the DNA component is also made from HIV subtype A strains. Evan Harris, a member of the British Parliament, led off the trial on 31 August when he

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then received the fusion protein (by twice-daily injections for 2 weeks after each of the first 2 vaccinations); 4 received a DNA plasmid encoding it (2 days after each of the first 2 vaccinations) and 4 received only saline with the DNA vaccine.

Characterization of the cellular immune responses after vaccination showed a clear enhancement by IL-2. (Some of this data was published previously in *PNAS 97*: 4192-4197, 2000). All 8 vaccinated animals had at least a 5-fold increase in the level of *gag*- and *env*-specific CD8+ cells, as well as an increase in their durability, relative to the 4 vaccinated animals that did not get IL-2. (*Gag*-specific CD4+ helper cell responses were also enhanced after challenge in animals that received IL-2.) All animals were challenged at week 46 with the highly pathogenic SHIV strain 89.6P.

Presenting the post-challenge data for the first time, Letvin said that IL-2 boosted the effects of the DNA vaccine according to several different criteria. IL-2-treated animals were able to control viral replication (by 2.7 and 3 logs in the IL-2 protein- and IL-2-plasmid-treated groups, respectively), resulting in undetectable viral loads, and remained clinically and immunologically healthy through 220 days of follow-up. They also showed complete preservation of CD4+ cells. In contrast, the 4 vaccinated animals without IL-2 showed a less pronounced reduction of viral load (1.9 logs) and, in a statistical comparison with controls, showed a trend towards preservation of CD4+ cell counts. Seven out of 8 non-vaccinated control animals displayed symptoms of simian AIDS, and 4 died within 140 days of challenge.

The researchers also found that ability to control viral load was correlated with the level of pre-challenge CD8+ T-cell responses to Env and Gag epitopes. Neutralizing antibodies were detected only several weeks post-challenge, leading Letvin to doubt their relevance to the biological outcome of this experiment.

In discussing the implications of these results, Letvin pointed out that the challenge was done using "the hottest virus we have available" at a high challenge dose (100 times the dose expected to infect half the animals). If these experiments would be done instead with the size and route of a typical human exposure to HIV, he speculated that "we might actually see frank protection." Letvin also said that responses like those seen in the macaques "would not be inconsequential" in terms of preventing disease and reducing the likelihood of HIV transmission in humans.

Tat-based Vaccines: An Overview

One session at the meeting focused on the potential of Tat as an immunogen. Interest in this approach has grown in the last few

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The Debate on Tat

The apparent contradictions among the results obtained by the Ensoli, Pauza and Shiver studies (see main article) continue to be a source of some controversy in the vaccine research community. Shortly after the Gallo meeting, Shiver's data showing a lack of protection with Merck's Tat protein vaccine was presented by Tong-ming Fu from Merck Research Laboratories at the "18th Annual Symposium on Nonhuman Primate Models for AIDS," held in Madison, Wisconsin on 4-7 October 2000.

The conflicting results could be at least partly due to the different study designs. Firstly, the immunization protocols were very different, with Ensoli's team using 8 immunizations over 32 weeks plus a booster containing immune-stimulating complexes (ISCOMs) at week 36. In contrast, Shiver immunized his rhesus macaques 3 times, at 0, 8 and 24 weeks using RIBI with aluminum salts as the adjuvant. The Pauza study used up to 5 vaccinations over 10 weeks, in the adjuvants IFA or polyphosphazene.

These varying regimens clearly generated different immune responses: high titers of anti-Tat antibodies and no CTLs in the Shiver work, compared with Tat-specific tumor necrosis factor α production and CTL activity reported in Ensoli's study. Tat-specific antibodies were detected in Ensoli's native Tat study, but not with their *tat*-DNA. Most of the animals in the Pauza study also generated anti-Tat antibodies, but CTL responses were not observed.

Ensoli maintains that CTLs are needed for protection by *tat*-based vaccines, so the lack of this response in Shiver's animals (which she thinks could be due to the shorter immunization schedule) would explain the absence of protection. As further support for the key role of CTLs, she points to a recent study on SIV-infected monkeys by Todd Allen and colleagues, who showed that Tat-specific CTLs helped reduce viral load (see article on p.13). She also cites reports that fully biologically active Tat can gain access to the MHC class I pathway of antigen presentation, which is necessary for CTL induction (e.g. Kim et al, *J. Immunol. 159*: 1666-1668, 1997). Subtle differences in the biological activity of the protein may also influence presentation to CTL and thus affect vaccination outcome, she says, and therefore her team routinely verifies that the Tat protein is fully active prior to each use.

John Shiver, however, believes that his protocol was a reasonable approach to inducing both anti-Tat antibody and CTL responses, based on past experience. While these results suggest that the ability to induce CTLs may not be a consistent property of Tat protein, his team is continuing to work with *tat* in the hope of clarifying this question.

Another potentially crucial difference between these studies is in the challenge. The Shiver study used a higher challenge dose of 50 m.i.d. 50 (50x the minimum dose required to infect half the animals exposed) compared to Ensoli's 10 m.i.d. 50. There is currently no consensus on what type of challenge most closely mirrors a typical human exposure. It is also unclear whether Ensoli's challenge virus is as highly pathogenic as Shiver's, although her team has just published evidence of high viral loads, CD4 T-cell decline and 70% mortality in challenged cynomolgus macaques that may address this issue (*J. Med. Primatol. 29*: 193-208, 2000). Each study also used a different dose of Tat protein (Ensoli gave 10 µg, Shiver 20 µg and Pauza used 3 doses of 10, 20 and 40 µg).

With the Shiver/Letvin paper now submitted for publication, the debate about the merits of *tat* in vaccines is likely to continue. Also unresolved by these studies, which all examined vaccines based on *tat* alone, is the question of whether *tat* can contribute to protection in a multi-antigen vaccine.

-R.J.

New Vaccine Study Seen as Significant Advance

But questions remain about whether approach will move into human trials

By David Gold

Few monkey studies have attracted more attention than one recently published in *Science* (20 October 2000; see also article on p.1). Conducted by Harvard researchers and funded by the U.S. National Institute of Allergy and Infectious Diseases (NIAID), the study showed that monkeys immunized with a DNA vaccine and the cytokine Interleukin-2 (IL-2) fused to an immunoglobulin molecule (Ig) appear to be protected against simian AIDS. It is, in the view of many observers, a major step forward in AIDS vaccine research. The work also raises a number of broader questions applicable to other vaccine studies, including whether and how the approach will move into human trials.

In the study, a team of scientists led by Norman Letvin and Dan Barouch immunized 4 monkeys with a DNA vaccine expressing SIV *gag* and HIV *env* at weeks 0,4,8,40. Another 8 monkeys received the same four DNA immunizations plus IL-2/Ig (either in the form of a protein or expressed in a plasmid) at weeks 0 and 4. A third group with 8 monkeys served as controls. Six weeks after the last immunization, all the monkeys were challenged intravenously with a pathogenic SHIV 89.6P. (SHIV viruses contain SIV core genes with the HIV envelope).

After challenge, all the monkeys become infected, but those vaccinated with DNA plus IL-2/Ig fared dramatically better: at 140 days, they had low or undetectable virus levels, significantly higher CD8+ T cells (an average of 5 times higher than controls), stable CD4 counts and no clinical disease or death. In contrast, the control animals had high viral loads and significant clinical disease; 4 of the 8 control monkeys died within this time.

IL-2 clearly boosted the effectiveness of the DNA vaccine, since monkeys receiving the DNA vaccine alone did not do nearly as well as those receiving the DNA plus IL-2/Ig. Two types of IL-2/Ig combinations were used. Of these, the plasmid expressing IL-2/Ig appeared to be more effective than the protein. Perhaps most significantly, the study, according to the researchers, "strongly suggests that the improved outcome of the monkeys receiving the cytokine-augmented DNA vaccine resulted from augmented vaccine-elicited CTLs."

The study adds to a growing body of data, from research in both monkeys and humans, that a potent cellular immune response can protect against AIDS. Some of these findings come from natural history studies of so-called "highly exposed but seronegative (ESN) individuals" and from HIV-infected, long-term non-progressors. Other studies (including one from Letvin's lab) have shown that when SIV-infected monkeys were depleted of their CD8 cells, virus levels showed a steep increase.

And in a field where researchers often complain about the way some groups conduct monkey studies (by using "weak" challenge viruses and a lack of standardization among different immunization regimens, etc.), this study appears to be rigorous, well-designed and well-executed. Moreover, the researchers involved, particularly Letvin and the Merck team (led by Emilio Emini), are credible and respected figures in the field.

By using a highly pathogenic challenge virus administered intravenously, the researchers were able to provide clear evidence of the vaccine's protective effect. Intrarectal challenges (which use a mucosal route more closely reflecting most transmission in humans) are considered far easier to protect against. In fact, most researchers now use intrarectal or intravaginal challenges in monkey studies.

This is clearly not the first vaccine that can protect monkeys against simian AIDS. In 1992, Harvard's Ron Desrosiers showed that a live attenuated SIV vaccine provides powerful protection against a pathogenic strain of SIV. But the live attenuated vaccines raised significant safety issues, particularly after some vaccinated monkeys began developing AIDS.

So far, no vaccine has conclusively demonstrated the ability to prevent infection in monkeys challenged with pathogenic SIV. However, in the last few years, a number of viral vector vaccines (used individually and in combination with a DNA vaccine) have begun to show some evidence of protecting monkeys against disease. The Letvin study adds strong new evidence that such protection is possible.

The study adds to a growing body of data that potent cellular immune response can protect against AIDS.

Yet these promising findings raise many questions, some of which are relevant to other vaccines in development. These include:

Will the vaccine work in humans, and if so, for how long?
The only way to know whether a vaccine works in humans is to test it in humans. Whether this particular approach can move into human studies, and if so, how rapidly, remains to be seen.

How long will the protected monkeys stay protected?
Letvin's data show that all 8 monkeys immunized with DNA and IL-2/Ig got infected with the challenge virus but remained healthy, with undetectable levels for 140 day of follow-up. But we don't know how long the animals will remain disease-free. Will the pathogenic challenge virus eventually break through and cause disease in the vaccinated monkeys? It is, of course, critically important to continue observing these monkeys to see how long they stay healthy.

How durable are the protective immune responses generated by the vaccine?
The monkeys in this study were challenged 6 weeks after receiving the last immunization. But how would the monkeys fare if they were challenged 6 months or 6 years after immunization, when higher cellular immune response levels are likely to have receded and protection would depend on memory? The only way to know is by doing more studies.

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One of the most important questions researchers face is how to maintain a potent HIV-specific CD8 T-cell response over the long-term. Some believe that doing so will require a vector (such as an attenuated herpes virus or an adeno-associated virus) that generates persistent antigen expression. It is now clear that some ESN sex workers became infected after stopping their work (see *IAVI Report*, Jan.-Mar. 2000), suggesting that without regular exposure to HIV antigens (through sex work, in these cases), the protective cellular immune responses might not be maintained. Yet Letvin believes that the vaccinated monkeys in his study might have fared even better if they had been challenged six months after the last immunization. "The longer the period between immunizations, the more likely you are to a get maximum CTL response," he told the *IAVI Report*. In fact, he thinks it may be beneficial to spread out the immunizations, but adds that the only way to know for sure is to do the studies. As for whether the vaccine will provide protection 6 years after the last immunization, he said, "that may be too long. But you never know."

Will the vaccine protect against diverse viral strains?

The Harvard researchers used the identical SHIV strain to produce both the vaccine and the challenge virus. This "homologous" challenge is much easier to protect against than a "heterologous" challenge that utilizes a different SHIV strain in the challenge. Given HIV's enormous genetic variability and the multitude of HIV strains circulating throughout the world, the question of whether a vaccine will protect against diverse strains of the virus is critically important. Studies to see how the DNA IL-2/Ig vaccine works against different challenges are now underway, says Letvin. While no one knows for sure what level of protection will be seen, he suggests that "if indeed it is a CTL response that is protecting these monkeys, the breadth of the protection should be substantial."

How does this vaccine compare with other vaccines in development?

A number of other vaccine approaches have demonstrated some ability to "blunt" disease in the SIV/SHIV monkey model. These include viral vectors such as modified vaccinia Ankara (MVA), MVA used with a DNA prime, Venezuelan equine encephalitis virus, Semliki forest virus and adenovirus. "There are now a lot of ways to make good CTL responses," says Letvin. And he told *The Wall Street Journal* on 20 October that "at least 3 or 4 other vaccines have achieved similar results in monkeys." Moreover, the *Science* paper concludes by noting that "the cytokine administration should be readily applicable to other vaccine modalities and for immunotherapeutic purposes."

But it is difficult to directly compare this DNA vaccine/IL-2/Ig regimen to other approaches in terms of its ability to generate immune responses and protect against disease. Comparative data barely exists because researchers generally do not use standardized animal models, immunization schedules or challenge regimens. Thus, it is still problematic to prioritize promising approaches.

What are the regulatory hurdles to testing a combination of an HIV DNA vaccine and IL-2/Ig in humans?

At least three different teams have tested HIV DNA vaccines in humans, so the DNA component should face few hurdles in moving into human studies. However, using IL-2 in healthy people will entail more significant regulatory considerations. The cytokine (which is naturally produced by the body) is currently approved as a treatment for certain types of cancer but can, at times, cause significant side effects. It is also being studied as a treatment in HIV-infected individuals on HAART. In Letvin's study he compared an IL-2/Ig protein to a plasmid expressing IL-2/Ig genes. The plasmid may present more safety concerns than the protein, since the protein is likely to disappear in the body, while the plasmid may continue to express IL-2 genes (with unknown long-term effects).

To further evaluate the impact of IL-2 on responses to the DNA vaccine, some researchers have suggested including another control arm in any further studies: IL-2 plasmid without vaccine. After challenge, would the IL-2/Ig give any protection, or even accelerate disease? Clearly, it is important to learn more about the potential biological effects of this cytokine.

Other research teams are also looking at testing cytokine-augmented HIV DNA vaccines. David Weiner at the University of Pennsylvania reports that his group, working with researchers from Wyeth Lederle Vaccines, hopes to move a second-generation HIV DNA vaccine administered with IL-2 into human studies.

With the intellectual property controlled by a number of different parties, will anyone take the lead in developing this vaccine and moving it into humans?

In news reports about the study, Merck officials appeared to be lukewarm about prospects for this particular vaccine. Safety and regulatory concerns are clearly a concern, and the company is known to be developing other candidate HIV vaccines. Another complication is that intellectual property rights to the vaccine components are owned by several different parties. Merck itself controls the DNA vaccine technology (licensed from Vical, the San Diego-based biotech company); the Chiron Corp. controls rights to IL-2; Genentech reportedly holds some rights to the use of Ig in a vaccine and Letvin's own team has patented some rights to the overall approach. While multiple patent rights often get sorted out in the end (as they did with the hepatitis B vaccine), such negotiations often take a lot of time.

On 26 October, an advisory committee of the NIAID AIDS vaccine program discussed how NIH can help move Letvin's approach into human studies. The NIH's newly created Vaccine Research Center (VRC) could be ideally suited to produce the vaccine for Phase I trials. The clinical trials could be conducted at the VRC or within NIAID's new HIV Vaccine Trials Network. It is unclear whether this can happen, and if so, how quickly. But, assuming safety issues can be adequately addressed, the field will benefit enormously if a clinical study can be initiated as fast as possible.

Might the vaccine work as a therapeutic vaccine in HIV-infected individuals?

A growing number of researchers are interested in testing HIV vaccines as therapies in HIV-infected individuals. In fact, on the day the paper was published, Merck representatives informed U.S. activists that the company had begun human trials of its HIV DNA and live vector vaccine (separately) in HIV-infected individuals.

Given the potent cellular immune response generated by the cytokine-augmented DNA vaccine, it would make sense to test the

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Vaccines at Durban: A Closer Look

Beyond the calls to action on behalf of PWAs with no medical care and poor countries struggling to turn the tide on AIDS, Durban offered abundant information relevant to vaccines

by Patricia Kahn

Even before the 12,700 delegates at this summer's XIII International Conference on AIDS had left for home, the meeting was already being hailed as a landmark event in the history of the epidemic. From the impassioned calls to bring treatment to PWAs in poor countries to the outrage over South African President Mbeki's espousal of AIDS "dissident" ideas, the meeting created a momentum which – if it truly lasts – will mark a turning point in the fight against AIDS.

The conference was also a landmark event for vaccine development, solidifying its place as a top priority in that battle, particularly in poor regions. Unlike earlier meetings of the series, where vaccines were largely a side issue, this year's event offered a profusion of vaccine-related sessions. With the dust now settled on the remarkable politics of Durban, the *IAVI Report* looks at some of the notable vaccine science and news presented there.

A vaccine by 2007?

In this succinctly-titled plenary talk on vaccine development, Margaret Liu, former head of the HIV vaccine program at the Chiron Corp. (and now vaccine advisor to the Gates Foundation) outlined what she sees as the field's major achievements and most promising ways forward. Key items on her list were:

- The demonstration of protection against SIV or SHIV in primate models by several different types of experimental vaccines, providing both proof of concept and systems for studying correlates of protection;
- Detection of HIV-specific immune responses in a small minority of people who seem resistant to either HIV infection (exposed but seronegative people) or disease (long-term non-progresssors). Such cases suggest that the immune system can contain the virus and pointed researchers towards strategies that target cellular immunity and mucosal responses;
- Illumination of viral structure, which is providing invaluable information for more rational vaccine design, especially of vaccines that might elicit neutralizing antibodies;
- Advances in vaccine technology and design concepts, such as prime-boost approaches, new gene delivery vehicles and adjuvants, vaccines directed to mucosal responses, and optimization of DNA vaccines.

Liu said that the question posed in the title of her talk could not yet be answered, although she expressed confidence that good candidates will be in advanced trials by 2007. But getting an effective vaccine on this timescale will take both a greatly increased effort and a commitment to be guided by scientific data and "rational empiricism" rather than by biases, which she said can creep into issues such as the debate on subtypes (see below). She also emphasized the importance of pushing multiple vaccine approaches forward in parallel and of preparing trial sites so they are ready when the candidates are.

AIDS vaccines for Africa

One major session focused on efforts to make vaccines targeted specifically to African needs and on vaccine work within Africa.

Immunologist Malegapuru William Makgoba, who heads South Africa's Medical Research Council, led with an overview of his country's HIV vaccine program, which has received strong government backing despite President Mbeki's questioning that HIV causes AIDS. The program is coordinated by the South African AIDS Vaccine Initiative (SAAVI), whose goal is the development of an affordable, subtype C-based vaccine owned by the public sector. SAAVI's scientific activities range from developing candidate vaccines (4 different approaches are now in the works) to immunological evaluation of vaccines and helping to prepare clinical trial sites. South Africa will also participate in testing candidates developed with international partners. The first one likely to enter clinical trials in the country is based on the Venezuelan equine encephalitis virus (VEE) vector made by AlphaVax, a North Carolina-based company, and funded by IAVI. Also under consideration are DNA vaccines from the Chiron Corporation and a vaccine from Targeted Genetics based on adeno-associated virus (AAV).

Carolyn Williamson of the University of Cape Town described how her lab derived subtype C sequences representative of southern African strains to use in the VEE vaccine. The researchers began by collecting samples from 14 commercial sex workers who were recent seroconverters and isolating virus from 10 of them. After confirming that these isolates infect cells via the CCR5 receptor, they sequenced 800 base pair regions from the *gag*, *pol* and *env* genes and compared them to over 70 known sequences from southern African subtype C strains, including some isolates from infected but asymptomatic individuals. In this way they arrived at 2 consensus sequences, which became the basis for this vaccine.

With Kenya now poised to launch Africa's second AIDS vaccine trial (see article, p.1), clinical investigator Dorothy Nbori-Ngacha of the University of Nairobi described the ongoing preparations. Efforts are now focused on finalizing the planning and oversight committees, such as the data safety and monitoring board, clinical steering committee and community advisory board, and getting the remaining scientific and ethical approvals. [Since the Durban conference, work is also focusing on patent and intellectual property issues; see *www.iavi.org*.]

Rounding out the session, Peter Mugyenyi, clinical director for Africa's first AIDS vaccine trial, described Uganda's long journey from the time of its early interest in AIDS vaccine development to the recently-completed trial at the Joint Clinical Research Center (JCRC) in Kampala (see below). Wayne Koff, who heads IAVI's R & D program, gave an update

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on IAVI-supported projects to develop AIDS vaccines geared to circulating African strains (see *IAVI Report*, Jan-Mar. 2000, p.5).

The Nairobi Declaration: An African Strategy

At a press conference following the above session, several African scientists, along with Jose Esparza of the WHO/UNAIDS HIV Vaccine Initiative, presented "The Nairobi Declaration: An African Appeal for an HIV Vaccine." They also unveiled the broad outline of a strategy to move the vaccine agenda forward within Africa and to achieve greater coordination across the continent. Signed by 38 African scientists, community advocates and policy makers, the statement came after a process that solicited the views of African researchers and then formulated a set of principles and proposals, which were adopted at a 14 June meeting in Nairobi under the auspices of WHO/UNAIDS, AfriCASO (an umbrella for African AIDS service organizations), the Southern African Development Community (SADC) and the Society on AIDS in Africa (SAA).

The strategy outlines 5 areas for activity: advocacy and education; coordination; promotion of promising candidate vaccines; building capacity to conduct trials; and ensuring access. It also lays out specific milestones, including the development of candidate vaccines based on African subtypes by 2002; completion of at least 4 Phase I/II trials by 2003; and initiation of at least one Phase III trial by 2005. South Africa's Makgoba described the initiative as a way for African scientists to "speak with one voice [and to] be responsible for our own future." Makgoba will help coordinate the effort, which is now working to turn the strategy into a specific action plan and to raise political support and funds for its activities.

Vaccine Trials: Tracking the VaxGen trial cohorts

VaxGen's first two HIV vaccine efficacy trials are now in full swing and have a combined enrollment of nearly 8000 volunteers. Several speakers presented data on the experiences and self-reported risk behaviors of these trial cohorts, with an apparent downward trend in risk behavior.

Kachit Choopanya of the Bangkok Metropolitan Administration gave an update on the VaxGen trial in Thailand. Participants were recruited among intravenous drug users at 17 methadone clinics in Bangkok. [On 31 August, VaxGen announced that enrollment of 2500 volunteers was complete.] The cohort is about 93% male and has an annual HIV seroincidence estimated at 6%, based on studies of a similar population over the years 1995-1999. Two-thirds of the volunteers will receive 7 doses of vaccine (made of recombinant gp120 subunit from two different HIV subtypes) over 24 months, while the rest will be given placebo. Choopanya also reported that retention in the trial is over 95% and that the vaccine is well-tolerated and immunogenic in all participants.

Another trial investigator, Suphak Vanichseni of the Bangkok Vaccine Evaluation Group, presented interesting findings on trends in risk behaviors. Based on data from the 1174 volunteers who had reached their 6-month follow-up, she reported that the frequencies of nearly all risk behaviors (except recent incarceration) had dropped - some dramatically so - since the trial began: the proportion of volunteers injecting drugs decreased from 72% to 57%; needle sharing fell from 32 to 13% and condom use increased from 51% to 62% with casual partners and from 8% to 12% with steady partners. Since such changes will lower HIV seroincidence, Vanichseni was asked whether the trial retains sufficient statistical power (it is designed to detect 30% vaccine efficacy). She responded that the study is powered for a reduction in seroincidence from 6% to 4% and that the cohort is still within that range, according to the Data Safety and Monitoring Board that is closely following the trial data.

Clayton Harro of Johns Hopkins University (Baltimore) reviewed the North America/Amsterdam trial, which is spread over more than 60 sites and is fully enrolled. Its 5414 volunteers are mostly men who have sex with men (MSM), along with 311 high-risk women. Annual seroincidence in the cohort is approximately 1.5%, and retention as of January 2000 was over 98%.

John Jermano of VaxGen presented data on the social impact of participation in the North America/Amsterdam trial, based on information reported by volunteers as of June 2000. The most frequent negative effects so far (reported by 7.5% of the participants at 6 months) are disturbances in relationships with family or friends, usually stemming from negative comments about participation or misperceptions that the volunteer is infected. Few volunteers (<1%) said they had experienced discrimination in employment or insurance due to their participation. Sexual risk behavior also decreased, with the median number of male partners reported by MSM during the past 6 months dropping from 5.0 (for the period prior to entering the trial) to 4.0 (during the first 6 months in the trial).

New adjuvant boosts gp120 immunogenicity

Jorge Flores (NIH, Bethesda) presented a potentially important finding concerning the adjuvant QS21, a saponin made from the soapbark tree and already used in veterinary vaccines. Reporting on a study by the NIH HIV trials network (study AVEG036) involving 60 volunteers, Flores said that 0.5 ?g of VaxGen's bivalent gp120 vaccine (subtypes B/E) prepared in QS21 gave the same immune response as 300 ?g in alum, the current adjuvant. This 600-fold reduction in the amount of antigen per dose would greatly cut the cost of the vaccine and make it far more economically feasible to produce polyvalent vaccines (containing gp120 subunits from multiple subtypes or strains, including breakthrough viruses). The trial also showed that a new formulation of QS21 reduced, but did not eliminate, the problem of relatively severe local reactions. According to study chair Tom Evans of the University of Rochester, a new trial is in planning to test whether reducing the amount of QS21 will reduce its reactogenicity but not its immune-enhancing effect.

Clinical studies on canarypox vaccines

In a late-breaker session, H. Cao of the Massachusetts General Hospital (Charlestown) presented results from a Phase I study of the ALVAC vCP205 canarypox HIV vaccine, conducted at the JCRC in Kampala and supported by the U.S. NIAID. The vaccine contains the *gag* and *pol* genes from HIV-1 subtype B. Several Phase I and II trials in the U.S.A. and France found it to be safe and to induce CTLs in a minority of volunteers. The Ugandan study enrolled 40 volunteers (20 immunized 4 times over 6 months with the test vaccine, 10 with rabies vaccine and 10

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The Developing World Debates Research Ethics

by Bob Huff

The ethics of clinical research in developing regions was the official topic of just four sessions at the Durban meeting. Yet the questions addressed there pervaded many other issues discussed at the conference, and are sure to come to the forefront as clinical studies of AIDS vaccines, microbicides and treatments move ahead internationally.

What emerged was an affirmation of the fundamental principle that successful and ethical collaborations in developing country settings must begin early in the research process and continue as an ongoing partnership among equals.

In his plenary address, the president of South Africa's Medical Research Council, Malegapuru William Makgoba, stressed that a true collaborative process is crucial as research relationships develop among parties with unequal power. Past collaborations have often been characterized by the mentality that "he who pays the piper chooses the tune," Makgoba said, so that the priorities of the more powerful partner dominated. Moving towards genuine partnerships and developing an ethical framework should not be done by imposing guidelines from the outside but by "engaging civil society in an open and transparent process." Makgoba noted that, during the past fifty years, the quality of clinical research has been vastly improved by greater attention to ethics. As this process continues, he said, we have a responsibility to "practice the best ethics within our constraints."

> *"Past collaborations have often been characterized by the mentality that 'he who pays the piper calls the tune.'"*
>
> — M. W. Makgoba, Medical Research Council, South Africa

Finally, addressing concerns about the commitment of the South African leadership to international collaboration, Makgoba assured the audience that "in our government you have a partner."

At a slide talk the following day, bioethicist Robert Levine of Yale University outlined some key points covered by the UNAIDS guidance document on HIV vaccine trials in developing regions. These include:

- the ethical uses of placebos;
- just and sustainable distribution of the benefits of the research;
- the avoidance of coercive influence over subjects and host governments;
- ethical considerations for including populations that may be considered vulnerable;
- the challenges of providing culturally sensitive informed consent; and
- the contentious issue of providing "best proven" treatment for trial subjects.

Engaging these issues, he said, requires a mutual understanding of the cultural context and scientific requirements of the study. For example, if no other proven vaccine exists, a placebo may be ethical. A better alternative, though, might be to give an unrelated vaccine as a control. Also, the terms of access to the test vaccine (if proven effective) and to knowledge gained from the trial should be discussed among all stakeholders in advance. By engaging the community in designing the protocol, informed consent process, and risk-reduction interventions, the scientific and ethical quality of the research is strengthened. Further-more, community acceptance is fostered and the capacity of the host country is enhanced. Levine concluded that "to ensure the scientific and ethical conduct of the trial, to support meaningful self-determination, and to strive for a partnership among equals is the foundation of ethical research."

One of these issues, that of providing "best proven" treatment to trial participants, was the subject of a heated debate on the proposition: "Research participants in developed and developing countries should receive the same standard of care." Speaking in favor was Jorge Beloqui of Brazil, a University of São Paulo mathematics professor, a member of the PWA advocacy organization GIV-SP, and an outspoken critic of international trade agreements that restrict access to affordable HIV treatments. Against the proposition was Solomon Benatar, professor of medicine and director of the Bioethics Centre at the University of Cape Town.

Beloqui argued that accepting different standards of care between poor and rich countries simply entrenches inequality as a principle and perpetuates the notion of two worlds—one that benefits from the best available care and one that must suffer. He also pointed out that failure to provide the best possible care is a breach of the doctor/patient relationship and violates the commitment to research subjects described in paragraph 11.3 of the Helsinki agreements. "How can an ethical physician knowingly provide suboptimal treatment for a patient?" he asked.

Beloqui believes that commercial interests are responsible for the conflict between the doctor/patient and researcher/volunteer relationships. Yet, since commercial sponsors have so much to gain in profit, he sees no reason for them not to provide the best standard of care to the individuals who sustain the risks of proving the drugs. "Clinical trials in developing countries are the first step to opening new markets," he said,

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yet he fears that the products tested will be unaffordable in the host countries. "Guarantees must be in place to insure future availability," Beloqui argued.

Solomon Benatar responded by questioning the meaning of "same standard of care," with its paternalistic implication that developed country standards represent the optimum. For example, a Western trial that failed to provide social support, continuing access to drug treatments initiated during the trial and adequate follow-up might be unacceptable by his criteria. For Benatar, "same standard" is not a clear-cut criterion; its meaning depends on who is speaking and where. Certainly, some standards set by developing countries have been mediocre and can be improved, but holding out for conditions equal in every way to those in rich countries would preclude research from ever being done in these settings. The solution, he feels, is to provide the highest attainable standard of care for the research setting in the host country. After all, Benatar noted, "we all come from developing countries," since even most wealthy nations have aspects of their health care provision that could be improved.

Benatar recommended that researchers have knowledge of the local social and economic situation, including an awareness of how the host country has historically been treated by the sponsor country. A point made by several speakers during the week-long conference was that Africans often emphasize community values and may understand their individual rights as emanating from membership in a community. Such a world view can be difficult to understand for Westerners, who give primacy to individual rights. This can result in paternalistic and insulting notions, such as the idea that home country institutional review boards (IRB) are necessary to protect host country subjects. Benatar also believes that the benefits of research conducted in a particular region should be seen to flow into the lives of the local people. In his experience, research participants in South Africa care less about access to antiretroviral drugs than that their participation contributes to a stronger community.

Benatar concluded that ethics should not mean policing researcher compliance to a disembodied set of international agreements. Rather, it should be about guiding people who want to work together to achieve ethical and excellent results.

Most of the arguments for and against the debate proposition are encompassed by the UNAIDS guidance document. For example, Beloqui's concern that host countries may never benefit from the test vaccine or drug is addressed by the principle that trial sponsors and hosts should negotiate the distribution of benefits beforehand. Benatar's willingness to trust local scientific and ethical oversight is supported by the guideline that clinical research should only be performed in countries with the capacity to provide that oversight.

Ultimately, Levine conceded, we all recognize that the distribution of wealth in the world is inequitable. But this fact should not "impede the efforts of the developed world to help low resource countries develop treatments and prevention that they can afford." ◆

Bob Huff works at the American Foundation for AIDS Research (amfAR) in New York as editor of their HIV/AIDS Treatment Directory. *He has been a community advocate for ethical HIV clinical research for twelve years.*

NIAID Announces International Sites for HIV Vaccine Trials Network

Ten sites in Africa, Asia, South America and the Caribbean will join nine U.S. centers in the new HIV Vaccine Trials Network (HVTN), according to an announcement in Durban by the U.S. National Institute of Allergy and Infectious Diseases (NIAID). Under the terms of a five-year, renewable grant, the network will receive US$29 million in its first year. Selection of the nine domestic sites, along with central facilities for administration, data management and immunological testing, were announced in late May (see *IAVI Report*, Jul.-Aug. 2000, p.1).

The following is a list of the ten new sites and their principal investigators:

- **Brazil**
  Mauro Schechter
  Hospital Escola, São Francisco de Assis
- **China**
  Jie Chen
  Guangxi Health and Anti-Epidemic Center, Guangxi
- **Haiti**
  Jean (Bill) W. Pape
  Cornell-GHESKIO, National Institute for Laboratory Research, Port-au-Prince
- **India**
  Ramesh Paranjape
  National AIDS Research Institute, Pune
- **Peru**
  Jorge Sanchez
  Universidad Peruana Cayetano Heredia, Lima
- **South Africa**
  Salim Abdool Karim
  South African Medical Research Council, Durban
- **Thailand**
  Chirasak Khamboonruang
  Research Institute for Health Sciences, Chiang Mai University
- **Trinidad**
  Courtenay Bartholomew
  Medical Research Foundation of Trinidad/Tobago, Port of Spain

IAVI Releases Blueprints for Speeding Vaccine Development...

At the Durban meeting, IAVI released two new documents that present detailed global strategies for key areas in AIDS vaccines.

The first one, "Scientific Blueprint 2000: Accelerating Global Efforts in AIDS Vaccine Development," starts from the premise that relatively few of the many potential vaccine approaches have been actively pursued. The Blueprint also maintains that, despite a major increase in resources for AIDS vaccines over the past two years – both from industry and a few governments – the level of commitment, investment and effort is still inadequate. That applies especially to work on vaccines aimed at HIV subtypes circulating in the developing world; only now are more such products entering clinical trials (see article on p.1).

Specifically, the Scientific Blueprint calls on the global community to take the following steps:

- **Introduce 25 new vaccine designs into the development pipeline, compare them in head-to-head clinical trials, and move 6-8 of the most promising ones into efficacy (Phase III) trials.** Since there is still no clear answer on which HIV antigens are needed to confer protective immunity, or which immune responses are required for protection, the fastest way forward is to pursue a variety of different designs in parallel. Head-to-head comparisons should form the basis for choosing the best 6-8 candidates to move forward into phase III trials.

- **Sharply compress timelines for vaccine development, especially by combining phase I and II trials into a single Phase I/II test and by conducting parallel (rather than sequential) clinical trials in high incidence areas of the world.** Other areas to accelerate include vaccine site preparedness and the myriad steps involved in getting regulatory, political and ethical approvals for vaccine trials.

- **Increase spending globally by roughly $US1 billion above current levels over the next seven years.**

IAVI's role in this accelerated effort will involve adding four to eight new "vaccine development partnerships" to the four already supported, each of which aims to move a different vaccine product (all based on strains predominant in developing countries) into human trials. These partnerships – first proposed in IAVI's original "Scientific Blueprint" two years ago – bring together researchers designing a vaccine with developing country partners where the vaccine can be developed further and tested, and with manufacturers who can produce it.

...and Ensuring Access

Another major challenge, beyond developing an effective vaccine, will be to make it immediately available to people in the countries hardest hit by the AIDS epidemic Clearly, it is imperative to avoid a situation like that which exists for anti-retroviral drugs, which are far beyond the means of the vast majority of people in developing countries. The second IAVI blueprint, entitled "AIDS Vaccines for the World: Preparing Now to Assure Access," presents a strategy for addressing the many economic, political and logistical obstacles to immediate and widescale access in the developing world. By beginning to plan and act now, the Blueprint argues, the typical ten or twenty year delay in introducing new vaccines to poor countries can be avoided.

The Blueprint's global action plan calls for the following five steps:

- **Development of effective pricing and global financing mechanisms**. This should begin with a system of tiered prices that make AIDS vaccines affordable in poor countries while allowing higher prices in wealthy ones, where companies can earn profits. In addition, mechanisms for ensuring that funds will be available to buy vaccines for developing countries should be established and supported now.

- **Development of mechanisms to reliably estimate demand for specific vaccines and to ensure sufficient production capacity to meet initial demand for an effective vaccine.** This could be accomplished through an international body that monitors vaccine candidates in clinical trials and, for the most promising ones, instigates studies on their use and distribution outside the region hosting the trial. Comprehensive efforts to predict demand for a given type of vaccine are also critically needed.

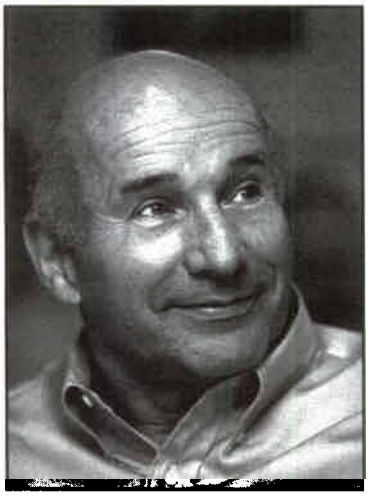
- **Development of appropriate delivery systems, policies, and procedures for the most at-risk populations, especially adolescents and sexually active adults.** This will be a major challenge for countries without well-developed health care systems, since their existing vaccine programs are aimed at infants and pregnant women; those at risk for HIV may have little contact with medical clinics. Creating systems for reaching these populations will demand strong commitment from international agencies, governments and other stakeholders.

- **Harmonization of national regulations and international guidelines governing vaccine approval and use.** While safety standards must obviously be upheld, the myriad of different regulations creates serious bureaucratic obstacles that complicate both international clinical trials and the widespread licensing of effective vaccines.

- **Establishment of a mass vaccination program in developing countries for at least one under-used pediatric vaccine.** This would demonstrate a global commitment to broad use of important vaccines and help establish confidence among those who will need to invest heavily in delivery systems for AIDS vaccines. ◆

AIDS Vaccine Work in Europe: An Interview with Marc Girard

Marc Girard has been in the AIDS vaccine research field since the mid-1980s, working first on chimpanzees and then on macaques as model systems for vaccine testing. Girard headed the French research agency's HIV Vaccine Task Force from 1988-1998 and recently finished a three-year stint on IAVI's Scientific Advisory Committee. In 1999, he left Paris' Pasteur Institute after 22 years to direct the European Research Center for Virology and Immunology (CERVI) in Lyon. Here Girard describes EUROVAC, a newly-launched European AIDS vaccine project he chairs, and discusses his views on primate models in AIDS vaccine development.



Can you tell us about the EUROVAC project and what its goals are?
It started from scratch, when a few European AIDS researchers met in a café during the 1998 Geneva AIDS conference to discuss what we Europeans can do on vaccines.

After much discussion, we decided on some key points. First, that our aim was Phase I trials in humans. Macaques might be useful for some challenge experiments, but our ultimate goal had to be human testing. This meant that we would need to prepare vaccines according to Good Manufacturing Practices (GMP), which takes an industrial partner. We then talked to Pasteur Mérieux [now Aventis Pasteur] and several smaller pharmaceutical companies in Europe, and they were willing to join us.

Next, we decided on a prime-boost combination of a pox virus and a glycoprotein. We will evaluate vaccines based on two attenuated pox viruses, MVA and NYVAC. That directly interested Aventis Pasteur, because NYVAC was their product.

Our choice of clades C and B came only after a lot of heated discussion between the immunologists and the industry people. The immunologists said, why work on clade C virus? We don't have the reagents we need - antibodies, peptides. For clade B we have everything. Industry people said, we want to make a vaccine for developing countries, and clade C causes the most disease globally. The immunologists responded that we're not making a vaccine, we're doing a prime-boost experiment to see what works. In the end we decided to compare clades B and C head-to-head, in CTL responses and cross-clade CTLs. Each of them will be boosted with a clade C gp140.

How did you get European Union support for your plan?
I went to discuss the broad outline of the project in Brussels. They said that it sounded very interesting but they would not pay for clinical vaccine batches. I then told them there would be no project, because we need GMP products to do human testing. Then they said that industry should pay for it. But I did not expect that industry would pay for this – they have their projects, and this work is outside of them.

We finally ended up winning EU support. Our project got the highest marks, and it's the biggest grant [8.8 million Euros] that the EU has ever given for this type of work.

Who else is involved?
Altogether there are 20 groups in 8 countries. [Editor's note: A complete list is available on IAVI's website at *www.iavi.org.*] From the industry side, besides Aventis Pasteur, IDT in Germany will make the MVA and the gp140 will come from an English company called Lonza.

Since we will use standardized assays and centralized labs to analyze the immune responses of the volunteers, we designed three networks – for T-cell analysis, chemokine analysis and antibodies. And we decided to incorporate parallel macaque studies, for two reasons: first, to see whether there is any difference between NYVAC and MVA in their ability to protect, and, second, to support some preclinical studies of other types of vaccines. Most of the animal work will be done by Jonathan Heeney in The Netherlands, plus some in England and Germany.

What is your time frame?
We have milestones for each of the steps, leading to human trials by early 2002. Beyond that, we are also thinking about Phase I/II in developing countries, since we will have the clinical vaccine batches available. There has been a working meeting in China, but it is premature for us to commit ourselves to any site. And we're applying for additional funds to move other candidates forward, such as DNA, Semliki Forest virus or Salmonella vaccine.

How do you see the longer-term role of the EU in this endeavor?
I don't want to sound un-European. But you should realize two things. First of all, it is very difficult to work as a unit in Europe. A united Europe is a nice image, but nations and governments have their own scientific programs. So it's not surprising that most of the support from Brussels so far has been for small research grants. EUROVAC is the first time that a grant of this size has gone to product-oriented research. It's all new for the EU, and the rules do not exist – they are being invented along the way.

Second, there is very little expertise in public health, vaccines, medicine at the European Commission level. There are meetings of the ministers for research, commerce, defense, whatever, from the member countries, but not of health ministers.

So to go beyond research and into vaccine development and clinical trials, we have to rely on the will, dynamism and energy of individuals. The director of research at the European level has a hard time. If he pushes vaccines too far, somebody is going to remind him about the problems in agriculture, or with the big radio telescope. There are many political difficulties and priorities in defining major themes. That's where I think IAVI has a beautiful role to play – as a catalyst and a leader in the field.

How can EUROVAC and IAVI best work together?
EUROVAC has good expertise, good scientists. The products we're developing are not that new. But I think the way we're working – as a group of coordinated networks – is a reasonable way to do this type of research. IAVI, on the other hand, can help with testing the vaccines in developing countries.

Shifting gears to the laboratory, can you tell us about your current research?
I work with non-human primates, mostly rhesus macaques, testing a variety of HIV antigens and DNA vaccines and doing SHIV protection experiments. We have a new SHIV, a CCR-5-dependent virus that has kept the native envelope of the primary isolate it was derived from. It should be interesting to use for challenging the macaques. We are now trying to make it pathogenic by passaging in monkeys.

The field needs a task force of people who agree to standardize assays, share reagents and perhaps exchange samples.

We are also testing whether vaccines with the *rev* and *tat* genes can protect macaques. There have been contradictory results about this – some people claim full protection using these antigens; others claim a partial effect on reducing viral load. So we are testing this using a Semliki Forest virus recombinant vaccine followed by an MVA recombinant, both made in our lab from primary isolates. We should have some results by the end of this year.

Why do protection data from different labs often seem to conflict?
Partly it comes from how the animals are challenged. In our case we use only intrarectal challenges, which test mucosal transmission. Some labs use intravenous challenges. There is also is a wide variety of SHIVs available for challenging. For example, SHIV-MN is a very wimpy virus – it grows only to very low levels, so it's relatively easy for a vaccine to protect against this challenge. At the other extreme, Norman Letvin's SHIV-89.6P is much more pathogenic, and I don't know anyone who can claim that they've protected more than 50% of the animals in a direct challenge.

Another difficulty is how you define protection. I define it to mean that no virus is found by co-cultivation and that viral load is only transient and usually less than, say, 6,000 RNA copies per milliliter. Also, that there are no visible immunological signs, such as a drop in CD4 cell count. But it is obviously not sterilizing protection, which is perhaps not achievable.

Arguably, a wimpy virus might mimic HIV transmission in humans better than a strong pathogen.
Absolutely right.

So how do you decide rationally what strain to use for challenge?
The classical way to develop vaccines has been to set up animal model assays, making clear that these do not necessarily reflect what actually happens in human. This approach goes back to the beginning of the century, with the development of the first bacterial vaccines.

For example, the model for testing pertussis or rabies vaccines is awful. You inject the challenge material intracerebrally into mice. These models have nothing to do with what you want to protect against. But they are still useful for standardizing vaccines, even though they're only a correlate of protection – they say nothing about how the vaccines work.

With HIV there's a tendency to ask a lot from animal protection experiments, to give us a huge margin of security in humans. So people tend to challenge animals with hot viruses.

Is any consensus emerging about standardizing some of these variables, so that data from different labs can be compared?
That is a key point. To be honest, we have been talking about this for several years, but there is still no real standardization. Actually, the trend is that new SHIV strains are being made, and we hear that so-and-so has protected some animals against these viruses whose properties nobody knows. So it's very difficult to assess and compare results.

How useful are monkey data in deciding whether to move a candidate vaccine into human testing?
No single criterion can tell us whether a vaccine should go into human testing. Different factors and priorities will play a role, and the monkey model is one of them. But I don't see primate models as a compulsory route to Phase I.

Still, we would obviously like to see good protection in monkeys. I would feel much more confident if a vaccine protected 80% of my macaques, especially against a strong challenge like 89.6P. But at this point we have no example of human and monkey data on the same vaccine from which to build a model that could guide these decisions. So for now I think it's reasonable to test humans and monkeys in parallel.

What else about candidate vaccines can we learn in macaques?
Macaque data are very important for going past Phase I, even though we cannot extrapolate directly to humans. After all, how else can we see what sort of efficacy is possible, short of

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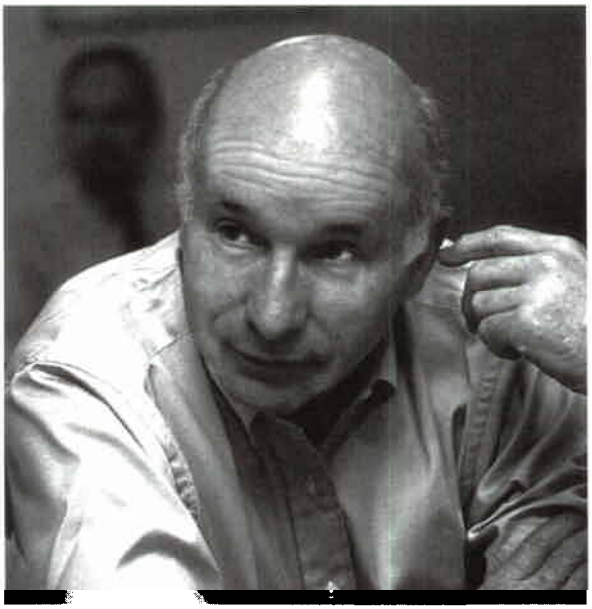
doing a Phase IIB or Phase III trial? You would feel much more confident launching such a trial in human volunteers if the same type of vaccine protects macaques against SIV or SHIV.

Another thing is that there are now SHIVs of clade E, clade A, clade C and of course, a lot with clade B. So you can start asking whether your vaccine provides some broad protection across clades.

To me this is a very important question. I'm a bit anxious seeing that some countries think they must use an isolate from their own country to make a vaccine. This has never happened before for any classical vaccine. To my knowledge, there is no evidence at this point that a vaccine derived from clade A could not work against viruses of other clades.

Why is it a problem to use local isolates?
It can slow down the field. It may also hamper some vaccine development if you have to remake your vaccine each time you change country. Let's not even think of the commercial problems, which could be very difficult.



Perhaps we will find that there are something like HIV 'super-types' that depend on a variety of virus properties, including neutralization type and serotype. But there is no evidence for this now.

There's lots of talk among researchers about shortages of monkeys for testing HIV vaccines.
I don't subscribe to that fear. We're getting our monkeys from China rather than from India, because India does not export macaques any more. Indian monkeys now come from U.S. breeding centers, which imported animals a long time ago. With the possible exception of the Dutch primate center, Europe has no such centers.

Our Chinese rhesus macaques are outbred animals captured from the wild and are less susceptible than Indian macaques to SIV disease. They tend to behave more like long-term non-progressors – they can stay infected for more than two years and nothing happens. No clinical signs. But you can measure viral load, and the CD4 cell counts stay very low. Clinically, animals usually survive for at least two years; many don't die at all, even after a strong challenge like SHIV89.6P that kills Indian macaques in 6 to 9 months.

How expensive is it to work with monkeys?
It's very expensive, although not nearly as much as working with chimpanzees, as we used to do.

But the cost of these experiments is not only the cost of the monkeys. Some of the reagents – for example, for measuring cytokines – are tremendously expensive.

One of the biggest limitations is space for housing the monkeys. At the Pasteur Institute, we have one facility that is now almost national, because it's subsidized by the ANRS. Anyone who wants to do HIV or SHIV experiments in France goes there. But we have only 150 cages, so it fills up fast. The Dutch Primate Research Center has a huge breeding center, but they are also limited by their facilities for housing animals. It's a logistical problem that could be solved with enough dollars, but nobody has made that investment.

Are there ways to make better use of the monkey model?
Yes, by having a task force of experienced people in the field who agree to standardize and validate their assays, share their reagents and perhaps exchange samples. In Europe, this has been done in part by Gerhard Hunsmann, for example, in the early tests of whole killed SIV vaccines. Different groups used the same batch of virus for challenge, but one group challenged the animals vaginally, another intramuscularly, and a third intravenously. So each group had a piece of the pie. It was a smart way of sharing resources and providing more facilities than anyone could have had alone.

So there are ways out. But it can take a lot of negotiation and discussion. And if you have an idea and would like to do a quick test, this approach doesn't work.

How do you see the overall prospects for an AIDS vaccine?
It's the perpetual question of the optimist or the pessimist – whether the glass is half full or half empty. If you're an optimist, like many Americans, then you say, gee, we've come such a long way, it's beautiful, we have many things in the pipeline. If you are pessimist – and perhaps we are more pessimistic in Europe – you say, yes, we've come a long way, but we still don't know how to induce neutralizing antibodies to primary isolates, and we've induced only transient CTL responses – with the possible exception of AAV, which is not yet made into a vaccine. Everything else tried so far, including DNA, does not give great long-term responses.

To get around this we combine different vaccines, and indeed this gives better immune responses. But then imagine the situation in the field. How can you go into a remote country and tell people that they need two shots with DNA, then two shots with MVA, then perhaps two more with gp120? That's just not going to work; it's not a practical vaccine.

It is certainly fantastic progress, but I would remain cautious and say that we have to work much, much harder before we will have a vaccine. ◆

Palm Beach Meeting Highlights New Vaccine Approaches

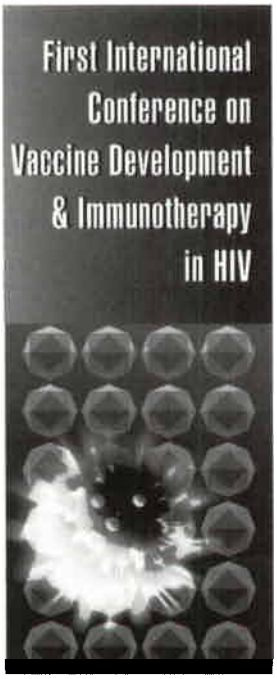
by Vicki Burkitt

A newcomer to the AIDS vaccine meeting scene, the "First International Conference on Vaccine Development and Immunotherapy" was held in Palm Beach, Florida from 28 June - 1 July. Sponsored by the International Medical Press and IAVI, it attracted about 150 researchers. Much of the focus was on vaccine design and testing, including novel vectors and macaques studies, and on immune evasion and correlates of protection. There was also a presentation on the VaxGen trial (see coverage in Durban report, p.3) and several reports about therapeutic vaccines.

Following are selected highlights from the meeting.

Vaccines based on Conserved CTL Epitopes

Mark Newman from Epimmune (San Diego) presented a novel approach to designing DNA vaccines against epitopes that are conserved in different HIV subtypes and recognized by multiple, common class I-restricted HLA alleles (to maximize immunogenicity across diverse populations).



To identify such epitopes, they began by scanning the sequences of different HIV-1 strains to find regions containing the known epitope-binding motif for three common class I HLA alleles (HLA-A2, A3 and B7). Motifs common to many virus strains were then synthesized as peptides of 8-11 amino acids and screened to find those which bound well to the HLA proteins. The 43 (out of 1000) selected peptides were then tested for immunogenicity in mice and humans, the latter according to whether they were recognized by CTLs from HIV-infected people with HLA-A2 or A3 phenotypes. Newman estimated that the resulting 43 peptides would be immunogenic in 87% of the general population.

He also predicted that this type of vaccine would require at least 16 CTL epitopes (2 Gag, 8 Pol, 3 Env, 1 Nef, 2 Vpr) for a single HLA haplotype, or 48 epitopes for three haplotypes (needed to achieve coverage across many populations). Epimmune has prepared a 20-epitope vaccine and is working towards one with 40.

Immune Escape

One concern about prospects for an HIV vaccine is the virus' ability to mutate rapidly and thereby escape host immune responses. But there have been few documented cases of this, especially when it comes to cellular immunity. Here Todd Allen of the Wisconsin Regional Primate Center (Madison, WI) reported a clear case of CTL escape, which revolved around a previously undefined epitope in the Tat protein. He also showed that it occurred several weeks earlier than other known cases. (The work is now published in *Nature 407:* 386-90, 2000).

Allen's team found that when 10 Mamu-A*01-positive rhesus macaques were infected with the pathogenic SIVmac239, they generated CTL responses against four out of six CTL epitopes tested (all from Gag or Tat). But 8 weeks later, all 10 animals harbored SIV variants with mutations in the same Tat epitope; in 5 animals, this variant had completely replaced the original strain. Complete viral sequencing from two animals at 4 weeks showed that, with one exception, the changes in Tat were the only ones present. In Mamu-A*01-negative macaques infected with SIV, 4/8 also showed Tat variants.

Allen also reported that the presence of variants correlated with initial control of viremia. By week 4, when wild-type virus began to be replaced by variant, all Mamu-A*01-positive and negative animals harboring Tat variants had begun to clear their initial, acute viremia. But the Mamu-A*01-negative animals without Tat variants were much less successful: 2/4 of these animals progressed to simian AIDS within 6 months, the only group to do so. Presumably this is because without CTL directed to Tat, there was neither immune pressure to generate escape variants, nor could the animals control viremia as well. Allen concluded that rapidly escaping CTL epitopes such as this one represent promising vaccine targets. (For more on Tat-based vaccines, see article on page 2).

Envelope modifications

Ron Desrosiers' group at Harvard Medical School has developed several classes of mutant SIVmac239 viruses that are more sensitive to neutralization and show altered properties in monkeys compared with the wild-type strain (which is difficult to neutralize). Their findings could therefore have important implications for vaccine design.

One set of mutants was created by removing 2 (out of 7) N-linked carbohydrate attachment sites in the V1-V2 loop of gp120, exposing the underlying protein domains. Macaques infected with one of 3 different mutants of this type (all fully replication-competent) initially showed high viral loads similar to wild-type virus. But by setpoint their load was significantly lower, suggesting that the animals can clear the mutant, neutralization-sensitive virus more effectively.

Similarly, macaques infected with an SIVmac239 strain lacking 5 N-linked carbohydrate attachment sites brought high post-challenge viral loads down to undetectable levels following the appearance of neutralizing antibodies. These monkeys were protected when challenged with SIVmac251, a highly related but heterogeneous and pathogenic virus strain.

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A third class of mutants contained a 101 amino acid deletion that removed the entire V1/V2 region, also resulting in a replication-competent virus that is cleared at viral setpoint, and which Desrosiers said is one of the most neutralization-sensitive SIV strains his lab has encountered. When questioned about the potential of this latter mutant as a live attenuated vaccine, he cautioned that it has not been well-characterized in terms of safety, and said that tests are planned in combination with *nef* deletions, which also attenuate SIV.

Leo Stomatatos from Aaron Diamond AIDS Research Center in New York presented results consistent with Desrosiers' studies. He removed 30 amino acids from the V2 loop of gp140 in a primary HIV isolate. Two rhesus macaques immunized with *env*-DNA from this virus (and boosted with Env protein) generated antibody higher titers against gp140 than did wild-type *env*-DNA. Sera from these monkeys neutralized heterologous primary HIV strains.

Herpes simplex vectors elicit persistent immune responses in humans and might have potential for dual HIV–herpes vaccines

Chiron's Alphavirus Vectors

Thomas Dubensky of Chiron Corporation (Emeryville, California) described the company's program to develop HIV vaccines based on alphavirus replicon particles, in particular Sindbis virus. The advantages of these vectors are that they target human dendritic cells (which play a key role in presenting antigen to the immune system) and that they induce more potent T-cell responses compared to conventional vectors. Chiron has isolated Sindbis variants that grow to high titers in human dendritic cells and is using them for prototype vaccine development. The company has also developed efficient packaging cell lines, overcoming a potential stumbling block for future large-scale vaccine production with this vector.

In further describing the HIV-Sindbis particles, Dubensky reported that constructs expressing HIV p55 *gag* or gp140 *env* infect immature human and mouse dendritic cells very efficiently *in vitro*. Moving to small animals, mice immunized intradermally with Sindbis particles showed infected, immature dendritic cells at the site of injection, and these cells migrated to the draining lymph nodes and underwent maturation. Intranasal immunization was also effective, eliciting high-level expression of CTLs systemically and in draining lymph nodes. Chiron researchers are now testing a variety of vector constructs (expressing *env* and *gag*) in prime-boost regimens in 44 macaques.

Recombinant Herpes Vectors

David Knipe of Harvard Medical School presented rhesus macaque data on two herpes simplex viruses (HSV-1) engineered to express SIV Env and Nef proteins. Interest in this approach stems from the fact that humans maintain persistent immune responses against herpes viruses, so HSV vectors may offer a way to generate long-lasting immunity. Knipe also speculated on their potential for a dual herpes-HIV vaccine, since replication-defective HSV strains have protected mice against HSV challenge.

Here he showed data on 7 macaques immunized (and boosted twice) with either a replication-competent construct, called K81, or the replication-deficient d81, plus 3 control animals. All animals were challenged rectally with homologous SIVmac239 22 weeks after the last boost. At 40 weeks post-challenge, 2 of the 7 immunized animals were completely protected (based on absence of viremia, viral p27 antigen and recoverable virus). Overall, peak viral load levels in immunized animals were 1.2 logs lower than control animals, a statistically significant difference. (These data are now published in *J. Virol. 74:* 7745, 2000).

Knipe's group is now immunizing monkeys with a second-generation HSV-2 recombinant that expresses higher levels of SIV proteins. He also reported that Avant Immunotherapeutics, a Massachusetts-based biotechnology company, is developing his modified HSV-2 strain as a genital herpes vaccine.

Some researchers in the audience expressed concern that vaccination with HSV vectors might result in latent infection. Knipe hasn't fully tested this premise but noted that no viral DNA was detected in mice injected with double-mutant HSV-2 viruses.

Improving the Potency of DNA Vaccines

Jeffrey Ulmer of the Chiron Corporation discussed potential ways to enhance the generally weak immune responses elicited by DNA vaccines. These include modifying vectors to target antigens to specific cellular compartments or using novel adjuvants. But even with improved vaccine designs, Ulmer said, inefficient uptake of antigen by host cells could result in suboptimal vaccines.

In one approach to improving DNA delivery, Chiron is investigating PLG microparticles pre-coated with a positively-charged surfactant that can then absorb negatively charged molecules like DNA (or proteins, virus particles or vectors). Animal studies show some promise. In one set of experiments, modified PLG particles adsorbed with HIV-*gag* DNA were used to immunize mice, which were then challenged with a lethal dose of vaccinia expressing HIV-*gag*. The result was 100-fold more potent CD8 T-cell responses, and 100-1000-fold more antibodies, than animals immunized with naked DNA alone. In macaques, animals primed with p55*gag*-DNA on PLG and then boosted with p55 protein on PLG showed both humoral and cellular responses, while immunization with only p55 protein on PLG failed to induce CTL. Ulmer also noted that DNA-PLG particle delivery targets antigen-presenting cells. ◆

Vicki Burkitt is a U.S.-based science writer whose work has appeared in GMHC Treatment Issues, HIV Plus *and other publications.*

IAVI Restructures its Scientific Advisory Committee

In Summer 2000, IAVI's Scientific Advisory Committee (SAC) was reorganized so it can better guide the organization's research and development program, which has grown considerably since IAVI's founding in 1996. In addition to the main advisory committee, there are now three sub-committees overseeing specific areas: HIV vaccine science; international clinical trials and project management for IAVI's vaccine development partnerships. Each sub-committee is chaired by a SAC member and consists of 5-10 additional scientists, along with an executive secretary from IAVI's Research and Development staff.

The following is a complete list of SAC and sub-committee members.

SCIENTIFIC ADVISORY COMMITTEE | Chair: **Jaap Goudsmit**, University of Amsterdam, Netherlands

Members:

- **Ian Gust**, University of Melbourne, Australia
- **Philip Johnson**, Children's Research Institute, Columbus, Ohio, USA
- **Norman Letvin**, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts, USA
- **Edward K. Mbidde**, Uganda Cancer Institute, Kampala, Uganda
- **Andrew McMichael**, University of Oxford, UK
- **Neal Nathanson**, University of Pennsylvania, Philadelphia, USA
- **Vulimiri Ramalingaswami**, All India Institute of Medical Sciences, New Delhi, India
- **Helen Rees**, Greater Johannesburg Metropolitan Reproductive Health Research, Johannesburg, South Africa
- **Jerald C. Sadoff**, Merck Research Laboratories, West Point, Pennsylvania, USA
- **Mauro Schechter**, Universidade Federal do Rio de Janero, Brazil
- **Hans Wigzell**, Karolinska Institute, Stockholm, Sweden

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- **Edward Mbidde**, Uganda Cancer Institute, Kampala, Uganda
- **Frank Plummer**, University of Nairobi, Kenya
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- **Haynes Sheppard**, California Dept. of Health Services, Berkeley, California, USA
- **Sriram Prasad Tripathy**, Pune, India
- **Hilton Whittle**, MRC Laboratories, Banjul, Gambia
- **Janet Wittes**, Statistics Collaborative, Washington, DC, USA

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- **Rafi Ahmed**, Emory University, Atlanta, USA
- **Frances Gotch**, Chelsea & Westminster Hospital, London, UK
- **Carl Hanson**, California Dept. of Health Services, Berkeley, USA
- **Shiu-Lok Hu**, University of Washington, Seattle, USA
- **Jan Holmgren**, Göteborg University, Göteborg, Sweden
- **Philip Johnson**, Children's Research Institute, Columbus, Ohio, USA
- **Norman Letvin**, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts, USA
- **Mike Levine**, Johns Hopkins University, Baltimore, Maryland, USA
- **Neal Nathanson**, University of Pennsylvania, Philadelphia, USA

PROJECT MANAGEMENT | Chair: **Ian Gust** | Executive Secretary: **Chip Carnathan**, IAVI

Members:

- **Marie-Paule Kieny**, INSERM, Strasbourg, France
- **Jack Melling**, IAVI consultant, Stroudsburg, Pennsylvania, USA
- **John Petricciani**, IAVI consultant, Palm Springs, California, USA
- **Stanley Plotkin**, University of Pennsylvania, Doylestown, USA
- **Jerald B. Sadoff,** Merck Research Laboratories, West Point, Pennsylvania, USA

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years, stemming from the fact that Tat is highly conserved (and therefore might induce cross-clade immune responses) and is expressed very early in the viral life cycle, before the more well-studied vaccine antigens such as *env* and *gag*.

IHV Director Gallo prefaced these talks by reviewing the ongoing debate surrounding Tat-based vaccines (see article on p.2). While Barbara Ensoli and colleagues at the Istituto Superiore di Sanità in Rome (the Italian NIH) reported that they have completely protected 5 out of 7 cynomolgus monkeys by immunizing with the native Tat protein (*Nature Med. 5*: 643-650, 1999), Norman Letvin (in a collaboration led by John Shiver at Merck Laboratories) has unpublished negative results with a Tat protein vaccine in rhesus macaques. In a third study, David Pauza of the University of Wisconsin, together with Gallo, reported results between these two extremes, using either a chemically inactivated form of Tat (called Tat toxoid) or native Tat protein to immunize rhesus macaques. In 15 vaccinated animals that became viremic after challenge, Tat-specific T-cell and neutralizing antibody responses appeared to partly control SHIV replication, resulting in lower viral load set points in immunized animals. However, differences in CD4+ T-cell declines between control and immunized animals did not reach statistical significance over 8 weeks of follow-up. These data were published earlier this year (*PNAS 97*: 3515-3519, 2000).

Cellular immune responses correlated with the ability of a DNA/MVA prime-boost combination to clear virus in challenged macaques.

Although not mentioned by Gallo, Albert Osterhaus and coworkers at Erasmus University (Rotterdam) have also published on a vector that incorporated the *tat* gene. Osterhaus's prime-boost regimen, utilizing the Semliki forest virus (SFV) and the modified vaccinia Ankara virus (MVA) as vectors and the SIV *rev* and *tat* as antigens, protected cynomolgus monkeys from a challenge with a pathogenic SIV (*Vaccine 17*: 2713-4, 1999).

New Data on Tat Vaccines

While these and other studies agree that vaccines can induce Tat-specific immune responses, the type of response and degree of protection they confer remains unclear. Aurelio Cafaro from Ensoli's group presented results on a *tat*-DNA vaccine from an unpublished part of the Nature Medicine study. Four cynomolgus monkeys were given 8 intramuscular (i.m.) immunizations while one animal was vaccinated intradermally (i.d.). Animals were then challenged intravenously (i.v.) with an SHIV 89.6P isolate adapted (by passage through one animal) to the cynomolgus macaque subspecies. Five control animals (2 naïve animals, 2 that received RIBI or alum adjuvant alone and one that received a DNA vector not encoding *tat*) were also challenged.

The researchers found that all i.m. vaccine recipients appeared to be completely protected. The i.d.-vaccinated animal and 4/5 controls all became infected, as determined by measurable plasma viremia, persistent antibodies to SHIV and a steep CD4 T-cell decline. In terms of immune responses, the i.m.vaccinated animals showed Tat-specific, Th1-type cellular immune responses (CTL and non-CTL CD8-mediated antiviral responses), which reportedly correlated with protection from overt SHIV infection. None of these animals had detectable Tat-specific antibodies. In contrast, the i.d. immunized animal (which was not protected) displayed anti-Tat antibodies but no detectable CTL prior to challenge. After challenge, antibodies to SHIV were detected in all the animals, which the researchers cited as confirmation that the challenge dose was sufficient to expose all animals to virus.

A confounding piece of data was that the control monkey given DNA vector alone also appeared to be protected, although unlike the i.m. vaccine recipients, virus could be isolated from the blood. In comments to the *IAVI Report*, Ensoli stated that the DNA vector sequence is rich in potent adjuvants called CpG motifs, and she suspects that a non-specific stimulation of innate immune function may explain this surprising protection. Some support for this notion comes from a possibly related phenomenon seen with malaria, where administering the cytokine IL-12 two days prior to challenge with the malaria-causing organism protected macaques against disease (*Nature Med. 3*: 80-83, 1997). A larger trial that includes monkeys immunized with either the unmethylated or methylated form of the CpG sequences is now in progress.

DNA/MVA prime boost vaccines in monkeys

Gunnel Biberfeld from the Karolinska Institute (Stockholm) presented new data from a prime-boost vaccination study with a DNA vaccine followed by an HIV/SIV-MVA. The DNA construct encoded multiple HIV-1 antigens, including *env, gag, nef, pol, rev* and *tat*; the MVA vector contained *nef, tat* and *rev* from HIV-1, along with *gag* and *pol* from SIVmac J5. Cynomolgus monkeys received the DNA vaccine either i.m. or by a mixture of routes (i.m., intrarectal and dental gun delivery to the oral mucosa) followed by an MVA boost and treatment with granulocyte-macrophage colony-stimulating factor (GM-CSF). One month later, they were challenged i.v. with SHIV 4, a non-pathogenic virus.

The results showed evidence of complete protection in one animal, based on the absence of detectable viral RNA and inability to isolate virus from the peripheral blood. One month after challenge, 3 more animals brought their viral load levels down to undetectable levels. These 4 animals came from the group that received vaccination via multiple routes. Two monkeys from the i.m.-only group also cleared virus in the weeks following challenge, while the third maintained low but detectable viremia. The fourth animal in this group had an outcome similar to controls: SHIV RNA stayed at high levels and virus could always be isolated from the blood.

The protective effects appear to be mediated by cellular and not humoral immune responses, according to Biberfeld, since neutralizing antibodies were "poor" in all animals prior to challenge. She also found that the combination of mucosal and i.m immunization induced stronger cellular responses than i.m.-only, both in terms of proliferative responses (which measures CD4+ helper T-cells) and ELISPOT assays of virus-specific CD4+ T-cells. Biberfeld also measured virus-specific CD8+ T-cells by ELISPOT, finding that 3/4 animals in the mixed-route immunization group had significant responses after the third vaccination.

Peptide-based vaccines

In a contrast to the many DNA vaccine studies, Jay Berzofsky of NIH presented studies on peptide vaccines containing an Env helper epitope portion and one Env CTL epitope. First, he reported that a mutated form of an adjuvant called LT (*E. coli* heat-labile enterotoxin) boosted responses to these peptides in mice by maintaining higher levels of IL-12, which appear to work synergistically with other cytokines in driving cell-mediated immune responses to the vaccine. In a macaque challenge experiment, animals given a similar vaccine (substituting the Env CTL epitope with 3 CTL epitopes from SIV Gag and Pol) plus adjuvant (intrarectally) developed Gag-specific CTL responses in the mesenteric lymph nodes and colon. After intrarectal challenge with SHIV KU, the immunized animals showed enhanced control of viral load. Berzofsky observed a correlation between virus-specific CD4 T-cell and CTL responses.

New approaches to live-attenuated vaccines

Barbara Felber from the National Cancer Institute (Frederick, MD) has created a novel attenuated SIV by blocking the activity of the Rev protein, which facilitates export of the virus from infected cells by binding to the Rev-responsive element (RRE) in the SIV genome. This process can be blocked by replacing RRE with a similar segment, called CTE, from type D retroviruses. Felber infected neonate macaques with an SIVMac239 isolate that had CTE substituted for RRE. After a transient spike in viremia, all animals appeared to clear circulating virus (by the criteria of PCR and virus isolation). CD4/CD8 ratios remained normal and the animals have stayed healthy for over 200 weeks. Six monkeys were then challenged with SIVMac251 and found to control viremia to less than 2,000 copies per ml of blood, which is the lower cutoff for the assay. Follow-up was out to 30 weeks at the time of the meeting and is still ongoing.

Ron Desrosiers, who pioneered live attenuated HIV vaccine research, presented data on genetically engineered SIV strains containing deletions in carbohydrate-binding sites within the *env* gene. These results were also described at the Palm Springs, Florida meeting in late June (see article on p.13).

Vaccines containing *vpr*

While negative results often fail to get published, David Weiner of the University of Pennsylvania (Philadephia) reported on an interesting phenomenon relating to the HIV *vpr* protein, which is incompletely understood but known to affect early events in T-cell activation. Using a DNA vaccine encoding *vpr*, *nef* and *gag/pol*, Weiner found that *vpr* reduced T-cell responses (both Th1 CD4+ and CTL) to all the vaccine antigens. Upon challenge, rhesus macaques that received *vpr*-containing vaccine developed high viral loads, rapidly lost CD4 cells and suffered a dramatic inversion of their CD4/CD8 T-cell ratio. In contrast, animals that received vaccine encoding HIV genes without *vpr* showed a 3-log drop in viral load and no CD4 cell loss post-challenge. While the take-home message may primarily be that *vpr* is detrimental for vaccine constructs, Weiner's results also raise interesting questions on the role of this gene in HIV pathogenesis.

Neutralizing antibodies in HIV-infected people

In one of the departures from challenge experiments, Guido van der Groen from the Institute of Tropical Medicine in Antwerp, Belgium gave a talk entitled, "To Neutralize or Not to Neutralize: That's the Question." He began by reviewing two published papers that reported finding antibodies with broad cross-neutralizing (BCN) activity in apparently rare HIV-infected individuals. The first study, published by Nyambi and colleagues (*J. Virol 70*:6235-43, 1996), analyzed sera from 27 individuals and discovered one with antibodies that neutralized several primary isolates from HIV-1 subtypes A-H and two from group O. A later study by van der Groen and colleagues (*J. Med Virol. 62*:14-24, 2000) found BCN antibodies in 7 out of 66 samples tested. Intriguingly, 6 of the 7 samples were from African women while just one was from a European male.

Van der Groen's group also looked at 168 different blood samples and found 16 with antibodies showing some degree of BCN (13/16 could neutralize 11 different primary isolates). African women accounted for 10 of these samples, while 5 were from African men and only one from a European male. Further analysis revealed that BCN activity was found more often in sera from people infected with recombinant HIV-1 strains. Van der Groen interprets these antibodies as evidence that "conserved linear and/or conformational epitopes exist!" (his emphasis) and that researchers need to look harder for immunogens that can elicit BCN antisera.

Susan Zolla-Pazner from New York University reached a similar conclusion, noting that HIV genotypes (subtypes) do not necessarily correspond with serotypes. In other words, antibodies derived from a person infected with one subtype of HIV-1 can sometimes cross-react with other subtypes but not with other isolates from the *same* subtype. Zolla-Pazner parsed isolates from subtypes A-H into three broad "immunotypes" based on binding antibody reactivity to various *env* epitopes and concluded that "there are epitopes that are shared by all immunotypes." ◆

Richard Jefferys is a treatment educator and director of the Access Project at the AIDS Treatment Data Network in New York. He has written about HIV research for several publications including CRIA Update, HIVPlus, POZ *and the* PRN Notebook.

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with placebo) and analyzed CTL responses against the vaccine antigens and against Gag and Pol from two non-matching subtypes, A and D.

Cao reported that immunogenicity was similar to earlier trials, with 4/20 of the volunteers positive for CTLs at some point during the study. In the 4 positive individuals, CTLs were no longer detectable 100 days after the last vaccination. CTLs in 2 of the 4 responders recognized subtypes A and/or D antigens (at about 80% of the level seen against B). Results were confirmed with the ELISPOT assay.

Luwy Musey from the University of Washington (Seattle) presented data showing that the ALVAC vCP205 vaccine can also induce mucosal responses. Although mucosal immunity is widely viewed as potentially important for protection, it has not been monitored so far in HIV vaccine trials. Musey analyzed immune responses in 12 participants of a Phase II study (AVEG202/HIVNET014), 6 of whom had CTLs in the blood at some earlier point in the trial. Seven of these volunteers received vaccine and 5 received placebo. HIV-specific mucosal responses (measured by CTL in rectal tissue) were seen in 4/7 vaccinated people, with 2 of the 7 showing both blood and mucosal CTLs; 3/7 had CTLs only in the blood. Mucosal response was not affected by a gp120 boost. Results were confirmed with the ELISPOT assay.

HIV Diversity

In two talks, including a plenary lecture, Francine McCutchan of the Henry M. Jackson Foundation (Rockville, Maryland) reviewed the current state of knowledge about HIV variation and the challenge that continuous, rapid evolution of the HIV genome poses for vaccine development.

McCutchan pointed out that new molecular data are changing the view of how this diversity arises. While previously attributed mostly to HIV's high mutation rate, it is now emerging (from analysis of the many new full-length genome sequences becoming available) that recombination also plays a major role. Moreover, the likelihood of inter-subtype recombination is rising as subtypes continue to spread worldwide and more regions have multiple subtypes in circulation. (For example, non-B subtypes are increasingly seen in western countries, while 4 or more subtypes are common in parts of western Africa).

Based on analysis of 145 full-length sequences, 65 of them previously unreported, McCutchan described some important patterns. Many recombinants seem to be unique to one person, and some show an unexpectedly high complexity (e.g., an AFGJK recombinant). Other recombinants have entered the circulation in certain regions and are as common as some local subtypes; for instance, 56% of the circulating HIV in Cameroon is a circulating recombinant form (CRF) called CRF02.AG. Other examples include the AE strain in southeast Asia, a BC recombinant in China and an AB form in Russia's IDU population. In the latter group, where infection rates are skyrocketing, McCutchan noted the highly puzzling fact that there is little genetic diversification of the transmitted strain - a stark contrast to all non-IDU transmission chains that have been followed, including blood-borne chains such as the Florida dentist group or the Sydney blood transfusion cohort.

Addressing the implications of these findings for vaccine development, McCutchan said that comprehensive data on the pool of circulating strains is crucial for designing vaccines based on local strains, especially in regions with multiple subtypes. Without it, there is a danger of basing designs on a unique recombinant isolate rather than an important circulating strain. But the central issue for vaccines remains just how much the diversity of HIV sequences affects immune recognition of the encoded proteins, and consequently, whether vaccines based on one strain will protect against others.

Should vaccines always match local strains?

Focusing on this uncertainty, an interesting debate session featured two speakers presenting opposing views on whether vaccines tested in the developing world should always be based on an important subtype in the host country.

Rosemary Musonda of the Tropical Diseases Research Center (Ndola, Zambia) made the case against requiring a match. There is ample evidence that vaccines based on one subtype can elicit immunity against other subtypes, she said, especially in terms of cellular immune responses. With the need for a vaccine so urgent, unnecessary restrictions on trials should be avoided. And it is only by testing such "mismatches" that the true relevance of subtype to vaccine efficacy can be clearly determined.

If a "matched" vaccine is found to be effective in a Phase III trial, will countries with other subtypes have to repeat the trial?

Arguing the other side, Carolyn Williamson of the University of Cape Town stated that neutralizing antisera from HIV-infected people appear to work better against virus of the same subtype than against unmatched subtypes. Such data suggest that matching will maximize the chances of success for a candidate vaccine. It may also contribute to community acceptance of vaccine trials, since it sends a message that the test vaccines are genuinely geared to local needs.

During the discussion, audience members raised some key questions. If a matched vaccine proves to be effective in a Phase III trial, what's next? Will countries with other subtypes in circulation have to repeat the trial? And what if new subtypes or recombinants enter the region, or vaccinated people move to areas where different subtypes predominate? Matched trials will not test whether there is protection under these circumstances. Session moderator Peggy Johnston of the U.S. NIAID made the crucial point that even if a vaccinee responds less strongly to an unmatched HIV subtype, this weaker response might still be enough to protect. Vaccinologist Don Burke of Johns Hopkins University (Baltimore) responded that a similar dilemma facing developers of a vaccine against Japanese encephalitis virus was addressed by a Phase III trial with three arms: matched vaccine, unmatched and placebo. It is likely that HIV vaccine trials will ultimately require a similarly systematic approach.

Immune correlates

Clinical studies of people exposed to HIV but seronegative (ESN), which have been done mostly in commercial sex workers, have pointed to key role for cellular immunity in this apparent protection. Lawal Garba of the University of North Carolina (Chapel Hill) presented new ESN data from a collaborative study of discordant couples in Zambia. Looking at 37 ESN individuals (all married or in steady partnerships with infected people for at least 3 years), the researchers found HIV-specific CTL in 9 of them. Intriguingly, the presence of CTL was correlated with high viral load in the infected partner, suggesting that total antigen dose may be an important aspect of stimulating CTLs.

Y.-M. Chen (Taipei) reported an observation which could imply the presence of enhancing antibodies to Tat in some HIV-positive people. (For a discussion of enhancing antibodies, see *IAVI Report*, April-June 2000, p.5). The researchers looked at transmission to the wives of 52 HIV-positive men drawn from blood bank donors who became infected primarily through contact with commercial sex workers. Among 52 men, 1 of 17 infected with subtype B, 14 of 33 with subtype E and 2 of 2 with subtype C transmitted virus to their wives. The transmitters had a higher level of anti-Tat antibody than non-transmitters (65% vs 26%), as determined by ELISA tests. The odds ratio of men infected with subtype E transmitting to their wives if they also had anti-Tat antibodies was extremely high (OR=18). The researchers are now analyzing whether anti-Tat antibodies might selectively enhance infectivity of different HIV subtypes. All current Tat vaccines have used subtype B *tat* genes, and enhancement of transmission has not been previously reported. ◆

OXFORD TRIALS
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became the first person to be injected with the vaccine. Pending approvals from the appropriate Kenyan authorities, it is hoped that the DNA vaccine will enter human trials in Nairobi early in 2001.

The vaccine candidates are being moved into clinical studies by the research teams of Andrew McMichael at Oxford University and J.J. Bwayo at the University of Nairobi. Both vaccines are designed to generate HIV-specific cellular immune responses, which researchers increasingly believe can provide some protection against HIV infection or disease progression. Koff made the announcement of the MVA vaccine approval in Bonn, Germany, noting that "two IAVI-sponsored vaccine candidates have now moved from concept to clinic in less than two years, near record time for these type of products." He added that the HIV MVA vaccine is the first of its kind to be approved for human testing. ◆

NEW VACCINE STUDY
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vaccine as an immune therapy, both in SIV-infected monkeys and HIV-infected individuals. Letvin himself supports the idea of such studies, but says he may be unable to do so himself. Yet there is clearly interest from the outside in seeing the therapeutic approach pursued: Greg Gonsalves of the New York-based Treatment Action Group has already written to Letvin to request that the vaccine approach be moved quickly into therapeutic trials.

On the whole, there is no question that Harvard study will have an impact on AIDS vaccine development. It also begins to show how the newer, more precise methods of quantifying T-cell responses will assist researchers in evaluating candidate HIV vaccines. These tests – known as tetramer binding and ELISPOT assays – will hopefully enable researchers to evaluate and compare a new generation of more potent vaccines, including cytokine-augmented HIV DNA vaccines in human studies.

"The study represents a major advance toward making a vaccine that really works," says Neal Nathanson, the former director of the U.S. NIH's Office of AIDS Research. And, he predicts, "it will help energize the whole field." ◆

IAVI REPORT

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The *IAVI Report* is published bi-monthly by the International AIDS Vaccine Initiative. To obtain a subscription to *IAVI Report*, send name and address, by e-mail to: iavi report@iavi.org; by fax to: 1-212-847-1112; by mail: IAVI, 110 William Street, 27th floor, New York, NY 10038, USA.

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 three key areas: accelerating scientific progress; education and advocacy and creating a more supportive environment for industrial involvement in HIV vaccine development.

IAVI is a UNAIDS Collaborating Centre. Its supporters include the Rockefeller, Alfred P. Sloan, Starr, Bill and Melinda Gates, Until There's A Cure and Vincent P. Beloussky, jr. Foundations, as well as the U.K., Dutch, Canadian, Irish and U.S. governments, the World Bank, UNAIDS, the National AIDS Trust and Fondation Marcel Mérieux. IAVI has also received support from Crusaid, the Elton John AIDS Foundation, Levi Strauss International, and other generous corporate and individual donors around the world.

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Industry Insider

SmithKline Bio Aiming For Phase III Vaccine Trials in Three Years

The president of SmithKline Biologicals, Jean Stephenne, reported that his company hopes to have vaccines for both HIV and malaria in Phase III trials within three years. Stephenne made these comments at the European Commission (EC)-sponsored "Roundtable on HIV/AIDS, Malaria, Tuberculosis and Poverty Reduction" on 28 September in Brussels. The Roundtable included representatives from the EC and its member states, and from a number of developing countries, multilateral agencies, research institutions, NGO's and industry.

SmithKline Herpes Vaccine May Work in Women Only

Two Phase III trials suggest that a vaccine developed by SmithKline Biologicals protects women, but not men, against genital herpes. According to data released at the Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) on 17-21 September in Toronto, the vaccine protected about 73% of women against genital herpes but did not appear to protect men or women who had previously been infected with HSV-1. The vaccine contains a recombinant glyocoprotein of herpes simplex virus-2 (HSV-2) and SmithKline's new SBAS4 adjuvant. These unexpected results highlight the need to enroll sufficient numbers of women in AIDS vaccine trials so that potential gender differences can be detected.

Targeted Genetics Announces New Deals

In August, Targeted Genetics Corp. announced that it would buy Genovo, a privately-held Pennsylvania-based gene therapy company. According to the Seattle-based Targeted, the acquisition will provide it with four new gene therapy candidates as well as valuable patents on the production and use of adeno-associated virus (AAV) vectors for gene therapy. The company is conducting human studies of gene therapy for cystic fibrosis and cancer and is working with IAVI and researcher Phil Johnson of the University of Ohio (Cincinnati) to develop an AAV vaccine for HIV.

Targeted also announced a collaboration with biotech company Biogen that will enable it to develop additional gene therapy products. Genovo collaborated with the University of Pennsylvania's Institute for Human Gene Therapy (Philadelphia) in a gene therapy study that led to the death of 18-year old participant Jesse Gelsinger last year, and which is now the subject of controversy and litigation.

AlphaVax Gets New Financing

AlphaVax announced that it has raised US$11 million in financing from U.S. and European-based investors. The North Carolina-based biotech company is developing an AIDS vaccine based on a Venezuelan equine encephalitis (VEE) vector, with backing from IAVI, the U.S. National Institute of Allergy and Infectious Diseases (NIAID) and the Walter Reed Army Institute for Research. AlphaVax hopes to begin human trials of its first HIV-VEE prototype vaccine in the first half of 2001. "With these funds, we can initiate new product programs, as well as begin taking up second-generation systems and new technologies," said Peter Young, the company's president.

ProdiGene to develop plant-based HIV vaccine

ProdiGene, a Texas-based biotechnology company that is developing a number of edible plant vaccines, has received an NIH grant to develop a plant-based HIV vaccine. The HIV grant will allow the company to engineer maize seed (corn) that expresses HIV gp120. According to ProdiGene's president John Howard, the corn seeds could also be designed to express codon-optimized genes and DNA vaccines and to be part of any corn product (cooked or uncooked), including cornmeal. Plant vaccines offer several potential advantages: they are cheap to produce, do not need refrigeration; can be grown in virtually every country and require no needles to administer.

Howard also presented data at the Millennium Second World Congress on Vaccines and Immunization, held on 29 August –1 September 2000 in Liege, Belgium, showing that a corn vaccine against porcine gastroenteritis virus seemed to offer some protection (measured as longer survival) in immunized pigs. At the same meeting, researchers from Cornell University (New York) reported that edible potato vaccines for *E. coli*, Norwalk virus and hepatitis B have entered human trials, and that all three generated measurable immune responses.

Four Companies Get NIAID "Team" Grants

Four companies were awarded "HIV Vaccine Design and Development Team" contracts by the U.S. NIAID to pursue different strategies for developing DNA vaccines. The contracts, totalling US$70 million over five years, were awarded to: Advanced BioScience Laboratories (for vaccines containing envelope proteins from multiple HIV strains); Chiron Corporation (to combine DNA vaccines with a protein or an alphavirus vector boost); the University of New South Wales (Sydney) and Virax (an Australian company developing a DNA vaccine to use with a fowlpox vector); and Wyeth Lederle Vaccines (for DNA vaccines that also containing a cytokine or other immunomodulatory protein, and for an HIV peptide vaccine).

NIAID also awarded a two year, US$4 million grant to Progenics (Tarrytown, New York) to develop novel envelope antigens.

In related news, Virax expects to begin trials of its fowlpox vaccine in HIV-infected volunteers by the end of this year. ◆