IAVI REPORT

A Newsletter on International AIDS Vaccine Research

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Canarypox HIV Vaccine Trial Set to Begin in Uganda

Delays and the death of a key Ugandan immunologist unlikely to derail Africa's first HIV vaccine trial

After years of preparation and discussion, the first HIV vaccine trial in Africa appears ready to begin. The trial, a Phase I study of Pasteur Merieux Connaught's ALVAC vCP205, is being conducted by the Ugandan Ministry of Health, Makerere University (MUK) and Case Western Reserve University (CWRU) and is sponsored by the U.S. National Institute of Allergy and Infectious Diseases (NIAID).

The study has been approved by the Ugandan Parliament and reviewed by the AIDS Research Subcommittee. The approval of two committees established by the Ministry of Health specifically for scientific and ethical review of vaccine trials is still needed. According to Roy Mugerwa, a researcher at MUK and one of the trial's principal investigators, "The trial is ready to go. We are just waiting for a few formalities."

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IAVI Launches Campaign for Global HIV Vaccine Purchase Fund

In an effort to stimulate private sector investment in HIV development and help ensure access to HIV vaccines once they are developed, the International AIDS Vaccine Initiative (IAVI) has launched an international campaign to create a Global HIV Vaccine Purchase Fund.

Over the past year, IAVI leaders have worked to build support for a Vaccine Purchase Fund among international agencies and G-8 and G-77 governments. The fund would provide developing countries with funds to purchase HIV vaccines, once candidate vaccines are demonstrated to be effective in humans.

The fund would also help assure pharmaceutical and vaccine companies of a commercially viable market for HIV vaccines in developing countries. Through grants and/or loans provided by industrial nations and international agencies, the Purchase Fund would, in effect, create a worldwide guaranteed paying market for HIV vaccines.

Historically, vaccines have been created primarily for industrialized country markets and have only "trickled down" to those in developing countries years after receiving marketing approval (and coming "off-patent"). If this paradigm were to continue, once an HIV vaccine is developed, the results would be disastrous for poorer countries hard-hit by the epidemic.

For this reason, a growing number of public health authorities are suggesting that mechanisms

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VACCINE BRIEFS

Still No Director for NIH's Vaccine Research Center; Search Reopened

The U.S. National Institutes of Health (NIH) is re-opening its search for a director of the new Vaccine Research Center. The center was announced with great fanfare by U.S. President Bill Clinton in May, 1997. At the time, NIH officials had predicted that a highly qualified vaccinologist from outside the agency would be selected within six months. After the initial search process stalled, a number of respected internal NIH researchers were seriously considered for the post and at least one was interviewed by NIH Director Harold Varmus. However, Varmus apparently decided against hiring the researcher and the search is now being reopened. In doing so, Varmus risks taking political heat for not having filled the post a full year after it was first announced. On the other hand, to his credit, it appears that the NIH Director is actively involved in the hiring process and committed to finding a respected outside candidate with experience in developing vaccines.

Will it be possible? Some insiders are skeptical about how much real authority the new position will control. But NIAID Director Anthony Fauci told the *IAVI Report* that NIH was prepared to offer the director the top salary available for a federal position (in the range of US$150,000 per year). Fauci added that "we are prepared to give the person the ball, the resources and the best financial package the U.S. government can offer."

Mann's Criticism of Varmus and Baltimore Stirs Controversy

Speaking before a meeting of the U.S. Presidential Advisory Council on HIV/AIDS (PACHA) in March 1998, Jonathan Mann, former director of the World Health Organization's Global Program on AIDS, accused the U.S. government of failing to proceed with trials of AIDS vaccines and said incompetent leadership at the NIH was directly responsible. He called these actions human rights violations.

Mann identified David Baltimore, head of the NIH's AIDS Vaccine Research Committee and Harold Varmus as two of the main barriers to progress in AIDS vaccine development, suggesting that they want "all the answers before preceding to trials. While David Baltimore has made tremendous contribution to science, he is not equipped to develop an AIDS vaccine," Mann stated. "It is as if the U.S. government has decided that the AIDS epidemic could be controlled without a vaccine. In contrast, progress towards Lyme vaccine development, to protect against a disease affecting middle and upper-class populations, has proceeded rapidly."

The former WHO official sent a copy of his remarks to Baltimore with a request for a response. In his reply, Baltimore called "making vaccine testing a human rights issue the ugliest form of rhetoric I can imagine." He noted that "in the debate over vaccine testing, instead of discussion of facts or probabilities, it is all rhetoric about empiricism and scientists who insist on all of the facts before moving. That is simply a way of avoiding talking about the issue."

Baltimore added that vaccine candidates moving along are focused on inducing CTL response "and I can't wait for them to reach the stage where they are ready for efficacy trials." He concluded by admitting that "there are problems with the vaccine effort, but there are people trying to grapple with them and they need help, not rhetoric."

According to Bruce Weniger, a member of PACHA, "there was some support on the Council for Mann's comments.". However, most of the scientific community, as well as activist groups, appear to consider the personal attack on Varmus and Baltimore to be unfair and inaccurate. Gordon Douglas, President of Merck Vaccines, stated that "Jonathan's comments appear to be made without any understanding of the scientific issues involved in vaccine development." He also described Mann's remarks about a Lyme vaccine as "inaccurate", noting that "Lyme disease was originally identified in 1975, well before HIV, and we still don't have an approved vaccine." Steve Wakefield of the AIDS Vaccine Advocacy Coalition (AVAC) said that "irresponsible accusations of incompetence and classism do not constitute effective research advocacy." And in a letter appearing in *Science* (8 May 1998), over fifty leading AIDS researchers and activists stated that "Slow progress is frustrating to all, but attacking the NIH and the scientists who are working on the problem serves no useful purpose."

New Trials Initiated in Thailand and the U.S.

The Royal Thai Army has begun a study of a clade E version of Chiron's gp120 vaccine. The vaccine is being tested alone and in combination with a clade B gp120 vaccine, and is based on primary isolates of HIV. The trial is being sponsored by Thailand's Armed Forces Research Institute of Medical Sciences, Mahidol University and the U.S. Walter Reed Army Institute of Research. A total of 386 volunteers will participate in the trial. To date, the study is 60% enrolled. In Thailand, clade E predominates, although clade B is also seen in particular sub-populations, primarily injection drug users.

In the U.S., three new vaccine trials were initiated by NIAID's AIDS Vaccine Evaluation Group. The trials will enroll a total of 167 human volunteers. The following vaccines are being studied: Mucosal vCP205: Pasteur Merieux Connaught's vCP205, is a weakened canarypox virus that has been genetically engineered to carry HIV proteins (see page 8). The vaccine will first be given as an injection and then swabbed or dripped onto

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Vaccines Low on the Agenda at 5th Retrovirus Meeting

by David Gold

On 1-5 February 1998, the 5th Conference on Retroviruses and Opportunistic Infections was held in Chicago. The conference, considered to be the most important annual scientific gathering on AIDS, is sponsored by the Foundation for Retrovirology and Human Health, in collaboration with the U.S. National Institute of Allergy and Infectious Diseases (NIAID) and Centers for Disease Control and Prevention.

This year's Retrovirus Conference was similar to previous year's gatherings in one key respect: very little new data was presented on HIV vaccines. To be sure, David Baltimore, head of the U.S. NIH AIDS Vaccine Research Committee (AVRC), gave a plenary presentation on vaccines. (In little more than a year, Baltimore has given plenary talks on vaccines at four AIDS meetings.)

But beyond Baltimore's generally well-received presentation, new data or discussion on HIV vaccine research was hard to find. Presentations by industry scientists and non-U.S. vaccine researchers were especially lacking. In total, out of thirty featured slide sessions, only two were devoted to HIV vaccine research.

Listed below is a summary of key data on HIV vaccines that was presented at the meeting.

Early CTL Response May be Key to Protection

In a state of the art lecture, Norman Letvin of the Harvard Medical School discussed why particular strains of simian immunodeficiency viruses (SIV) do not cause disease in some monkey species. Letvin's research team inoculated monkeys intravaginally with a strain of SIVmac. After the animals were sacrificed, the researchers performed intensive immunological studies, looking for evidence of SIV-specific cellular immune responses in various tissues. As early as 72 hours after infection, CTLs could be identified from vaginal, local, and distant lymph nodes, but not from the peripheral blood. The CTLs were present in areas with the highest levels of SIV replication. Letvin suggested that the SIV-specific CD8(+) CTL is an early immune response to SIVmac infection that appears to play a key role in controlling early infection. This type of rapid and protective response, according to Letvin, may help researchers in designing effective candidate HIV vaccines.

CD8 Antiviral Activity May Help Vaccine Protect

Researchers at the National Cancer Institute (NCI) presented data suggesting that a CD8 associated anti-viral factor may be important in a candidate vaccine's ability to protect against HIV. A CD8 cell antiviral factor (CAF) has long been proposed by University of California at San Francisco researcher Jay Levy and others, but not yet identified or purified.

Michel Leno of the NCI described a study utilizing an attenuated strain of vaccinia virus known as NYVAC produced by Virogenetics Corp. of Troy, New York. A total of 20 rhesus monkeys were immunized with: NYVAC-SIV (expressing SIV antigens); NYVAC-SIV with Interleukin-2 (IL-2) and Interleukin-12 (IL-12); or IL-2 and IL-12 alone as a control. Following challenge with a pathogenic strain of SIV, all animals became infected. However, at 8 and 21 weeks after challenge, according to Leno, high levels of the CD8 anti-viral activity were associated with protection against disease. Slower disease progression was seen in the immunized animals. In addition, there was also a significant correlation between CD8 antiviral activity and level of plasma viremia. The inclusion of IL-2 and IL-12 to the NYVAC SIV had a minimal effect on CD8 anti-viral activity.

The results, according to NCI researchers, suggest that vaccination-induced CD8 antiviral activity may be associated with protective immunity. Leno suggested that "in addition to neutralizing antibody, CTL and mucosal immune responses; the ability of CD8 cells to suppress HIV should be considered in evaluating candidate vaccines". (Abstract 536.)

Simultaneous Canarypox/gp120 Better than Sequential

Researchers from the University of Rochester, working with NIAID's AIDS Vaccine Evaluation Group (AVEG) presented data on a study of Pasteur Mérieux Connaught's (PMC) canarypox vCP300 vaccine. (vCP300 is similar to PMC's earlier canarypox construct vCP205, but with sequences of *nef* and *pol* added). In total, 140 uninfected individuals were randomized to a number of different combinations: vCP300 alone, vCP300 and Chiron's gp120 vaccine given sequentially or vCP300 and gp120 given simultaneously. Immunizations were given at 0,1,3 and 6 months. In three of the regimens an additional immunization was given at 9 months. The overall results for vCP300 were disappointing (CTL levels were no better than those seen with vCP205 in previous trials). Yet interestingly, the simultaneous immunization of vCP300 and gp120 was found to induce higher levels of antibodies than sequential immunization. Regimens that included an additional immunization at nine months seemed to induce higher levels of CTLs although this did not reach a statistically significant level. (Abstract 533.)

QS-21 May be a Superior Adjuvant

Researchers from VaxGen, a California-based biotechnology company, reported that

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To Our Readers:

The *IAVI Report* continues to reach new milestones. With this 16-page issue, our circulation has now surpassed 10,000 subscribers in more than 95 countries around the world. We are also pleased to note that a French-language version of this issue will be printed in the journal *Médicin et Santé*.

The readership of the *IAVI Report* includes leading researchers, clinicians, public health authorities, industry scientists and executives, journalists, leaders of non-government organizations and activists interested in international HIV vaccine research and development.

The *IAVI Report* is provided free of charge to assure its availability, particularly to those living in developing countries. We thank you for your continued readership and contributions to help support its distribution.

We also ask that you direct any questions, comments or suggestions to iavireport@iavi.org.

David Gold
Editor

QS-21 appears to be superior to alum as an adjuvant for gp120 vaccines in terms of inducing antibody and cellular responses. Uninfected human volunteers were given three immunizations of gp120 in either alum or QS-21 or a placebo. The VaxGen researchers found that antibody levels were consistently higher in QS-21 groups. The Q-S21 groups also had evidence of a greater lymphoproliferative response. No significant side effects were seen. (Abstract 88.)

U.S. Army's Oligomeric gp160

Tom VanCott of U.S. Army's Walter Reed Army Institute of Research (WRAIR) presented data on WRAIR's oligomeric gp160 vaccine (ogp160) and an SIV DNA construct (*env*, *rev*) in macaques. The researchers hope that the ogp160 can elicit more potent immune responses than monomeric gp120 constructs. In total, four groups of monkeys were immunized with either: ogp160 in two different adjuvants, SIV DNA/ogp160 or SIV DNA alone. The monkeys were challenged with a non-pathogenic SHIV strain after the fourth immunization. All 22 monkeys that received the ogp160 vaccine were virus isolation-negative. Virus could be isolated from 4/5 monkeys in the DNA alone arm and in all control animals.

VanCott reported that DNA/opg160 prime boost appeared to increase the levels of antibody response compared to the ogp160-only arm. WRAIR researchers are now preparing to re-challenge the monkeys with a pathogenic SHIV strain. (Abstract 535.)

NCI Reports on "Zinc Fingers" Mutant Virus Vaccine

Larry Arthur of the NCI presented information on a novel vaccine strategy that uses full length mutant DNA with genetic alterations in a part of the nucleocapsid (NC) protein of HIV known as the "zinc fingers" domain. NCI researchers believe that NC region may be key to HIV's ability to replicate. By altering the "zinc fingers" of SIV a mutant, non-infectious strain of SIV was created. Five macaques were then immunized with this mutant virus. Four control animals received DNA without the mutant SIV. After challenge with pathogenic SIV, all controls animals became infected with high viral loads. Two of these died, another showed CD4 decline. In comparison, only one of five of the immunized animals developed high levels of virus. In the other four monkeys, virus was either undetectable or significantly lower and CD4 levels were normal. NCI researchers believe that the mutant virus may mimic real infection, but with a less pathogenic effect. Future plans are to try to enhance the immune response to the mutant virus, perhaps by increasing expression by using a CMV promoter, gene gun delivery or liposomal encapsulation of the virus (Abstract 536).

Using Semlike Forest Virus as a Vector

In a poster presentation, researchers from the Centre National de la Recherche Scientifique in France reported that a recombinantly produced Semlike Forest virus (SFV) vector induced impressive immune responses in mice. The French researchers compared four different HIV vaccine constructs. The constructs included: a DNA vaccine encoding *env*, a self-replicating recombinant RNA vaccine encoding *env*, a gp160 vaccine and a recombinant Semlike Forest virus expressing *env*. The highest levels of antibodies were seen in mice immunized with SFV. The researchers concluded that among the different HIV *env* vaccines tested, the SFV recombinant vector induced qualitatively and quantitatively the best antibody response. (Abstract 85)

Anti-HIV Drugs for Live Attenuated Trial Participants

In an announcement timed with the opening of the Retrovirus Meeting, the International Association of Physicians for AIDS Care (IAPAC) disclosed that three drug companies have agreed to provide free AIDS medications to participants in a study of a live attenuated HIV vaccine, if needed. In 1997, IAPAC proposed the initiation of a small Phase I trial of a live attenuated HIV vaccine based on research conducted by Ronald Desrosiers of the Harvard Medical School.

The group also reported that hundreds of volunteers have signed up to participate in the study. The companies that have agreed to provide immediate, free medicines to study volunteers, if needed, are Abbott Laboratories, Bristol-Myers Squibb and Hoffmann-La Roche. Roche also has agreed to donate testing systems to monitor the

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Vaccine Research and the Retrovirus Meeting

Why has HIV vaccine research historically had such a low profile at the Retrovirus Conferences?

According to Robert "Chip" Schooley, a leading AIDS researcher at the University of Colorado and influential member of the conference's scientific program committee, only a small amount of vaccine data was submitted for inclusion in the program. Meeting organizers, he stated, "cannot include data that is not submitted." But for next year's gathering, Schooley reports that conference organizers "will actively seek out presentations on HIV vaccine research."

One obvious step would be to add more vaccine researchers to the conference's program committee. Schooley notes that this year, Harvard researcher Norm Letvin, a well-known vaccine researcher (and a member of IAVI's Scientific Advisory Committee) was added to the program committee. (On the other hand, Ron Desrosiers, another leading vaccine researcher, recently left the committee after his term expired.)

Conference organizers are also considering the inclusion of a special satellite meeting on HIV vaccines as part of next year's Retrovirus Conference. But Schooley for one, would prefer that vaccine research be integrated into the regular scientific program. He urges vaccine researchers to submit data for next year's Retrovirus Conference. Information on the meeting is available at: www.retroconference.org.

–DG

Using Viral Load as an Endpoint in HIV Vaccine Trials

by Chris Collins

Advances in HIV treatment and viral load measurement could have a profound impact on some HIV vaccine trials. Last year, two organizations – the Joint United Nations Programme on HIV/AIDS (UNAIDS) and the U.S. National Institute of Allergy and Infectious Diseases (NIAID) – each began looking at the potential use of viral load measurements as a secondary endpoint in HIV vaccine efficacy studies.

The efforts of UNAIDS are part of a broader effort by the organization to examine ethical isues and standards of care to be used in HIV vaccine trials. On 23-24 September 1997, UNAIDS's Ethical Review Committee met in Geneva to develop plans for the drafting of international guidelines on ethics in HIV vaccine trials (see box). Among the key issues discussed were the use of viral load measurements as "secondary endpoints" and how new antiretroviral treatments could impact the ability to utilize such measurements.

NIAID has also begun looking at how HIV treatment advances may impact the design of vaccine trials. At a one day conference, held on 19 September 1997 at the headquarters of the U.S. National Institutes of Health in Bethesda, NIAID brought together representatives from government, industry, research institutions, non-profit organizations and affected communities to discuss the issue.

The use of post-exposure prophylaxis and early treatment by participants in HIV vaccine trials could make it difficult to use viral load as an endpoint.

The key issue considered at the NIAID meeting was whether viral load can be used as a surrogate marker for vaccine effectiveness. In HIV vaccine trials, unless a candidate vaccine provides 100 percent protection after the first immunization (which is highly unlikely), some vaccine recipients will likely become infected through sexual exposure or needle use. While the ideal HIV vaccine would prevent infection (provide "sterilizing immunity"), many researchers believe that preventing or delaying disease progression with reduced infectivity may be more attainable goals. Thus, measuring the viral load of trial participants who become infected could be a more rapid way of assessing the effectiveness of a vaccine. Conclusions could be made far earlier than by relying solely on differences in clinical outcomes (disease progression or death).

Research reviewed at the NIAID meeting indicated that the viral load "setpoint" correlates with disease progression in HIV-infected U.S. populations. (The setpoint, or plateau, is the point at which viral load levels become relatively steady. It is usually reached months after infection.) In comparison, data is still inconclusive as to whether viral load at peak (the viral spike which occurs soon after infection) or the rate of increase of viral load after infection correlate with the viral setpoint or disease progression.

Animal study data presented at the meeting demonstrated a range of possible outcomes after vaccination, from complete protection against infection to viral clearance to reduced viral load with slow disease progression. However, the studies did not provide conclusive information about the relationship between viral peak and plateau levels and vaccine effectiveness.

Validating viral load as a marker

Many researchers believe that additional studies are needed to determine the appropriateness of viral load as a surrogate of vaccine effectiveness. According to Tom Fleming of the University of Washington, for a surrogate to be valid it must correlate with vaccine efficacy. Endpoints in a vaccine trial could be the rates of infection, disease, death or levels of infectiousness in vaccine recipients. As noted earlier, current research suggests a correlation between the viral setpoint and disease progression in HIV-infected individuals. It is still unknown whether this will also correlate in vaccinated individuals.

Fleming noted that the validation of viral load as a surrogate marker of vaccine effectiveness may ultimately require large trials or a meta-analysis of multiple trials. But others suggested that larger efficacy trials may not necessarily be required. Steve Self, also of the University of Washington, said that an "intermediate-sized trial" could detect a reduction in virus load of less than one log at six months post-infection. (A "log" represents a change in viral load by a factor of ten; for example, the difference between 10,000 and 100,000 copies of HIV RNA per ml.) Thus, intermediate-sized trials currently being considered might have sufficient statistical power to demonstrate meaningful differences in viral load levels among vaccinees and controls.

Early Treatment and PEP

The use of viral load as a surrogate measure of effectiveness in vaccine trials is complicated by the likelihood that many vaccine trial participants in industrialized nations may choose to receive "post exposure prophylaxis" (PEP) immediately after exposure or "early treatment" soon after infection and seroconversion. PEP therapy includes a combination of antivirals taken right after exposure to HIV is believed to have occurred (usually within 24 hours), with the goal of preventing infection from taking hold in the body. Early infection treatment utilizes antiviral therapy within days or weeks after infection has been diagnosed in an attempt to reduce damage done early in the infection process and improve the course of HIV disease. While studies in small numbers of animals suggest benefits in utilizing PEP, there are, as of yet, no data from controlled clinical trials in humans to prove the effectiveness of PEP or early treatment.

Because combination antiviral therapy can have such a profound

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USING VIRAL LOAD *continued from page 5*

impact on viral load, it is not clear that viral load measurements would be a valid surrogate marker of vaccine effects if participants utilize PEP and/or early infection treatment. In addition, the use of early treatment would allow for only one or two opportunities to take viral load measurements prior to the initiation of such treatment. Therefore, if PEP or other HIV prevention strategies succeed in reducing HIV incidence in a vaccine trial cohort, much larger sample sizes would be required to assess a vaccine's effects. Dr. Self noted that it may be possible to incorporate early antiviral therapy into a vaccine trial by using factorial or other designs.

Related Issues

Attendees at the conference also discussed implementation issues involved in using viral load as a surrogate. Freya Spielberg of the University of Washington reported that the use of "finger prick" collection of blood by trial participants provides accurate samples for viral load measurement and may allow for more frequent measurement during a study. In addition, finger prick collection may be particularly useful in developing countries. Oral fluid collection may be used for detecting antibodies. Spielberg said that these collection methods were well accepted by HIVNET vaccine preparedness study participants in the U.S.

Anne-Marie Duliege of Chiron, the California-based biotechnology company, raised several questions of concern to industry. Vaccine manufacturers, she noted, need clarification from government officials and researchers regarding the primary endpoints in U.S. government-sponsored HIV vaccine trials. Among the uncertainties: Will viral load be used? Is sterilizing immunity still being considered as a primary endpoint? What level of efficacy will be required for vaccine licensure and use? And, have NIAID's criteria for advancing from Phase II to Phase III trials changed in light of recent research developments?

Officials from NIAID and the U.S. Food and Drug Administration indicated that data are not yet conclusive enough to decide whether early virus load measurements could be used as an endpoint for a vaccine trial. In the meantime, a future workshop will address the possibility of utilizing viral load measurements in a standard fashion in HIV vaccine efficacy trials, thus allowing researchers to "bridge" results from trials in different countries. ◆

Chris Collins is a member of the AIDS Vaccine Advocacy Coalition (AVAC)

VACCINE BRIEFS *continued from page 2*

the mucosal surfaces of the nose, rectum, or vagina of volunteers. Most HIV infections are transmitted via mucosal surfaces. vCP205 + GM-CSF Adjuvant: Researchers are examining whether adding a cytokine known as human granulocyte-macrophage colony stimulating factor (GM-CSF) to vCP205, will increase the immune response. Cytokines are chemicals that signal the immune system. GM-CSF is commonly used to improve blood cell production in cancer patients. VVG203: University of Maryland at Baltimore and Johns Hopkins University researchers will study whether a weakened form of salmonella, carrying HIV antigens, can help generate a potent response to HIV. The researchers hope that since the bacteria normally reproduces only inside human cells, it can present HIV proteins (*env*) in a way that induces good immune responses.

New NIH AIDS Vaccine Website

The NIH has launched a new website devoted to HIV vaccines. The site, which contains information on funding mechanisms for researchers can be found at: http://www.niaid.nih.gov/daids/vaccine.

Thailand, Japan Prepare BCG Vaccine for Human Trials

Thailand's Permanent Secretary of Health, Prakrom Vuthiphong, announced that his country will produce a candidate HIV vaccine in cooperation with Japan's Science and Technology Corporation. The vaccine uses a Bacille Calmette-Gurein (BCG) vector expressing the V3 loop of clade B and E strains of HIV. BCG is the strain of mycobacteria that is used as a TB vaccine. (See "Using Recombinant Vectors as HIV Vaccines", *IAVI Report*, vol.3, no.1.) The BCG construct being developed by the two countries is currently in animal studies and reportedly could be ready for human trials within two years. ◆

UNAIDS Organizes Meetings on Ethics in HIV Vaccine Trials

On 24-26 June 1998, UNAIDS will hold an international meeting in Geneva to consider a guidance document on ethical issues in HIV vaccine trials. The meeting will precede the XII International Conference on AIDS, which will be held on 28 June to 3 July.

In preparation for the meeting, UNAIDS organized a series of regional community workshops on ethics of HIV vaccine trials. The first regional workshop was held in Ouro Preto, Brazil on 1-3 April 1998. It was co-sponsored by the Brazil's Ministry of Health (PNDS/AIDS Ministério da Saúde). On 20-22 April, another regional meeting was held in Bangkok, Thailand. A third regional meeting was held in Entebbe, Uganda on 27-29 April. A final community meeting will be held in Washington, DC on 11 May.

Plans to draft an international consensus statement on ethical issues related to HIV vaccine trials were originally discussed at a meeting held in September 1997 in Geneva. At that meeting, researchers, ethicists, human rights experts, clinicians, and community representatives from 11 countries as well as UNAIDS and CIOMS (Council for International Organizations of Medical Sciences) gathered to review the overall question of ethics and the design of HIV vaccine trials.

Jose Esparza, UNAIDS Vaccine Development Advisor, is overseeing the entire effort to develop the guidance document on the ethics of HIV vaccine trials. Dale Gautner, a Canadian physician and researcher, is helping to organize the community workshops.

An Interview with Pasteur Mérieux Connaught's Michel Klein

In January of this year, Michel Klein was appointed Corporate Vice President for Science and Technology at Pasteur Mérieux Connaught (PMC). In this position, Klein oversees a broad range of research and development activity for the company, including its HIV vaccine program. PMC, a subsidiary of the giant French pharmaceutical company Rhône-Poulenc Group, is considered by many observers to be the only company in the world that has a truly broad-based HIV vaccine program. Klein can thus be expected to have a significant impact on global efforts to develop an HIV vaccine.

IAVI REPORT: Can you give us an overview of PMC's HIV vaccine program?

MICHEL KLEIN: We are testing a series of candidate vaccines to validate the prime boost concept by using canarypox virus vectors as priming antigens and gp120 or gp160 constructs as boosters. So far, this has been the only protocol which has induced neutralizing antibodies against laboratory isolates in one hundred percent of human vaccinees and measurable CTL responses in between forty and sixty percent.

Our goal now is to try to induce even stronger and more polymorphic CTL responses. To this end, we are developing vectors that are improved in two ways — they express more CTL targets, including additional HIV proteins and secrete much better, more sustained antigen. We believe that these improvements will make a difference, in terms of CTL response. Phase I trials of the newer constructs will begin in the United States very soon.

IAVI REPORT: Which candidate vaccines are these?

KLEIN: Our two new canarypox constructs are ALVAC vCP1433 and vCP1452. vCP1433 is an improved version of vCP300 and is designed to be more stable. vCP1452, our lead canarypox candidate, is an even better producer of antigen. And we continue to test an earlier immunogen, vCP205, in a number of different studies.

The second generation vCP1452 vaccines will express *env* from a primary isolate of HIV. And ultimately, we hope to use it as the backbone to make vectors which would express HIV proteins from clades A, E, C and D.

We are also trying to improve CTL responses by boosting the vCP1452 vector with a cocktail of five lipopeptides, which contain CTL epitopes of HIV that are recognized by eighty-five percent of all human HLA types. A trial of this combination will start in France this year in collaboration with the ANRS (Agence Nationale de Recherches sur le SIDA). And the trial of vCP1452 with a sub-unit envelope boost will start in the States next month under the aegis of NIAID.

IAVI REPORT: These vaccines are designed to boost CTL response, but what about neutralizing antibodies?

KLEIN: Our program is also focused on inducing neutralizing antibodies against the envelopes of primary isolates. To this end, we are initiating a study in Thailand, with the Royal Thai Army, Walter Reed Army Institute of Research and Mahidol University to test a combination of the vCP205 expressing a clade E envelope, boosted with either a clade E gp120 (by Chiron), or our own clade E oligomeric gp160 derived from primary isolates. The trial, which should start in early 1999, will examine whether priming with an immunogen containing a clade E *env*, boosted with an envelope from a clade E primary isolate, can induce neutralizing antibodies against at least the autologous primary isolate, which has never been achieved, yet, in humans. We will also compare our oligomeric gp160 with Chiron's monomeric gp120 as boosts.

We expect that our best canarypox vector, vCP1452, could be ready for a Phase III trial in late 1999.

Our gp160 contains a deletion in the gp41 region. We are hoping that by not inducing certain gp41 antibodies, recipients of this vaccine will test negative on HIV diagnostic tests.

IAVI REPORT: What about the status of PMC's pseudovirion HIV vaccines?

KLEIN: That's the other line of research we're developing to induce neutralizing antibodies. The trial should start next year with a clade B construct in the United States.

IAVI REPORT: In terms of the canary pox constructs, are you concerned that there are no non-clade E core proteins? Or is everyone now assuming that for the core proteins, one clade will work for all?

KLEIN: That's a very good question. The current poxvirus vaccines include clade B core proteins. And we are encouraged because they are capable of inducing cross-clade-reactive CTL, against some clade A, E and C HIV isolates, in some vaccine recipients.

We also know that clade B HIV can induce cross-reactive CTL against other clades of HIV, and people infected with clade E and A can have CTLs against clade B. So, we were quite happy to learn that clade B immunogens could induce cross-clade-reactive CTL against other clades, up to seventy percent, in some cases.

Now with the next generation of vaccines, it may be preferable to have core proteins from the clade circulating in the country, because they may contain different CTL epitopes which could play a role in protection. However, as a first step, having a vaccine which could at least induce cross-reactive CTL in a significant percentage of vaccine recipients is a significant achievement.

IAVI REPORT: What about PMC's HIV DNA vaccine program?

KLEIN: We are developing plasmid DNA vaccines that express either the envelope or the core proteins; or both in a single construct. We also are developing plasmids which express HIV-like particles. And all these plasmids are derived from primary isolates, with respect to the envelope.

IAVI REPORT: How far are you from clinical trials of an HIV DNA vaccine?

KLEIN: We are working with Harriet Robinson of Emory University in doing preclinical studies in monkeys. She is going to compare different vectors, routes of administration and combinations including canarypox/ DNA combinations. Human studies could begin within eighteen months, probably in both the U.S. and France.

IAVI REPORT: What is your current timeline for Phase III efficacy studies of the canarypox prime boost?

KLEIN: We expect that our best canarypox vector, which is vCP1452, could be ready in late 1999 for a Phase III trial. This vector does not express an envelope from a primary isolate, that will come later. But in terms of CTL, vCP1452 is potentially the best vector we have engineered.

IAVI REPORT: Some people were hoping that a Phase III study with vCP205 would be proposed. Why not do two different efficacy studies, one with vCP205 and the second one with a later candidate like vCP1452

KLEIN: There are a number of issues. First of all, it seems unnecessary to enter a Phase III with vCP205 if six months or even nine months later a potentially improved vector, in terms of CTL induction, could be produced. Secondly, doing a staggered Phase III trial introduces many logistical problems. We would need 4,500 individual volunteers for vCP205, and then six months later another 4,500 volunteers. So, if the two trials could not be staggered, vCP1452 would have to be evaluated three years later.

IAVI REPORT: Are you seeing any surprises in

Pasteur Mérieux Connaught's HIV Vaccine Pipeline

Canarypox/ALVAC vCP125 The ALVAC vaccines are recombinant canarypox virus vectors with one or more portions of HIV genes inserted. vCP125, the first generation canarypox vector, contained a gp160 (clade B) gene insertion. It was tested in Phase I trials in the U.S. and France and is no longer in development.

ALVAC vCP205 PMC's second canarypox vector, contains genes encoding for *env, gag* and protease (clade B). It is currently in Phase II trial in the U.S. (with Chiron's gp120 as a boost). A Phase I study is planned in Thailand to evaluate vCP205 (clade E env) with PMC's oligomeric gp160 (ogp160, clade E) and a Phase I study of the vector alone is about to start in Uganda *(see page 1)*. Two other Phase I trials of vCP205 recently began in the US: vCP205 administered via mucosal surfaces and vCP205 given with GM-CSF, a cytokine commonly used to improve blood cell production, as an adjuvant.

ALVAC vCP300 PMC's third canarypox vector includes everything in vCP205, plus pieces of additional HIV genes *(pol* and *nef)*. In a Phase I study in the U.S., vCP300 generated disappointing results. CTL responses in vaccine recipients were not significantly better than vCP205 despite the additional genes. PMC researchers believe that vector's lack of stability was responsible for the lack of enhanced immune responses.

ALVAC vCP1433 One of PMC's newest canaryox vectors, vCP1433 includes the same genes as vCP300 but is designed to be more stable. A three arm study in the U.S. will compare vCP1433, vCP1452 and vCP205 all given with PMC's ogp160.

ALVAC vCP1452 PMC's most advanced canarypox vector. Company researchers believe that vCP1452 has the greatest promise of all the canarypox vectors and hope to take it to Phase III studies by the year 2000. The vector contains the same genes as vCP1433, but has been designed to increase antigen expression. If Phase I studies show potent immune responses, PMC plans to produce a second generation vCP1452 which will contain *env* from primary isolate of HIV, and eventually, include genes from different clades in the vector.

Oligomeric gp160 (ogp160) PMC's envelope protein is an almost full length envelope ogp160 (compared to the monomeric gp120 developed by VaxGen and Chiron). It is derived from a clade B (MN/LAI-2) strain of HIV. The gp41 region contains a deletion that researchers hope will enable vacine recipients to test negative on HIV diagnostic tests. The ogp160 will be tested in combination with canarypox vectors the U.S. and Thailand. PMC is also developing an ogp160 based on primary isolates of HIV (in both clade B and E).

Pseudovirions Pseudovirion vaccines (also known as virus-like particle vaccines) consist of genetically engineered, non-infectious HIV particles that contain most, but not all HIV proteins. The vaccines are produced by constructing a viral particle that is non-infectious but contain envelope and internal viral proteins that make it immunogenic. PMC's pseudovirion vaccine contains gp160, p55 and p24, but lacks *pol* and *nef* proteins. Efforts to initiate a human study of the candidate vaccine have taken significantly longer than expected, but the company hopes to begin a Phase I trial in the U.S. sometime in 1999.

Lipopeptides Lipopeptide vaccines consist of HIV peptides (chemically synthesized pieces of HIV proteins that are known to stimulate HIV-specific immunity) with lipids added to increase CTL responses. The ANRS has developed a cocktail of five lipopeptides which contains several known human CTL epitopes of HIV. It is hoped that the lipopeptide vaccine, which will be used as a boost after priming with vCP1452, will significantly augment CTL responses. A Phase I trial of the canarypox/lipopeptide combination will begin in France sometime in 1998.

HIV DNA Vaccines HIV DNA vaccines utilize the injection of selected HIV genes into the body. It is hoped that the genes, when taken up by the cells, will enable the vaccine recipient's own cells to make selected HIV proteins. PMC is working with Harriet Robinson of Emory University in studying a broad range of DNA constructs. The preclinical studies will compare different vectors, routes of administration and combinations including canarypox/DNA combinations.

the Phase II canarypox study underway in the U.S.?

KLEIN: The only surprise was that initially the CTL assay had a very high background which did not allow measuring CTL-specific lysis (cell killing). But this has been corrected and we are now seeing the same percentage of CTL activity as in earlier trials.

IAVI REPORT: But if you look at CTL activity after one year — at least with the vCP205 — only a minority of vaccine recipients still have a measurable response.

KLEIN: Yes. But I would put it the other way around. I was very encouraged to see such activity, one year after the last injection. There had been no indication that the canarypox would give such a long-lasting CTL response, because, basically, pox virus disappears very quickly.

HIV vaccine development is still a very high-risk investment.

IAVI REPORT: What do you think should be the standard for moving a product into Phase III trials?

KLEIN: The availability of a vaccine which could induce both neutralizing antibodies and broad CTL responses would represent a significant step torwards a Phase III study.

The fact that the sero-negative sex workers, in Kenya, have very good CTL responses; that long-term non-progression is associated with a good cellular immunity and that we can prevent infection in chimpanzees immunized with envelopes inducing neutralizing antibodies, all suggest that both types of responses are desirable.

IAVI REPORT: Have you had any discussions with VaxGen about using their new bivalent gp120 with the canarypox constructs?

KLEIN: Yes, our discussions with VaxGen are progressing. The inclusion of a bivalent envelope vaccine derived from both SI (syncytium inducing) and NSI (non-syncytium inducing) isolates of HIV is attractive

IAVI REPORT: Where are plans for the Phase I canarypox trial in Uganda?

KLEIN: The Ugandan trial is going to start this year. The infrastructure is ready. There is great interest in the trial. The objective is to demonstrate that a clade B immunogen is capable of inducing CTLs against clades A, D and C of HIV, in a non-Caucasian population with a different HLA makeup. So, it would be the proof, in vivo, of what we have observed in the laboratory.

IAVI REPORT: Do you have any plans for a follow-up study to that?

KLEIN: Yes. We are developing canarypox constructs based on clades found in Uganda and other countries in Africa, such as the Ivory Coast. Specifically the A and C clades of HIV.

IAVI REPORT: What is PMC's relationship with ANRS?

KLEIN: It's an excellent, long-standing relationship. The ANRS, led by Jean-Paul Levy, has been a very strong supporter of PMC's HIV vaccine program. And it has sponsored all the clinical trials that we have performed in France.

IAVI REPORT: What is the level of financial support that they provide in terms of financial aid?

KLEIN: Let me say that it has been and remains essential to the PMC program.

IAVI REPORT: Are they involved in development decisions?

KLEIN: Obviously, we collaborate, share ideas and have regular meetings with the ANRS. Our R&D is done in a very transparent manner and our common goal is to develop an efficacious vaccine.

IAVI REPORT: How is your working relationship with the NIH?

KLEIN: It is also an excellent relationship. The NIH has played a critical role in helping us evaluate our vaccines in a very expeditious manner.

IAVI REPORT: You have the experience of dealing with the world's two largest government-funded biomedical agencies, in terms of AIDS research. Do you see sort of any major differences in the way they operate?

KLEIN: That's an interesting question. In regard to conducting HIV vaccine trials, both governments are particularly interested in two issues, the scientific rationale and ethical concerns. Without the ANRS and the NIH, we couldn't have launched or sustained our HIV vaccine program. And both governments deserve a great deal of praise their efforts.

IAVI REPORT: Well, they're really the only two industrialized countries doing so.

KLEIN: That's right. You could say that the rest of Europe has not shown the same commitment.

IAVI REPORT: I've heard there are also plans to test the canarypox constructs as a therapeutic HIV vaccine.

KLEIN: Yes, we believe that therapeutic vaccination may be a valuable adjunct to combination anti-retroviral therapy. We've been talking to a number of scientists including Jean-Paul Levy in France and David Ho and Dani Bolognesi in the U.S. to determine how we should proceed with clinical trials.

IAVI REPORT: How do you ensure worldwide access once HIV vaccines are developed?

KLEIN: Short of having a live attenuated HIV vaccine, which companies are reluctant to pursue because of safety issues, an HIV vaccine is going to be complex. We will probably need more than one immunogen. In addition, production yields are likely to be low and not amenable, at this point, to large-scale production. So we will have complex, recombinant products, low production yields and costly manufacturing. As a consequence, an HIV vaccine is not going to be cheap to produce.

Although companies like PMC are prepared to help, governments, international agencies like the World Bank and foundations will all need to play a role. A single company is unlikely to be able to evaluate, produce and distribute a vaccine on its own without external support.

IAVI REPORT: Why do you think so few companies around the world are investing in HIV vaccine development?

KLEIN: HIV vaccine development is still a very high-risk investment. We still don't know the biological correlates of immunity, there is no good animal model to test candidate vaccines, we will probably need more than one immunogen, production will be expensive and yields are likely to be low, and efficacy studies of HIV vaccines will require very large investments of financial and human resources.

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Palm Springs Symposium Looks at HIV Vaccines

On 5-8 May, 1998, the University of California centers at Los Angeles and Irvine co-sponsored a three-day meeting "Towards an HIV Vaccine: Immunopathogenesis of HIV Infection" in Palm Springs, California. Listed below are some of the highlights of the meeting:

VaxGen Hopes to Move Bivalent gp120 Into Phase III

Phil Berman of VaxGen presented his company's plans to move its new and improved gp120 (AIDSVAX) into Phase III studies. VaxGen has prepared bivalent gp120 vaccines (based on two HIV strains) for study in the U.S. and Thailand. The vaccine to be tested in the U.S contains gp120 from two strains of clade B virus (one is the MN strain used in the earlier gp120, the other strain is known as "GNE8"). The construct that the company hopes to test in Thailand is based on the clade B/MN strain and a clade E strain known as "A244". Each of the new candidate vaccines are derived from macrophage-tropic strains, while the MN strain of HIV is T-cell-tropic. Berman believes that the addition of the new strains will "increase the likelihood of protection." The dosing schedule will include immunizations at 0,1,6, and 12 months.

The VaxGen researcher also suggested that the breakthrough infections observed in Phase I and Phase II studies of the earlier gp120 construct were too small to be able to draw conclusions about. However, he noted, that the data may provide some useful information about designing more effective vaccine constructs. According to Berman, 5 of 7 breakthrough infections that occurred after immunization with the earlier MN-strain gp120 construct were with non-MN strains of HIV. VaxGen believes that by combining the MN gp120 and the newer GNE8 gp120, it can increase the likelihood of generating a protective response. Berman also reported that in studies in rabbits, the bivalent gp120 vaccine neutralizes some primary isolates of HIV.

According to Berman, VaxGen is planning to enroll 5000 sexually active gay men and serodiscordant couples in a VaxGen-supported Phase III study in the U.S. and 2,500 injection drug users in its trial in Thailand.

In the February 1998 issue of *The Journal of Virology*, a group of researchers led by Steven Wolinsky of Northwestern University Medical School reviewed the breakthrough infections seen in the Phase I and Phase II gp120 studies in the U.S. The researchers concluded that there were no significant differences in terms of HIV levels between the 18 patients who had received gp120 vaccines and later became infected with HIV and unvaccinated HIV-infected patients.

New Inactivation Method May Hold Promise

Jeff Lifson of the NCI presented data on a new method of inactivating HIV that appears to eliminate the infectivity of the virus while preserving the functional integrity of its surface proteins. Lifson's research team inactivated the virus by using a compound that targets the "zinc fingers" of the HIV nucleocapsid protein. NCI researchers believe that the nucleocapsid protein plays a critical role in infectivity. (NCI researchers are also developing a full length DNA mutant virus with genetic alterations in the "zinc fingers" as a candidate vaccine. See page 4.)

NCI researchers have developed a method of inactivating HIV which seems to preserve the viral envelope. This may be useful in developing whole-killed HIV vaccines.

Lifson's team compared various methods of inactivating HIV (including using formalin or heat). Most methods that have been used to inactivate HIV strip the virus of its glycoprotein (envelope). To avoid this, the NCI researchers used a compound, "aldrithiol-2", to modify HIV's "zinc fingers". While the compound appears to inactivate the virus, immunolgical and functional studies suggest that the conformational and functional integrity of the envelope proteins are preserved. If, after viral inactivation, the glycoprotein of HIV can be preserved, the likelihood of the vaccine generating a protective immune response could be significantly greater. Lifson concluded that the inactivated virus could be an interesting candidate HIV vaccine, used alone or as a boost with other candidate vaccines. Because of safety concerns regarding the use of inactivated HIV as a vaccine, human studies of this approach will likely require a significant amount of preclinical safety data.

Carbohydrates on HIV Envelope May Limit Immune Response

Ron Desrosiers of the New England Primate Research Center presented data suggesting that carbohydrates on the envelope of HIV may limit the immune response to the virus. His lab infected monkeys with mutant forms of SIV lacking specific "N-linked glycosylation sites" in the viral envelope. (The envelope is heavily "glycosolated" meaning that it contains high levels of sugar attached to the amino acid backbone of the protein molecule.) Desrosiers's team found that the monkeys infected with the mutant SIV had increased levels of neutralizing antibodies. At two weeks post infection these monkeys had similar levels of SIV to those infected with wild-type virus. However, levels of the mutant SIV then began decreasing dramatically until they became undetectable. Desrosiers suggested that the more effective antibody response seems to limit SIV replication in monkeys infected with the mutant virus lacking N-glycosylation sites. He believes that glycosylation in the HIV envelope may be shielding the virus from immune recognition.

New Data on Live Attenuated SIV Vaccines

Desrosiers and Paul Johnson, also of the New England Primate Research Center, presented updated data on macaques immunized with live attenuated SIV. Studies now suggest that some of the more attenuated SIV vaccines (including the "delta-4" construct) are able to protect against a

mucosal (vaginal) but not intravenous challenge. Desrosiers also discussed data showing that live attenuated SIV vaccines can protect against challenge with a pathogenic SHIV 89.6 (SHIV viruses are genetically created by substituting the HIV envelope for SIV env protein). While monkeys infected with SHIV 89.6 usually die within 8 weeks, vaccinated monkeys challenged with the SHIV strain, remain healthy after 3 years. Desrosiers concluded that strong protection was seen against SHIV 89.6, even though it had a totally mismatched envelope relative to the vaccine. He concluded that factors other than an anti-envelope immune response appear to be responsible for this protection.

Combining DNA Vaccines with Cytokines

David Weiner of the University of Pennsylvania presented data on his lab's work in developing different DNA vaccine constructs in collaboration with Apollon, a Pennsylvania-based biotechnology company. To date, Apollon has initiated four human studies of candidate HIV DNA vaccines. Weiner's lab is now studying whether combining various cytokines with the DNA vaccines can increase immune responses and drive specific types of responses. Data presented suggest that: interleukin-12 increases CTL response, interleukin-2 induces stronger proliferative response and interleukin 4 and interferon gamma increase antibody response. Weiner noted that much is still unknown about how the different cytokines impact immune responses. He presented data on a study in which his lab immunized macaques with a low dose of SIV DNA (*env, gag* and *pol*) and different combinations of IL-2, IL-4 and interferon gamma. High levels of CTL and some neutralizing antibodies were seen. After challenge with SHIV-IIIB, 3 of 6 animals were protected. According to Weiner, development of neutralizing antibody response showed no correlation with protection.

Studying Peptide Vaccines with Cytokines

Jay Berzofsky of the NCI presented data on his lab's work in engineering different HIV peptide vaccines to impact CTL responses. Berzofsky described how modifying the amino acid sequence of a helper epitope and attaching it to a CTL

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Meeting on Vectors for Delivering HIV Vaccines Held by NIAID

By Sam Avrett

On 5 March, 1998, the U.S. National Institute of Allergy and Infectious Diseases (NIAID) organized a workshop on viral and bacterial vectors for HIV vaccine delivery. The workshop, which preceded the Palm Springs Symposium on HIV Vaccines, featured presentations on a range of vectors by researchers from the vaccine, gene therapy and cancer fields.

The focus of the meeting was on potential HIV vaccine vectors that are early in development. Vectors further along in the development pipeline, such as canarypox, vaccinia, modified vaccinia Ankara (MVA) and BCG, were not discussed at the meeting. (For a review of these vectors, see *IAVI Report*, vol.3, no.1.)

Listed below are some presentations at the meeting:

Adeno-Associated Virus (AAV)

Barrie Carter of Targeted Genetics Corporation presented information on the use of AAV, primarily as a gene vector. AAV is a parvovirus that does not cause disease. It infects epithelial cells (the cells that line the mucosa), and thus might be a useful vector for generating mucosal immune responses against HIV. Most of the population has been naturally exposed to AAV, but there is little data on whether the immune response from prior exposure prevents re-infection with or rapid *in vivo* clearance of an AAV vaccine. As a vector, AAV can handle a reasonable-sized gene insertion of about 4.5 kbp ("kbp" means a thousand base pairs, a measurement of the length of a strand of DNA or RNA). AAV seems to replicate well and can provide prolonged, persistent expression in human cells (two months or more). Research teams at Ohio State, the University of Pennsylvania, Johns Hopkins and Stanford have studied AAV as a gene vector, and Chiron researchers are examining AAV as a vector for a herpes simplex vaccine. As a gene vector, AAV is in a Phase I trial as a treatment for cystic fibrosis and in Phase II studies for chronic sinusitis. Studies of AAV vaccine vectors in mice show detectable serum antibody against a number of pathogens, including HSV-2. Researchers plan to develop HIV and SIV recombinant AAV vectors and test them in small animals and then non-human primates.

Herpes Simplex Viruses (HSV)

David Knipe of Harvard University presented information on a herpes simplex virus vector, genetically altered to be incapable of replication. A replication-defective HSV-2 virus is being developed by the Virus Research Institute as a candidate genital herpes vaccine. The HSV vector has protected small animals against herpes disease. Intranasal plus subcutaneous administration appeared to generate better protection against genital herpes in animal studies. The Harvard researchers have inserted SIV *env* coding sequences in an HSV-1 vector and generated moderate levels of *env* expression. Of two macaques immunized with a replication-defective HSV-1 vector, one showed SIV-specific antibody response, and the other showed a definitive CTL response. Surprisingly, two others injected with a replication-competent, live attenuated HSV-1 vector showed neither SIV antibody or CTL response. No primate protection data was reported.

Polio Replicons

Casey D. Morrow of University of Alabama at Birmingham presented data on polio virus replicons. Although polio is a relatively small virus, Morrow's laboratory has developed methods to insert up to 2.0 kbp of genetic material into a modified genome. Lacking the genes encoding the poliovirus capsid, these so-called "replicons" are encapsidated by providing the capsid proteins in trans. The resulting replicons lack the genes for the capsid and can only undergo a single round of replication. The infection thus cannot spread. In a study of mice immunized with a replicon expressing a fragment of tetanus toxin, half were protected against challenge with tetanus; the other half

had reduced susceptibility. When macaques immunized with a replicon encoding HIV *env/gag* and SIV *env/gag/pol/nef* protein, all responded with antibodies to the HIV/SIV proteins. Following challenge with pathogenic SIV virus though, all the animals succumbed to disease. More recently, macaques were inoculated intramuscularly and intranasally with polio replicons encoding SIV proteins. The animals will be challenged with a less pathogenic strain of SIV shortly. Morrow noted that mice immunized with the Salk polio vaccine and then given replicons encoding tetanus toxin were protected against tetanus challenge despite the prior polio exposure. This result is consistent with earlier studies in humans showing that prior immunization did not prevent re-infection with poliovirus. Thus, Morrow suggests it could be possible to use replicons as recombinant vaccines in a population that has been previously vaccinated against poliovirus.

The focus of the meeting was on potential HIV vaccine vectors that are early in development.

Salmonella and Shigella

George Lewis of the Institute of Human Virology (IHV) at the University of Maryland presented studies carried out in collaboration with David Hone, also of the IHV, on the use of Salmonella and Shigella to deliver both conventional HIV protein antigens and DNA vaccines. The advantages of Salmonella are that it can be given orally, is easy to produce and can encode large gene insertions (more than 10 kbp). One problem has been the absence of an animal model of the *S. typhi* strain of salmonella to do pilot immunogenicity studies. For this reason, studies have been carried out in mice using a related species encoding gp120. In these studies, antigen-specific T-cell proliferative responses, Th1 cytokine responses, and beta-chemokine responses were obtained after a single oral dose of the vaccine. CTL responses were obtained inconsistently. In addition, strong mucosal anti-gp120 antibody responses were observed. Based on these results, a Phase I study has been initiated in human volunteers with the same gp120 gene encoded by an attenuated *S.typhi* carrier strain. Immunogenicity data are not yet available. Data were also presented on the use of attenuated Shigella to deliver DNA vaccines. In addition, the results of three other groups who have used Shigella or Salmonella to deliver such vaccines were summarized emphasizing the emerging importance of live attenuated bacterial vectors as DNA delivery vehicles. Finally, results of another collaboration were presented on a new targeting system developed by Nicholas Carbonetti, also of the IHV and University of Maryland, which uses attenuated bacterial toxins such as pertussis toxin and cholera toxin to deliver CTL epitopes.

Simian Virus 40 (SV40)

David Strayer of Thomas Jefferson University described the potential of SV40 as a vector for immunizing against HIV and other viruses. SV40 is a papova virus with a genome size of 5.2 kbp, which has been modified for gene transfer purposes to accommodate as much as 4.7 kbp of foreign DNA. The virus is easy to make and manipulate, and is relatively stable at room temperature. Strayer reports that SV40 is likely to be safe; follow-up studies of people infected with live SV40 by the early Salk vaccine do not show any higher rates of tumor-associated disease. To assess its usefulness as an immunizing vehicle, researchers inoculated mice with an SV40 derivative containing a gene for hepatitis B surface antigen. High titers of hepatitis B antibody were found, but no neutralizing antibody against SV40 was detected in any animals, even following eight immunizations. Strayer suggests that SV40 is, therefore, a potential vector for delivery of viral and other antigens, although studies have yet to assess immunogenicity and clearance of the vector in primates. Currently, no company is known to be involved in developing this vector.

Varicella Zoster Virus (VZV)

Jeffrey Cohen of NIAID presented data on a live attenuated VZV vaccine vector. VZV, the virus that causes chickenpox, can reactivate to cause herpes zoster. The vaccine is based on a live attenuated strain of VZV that was developed by Japanese researchers in 1985 and was approved in the U.S. (trade name Varivax) in 1995. The attenuated VZV is a large vector that can accomodate at least 5.0 kbp of genetic insertions. Japanese researchers are planning to study a hepatitis B VZV recombinant vector in humans. Because VZV infects CD4 and CD8 cells, researchers hope that the attenuated virus can generate potent immune responses. Cohen noted that the vaccine is relatively safe in mildly immunocompromised people. Guinea pigs immunized with a VZV vaccine containing a herpes simplex virus gene and challenged with herpes simplex virus had significantly less disease than non-immunized animals. However, to date, no animal model reproduces the signs and symptoms of chickenpox or herpes zoster. Primate studies to evaluate VZV vectors expressing HIV/SIV genes are planned. Potential concerns include the impact of pre-existing immunity that most humans have to VZV and the potential of VZV to reactivate in nerve cells after a long period of latency.

Venezuelan Equine Encephalitis Virus (VEE)

Nancy Davis of the University of North Carolina at Chapel Hill (UNC) presented data obtained in collaboration with scientists at UNC, U.S. Army Research Institute of Infectious Diseases, Ohio State University and Duke University on attenuated VEE as a vector. The vector consists of VEE with key genes deleted so that it can infect but not produce progeny particles. The VEE vaccine vector is attractive because: 1) it targets professional antigen presenting cells, or dendritic cells, in the draining lymph node; 2) it expresses high levels of protein from the inserted foreign gene; and 3) it induces mucosal as well as systemic immunity. The vector can accept up to 5.0 kbp of genetic insertions. VEE replicon particles are safe because they cannot propagate, and in addition they carry attenuating mutations as a redundant safety feature. No observable side effects of vaccination have been detected in over 1,000 mice or in 40 monkeys. In mice, a VEE vector expressing influenza genes induced high levels of neutralizing antibody and protection against intranasal challenge. VEE vectors expressing HIV antigens generated mucosal and humoral antibody, as well as HIV-specific CTL in mice. Rhesus macaques immunized with a cocktail of VEE vectors expressing SIV *gag* and SIV *env* showed SIV-specific neutralizing antibody and strong CTL responses. Results of recent challenge with SIV strain E660 are being monitored. A private company, AlphaVax LLC, has been formed by Davis, Robert E. Johnston and Jonathan Smith to develop the VEE vector technology. ◆

The Gender of HIV Vaccines

By Daniel Whelan and Geeta Rao Gupta

According to the Joint United Nations Programme on HIV/AIDS (UNAIDS), 42 percent of all new HIV infections now occur among women. In some parts of the developing world, this number is believed to be 50 percent or higher. Beyond the obvious impact that HIV will have on the lives of women, most of whom are infected during their reproductive years, neither the economic nor the social impact of the epidemic have been adequately measured, especially from a gender perspective. Nevertheless, the impact of these issues on women's lives and productivity will be devastating to many societies.

As with any population that is disproportionately affected by the HIV/AIDS epidemic, the prospect of a vaccine to prevent infection offers new hope for women. A safe and effective HIV vaccine could play a key role in improving the economic, social and political status of women in developing countries. However, there are many lessons that have been learned since the onset of the HIV pandemic 17 years ago. It took more than a decade for research to begin to reveal that the cornerstones of HIV prevention initiatives—namely the promotion of abstinence, mutual monogamy, sexual partner reduction, treatment for STDs and condom use—were not always as appropriate for women as they may have been for men. The gender-neutral assumptions that these strategies turned out to be precisely that: assumptions. Inherent in all these strategies was confidence in every individual's power to make choices and decisions on their own behalf, and take appropriate action to avoid infection.

What expectations are we making about women's ability to get vaccinated against HIV even if a product was available?

However, research collected throughout the world has painted quite a different picture of our vulnerability to HIV. In many cases, women are unable to take matters into their own hands, due to economic, social, political and even legal realities that compel them to make choices based on different needs. Women who are economically dependent on a male partner may not be able to change the behavior of that partner if it would result in violence, divorce or loss of support. The ways in which societies instill gender roles in men and women, whereby women are to remain ignorant of sexual matters and are expected to fully comply with male sexual needs, and whereby men are expected to demonstrate sexual prowess and domination over their female partners, further contribute to women's inability to control the situations under which they may be at risk of infection. In sum, the driving forces behind the epidemic among women reflect expectations of gender roles in economic, social and sexual relations.

Insofar as a vaccine is a type of "technology" that can be used to prevent HIV infection, there are many parallels that can be drawn between a vaccine's potential accessibility to women and the access they have to already-available technologies, such as male and female condoms or vaginal microbicides that may be developed. What expectations are we making about women's ability to get vaccinated against HIV?

The first important point is to look at populations that would realistically be targeted for vaccination. With contagious diseases, such as influenza, polio or smallpox, everyone is vulnerable, so there are no stigmas attached to seeking out and receiving vaccinations. Will this be true of an HIV vaccine? Research has shown that men sometimes become suspicious of wives or other female partners who are knowledgeable about, or seek information on, sexual matters. If a wife were to seek out vaccination in an effort to protect herself from infection by a husband who has multiple sexual partners, might her husband "suspect" that *she* is the one who is sexually active outside the marriage? This is not a whimsical assumption: women around the world have pointed out that when they seek information or technologies to protect themselves from HIV or STDs, it interferes with the intimacy of the relationship, and causes doubts as to the motivation for seeking such things. When women try to encourage their male sexual partners to "love safely," it is sometimes considered tantamount to an accusation on the part of the woman, and can lead to violent interactions.

This situation is especially true for adolescents, who are not "supposed" to be sexually active. Research conducted with adolescent girls has revealed the many barriers they face in seeking advice and information on sexuality and contraception. As is true with conventional HIV prevention strategies, young girls remain silent about their sexual activity or knowledge because, in the words of one girl from Thailand, "otherwise people might think badly of us." In looking at the prospect of a vaccine, how will adolescent girls and boys avoid the stigmatization of being labeled as sexually active if they seek vaccination?

These examples highlight the necessity of continuing efforts to reduce the barriers that prevent women and adolescents from accessing currently-available HIV prevention tools. These efforts must continue while HIV vaccine developments move forward. We must also support community-based social science research to determine how people, especially women and adolescents, will react to the availability of a vaccine. This will provide insights into how we can best promote vaccination once a product is available, and ensure that all who are at-risk are vaccinated as soon as it is feasible. ◆

The authors work with the International Center for Research on Women (ICRW). Geeta Rao Gupta is also a member of IAVI's board of directors

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must be created so that HIV vaccines can be made available in developing countries at the same time they are made available in industrialized countries (where HIV treatment and other forms of prevention are readily available).

According to Seth Berkley, IAVI's President, creation of the fund will stimulate industrial investment in HIV vaccine development. "A Global HIV Vaccine Purchase Fund would help mobilize private capital and provide real incentives for companies that have the experience and expertise to produce vaccines. It is absolutely vital that we encourage real market forces to develop in the area of HIV vaccines," says Berkley.

The World Bank is currently exploring different potential models for structuring an HIV Vaccine Purchase Fund.

As envisioned, funding for the HIV Vaccine Purchase Fund would be provided by developing country contributions, industrialized country grants and/or guaranteed lines of credit arranged through international agencies such as the World Bank. According to Berkley, if 10 to 15 countries each provide US$100 million, a US$1.5 billion guaranteed market for HIV vaccines would be created in developing countries.

The World Bank is currently exploring different potential models for structuring the Purchase Fund. One model would be to obtain commitments from wealthy nations to provide direct grants or low cost financing for the purchase of HIV vaccines once they are developed. Funds and credit would be provided to countries according to need with the poorest, and those hardest hit by HIV, receiving the most funds. Another more innovative and attractive model would be to create an approved line of credit for countries at the World Bank which would be used to purchase vaccines of a specified nature when they became available. A key advantage of this plan would be that funds need not be made available until a safe and effective HIV vaccine is developed. Thus current funds could continue to be used for short-term prevention and treatment needs.

The senior management of the World Bank has embraced the idea of creating an environment more conducive to industrial investment in HIV vaccines and convened a Bank-wide task force to look at potential financing options. The European Commission has also indicated support for such an idea and is investigating possible participation.

In order to further stimulate HIV vaccine development, IAVI is also pushing the G-8 and G-77 nations to create an HIV vaccine development fund or provide direct funding for HIV vaccine research and development. Such funding would stimulate the development of approaches that may not have a viable commercial market at this time because of intellectual property issues or unfavorable risk-benefit profiles for industrialized countries with relatively low rates of HIV infection. Moreover, issues such as ease of use, temperature stability and the cost of production may be critical in some of the more impoverished markets and, therefore, vaccines with these characteristics, even if shown to have lower efficacy, may have an important role to play.

In the Final Communiqué at the Denver Summit of the Eight in June 1997, the G-8 nations agreed that "the development of a safe, accessible and effective vaccines against AIDS holds the best chance of limiting and eventually eliminating the threat of this disease. We will work to provide the resources necessary to accelerate AIDS vaccine research, and together will enhance international scientific cooperation and collaboration. We call on other states to join us in this endeavor." (Denver Summit of the Eight Communiqué, Paragraph 33, 22 June 1997).

The G-8's agreement to act on HIV vaccine development was, according to many observers, a response to U.S. President Bill Clinton's call for development of an AIDS vaccine by 2007 just one month before the meeting. It was also brought about by international pressure generated by IAVI's Call to Action, an international consensus statement endorsed by more than 80 leading organizations in 52 countries. However, since the Denver Summit, the G-8 nations have done little, if anything, in the area of joint action on HIV vaccines.

IAVI is marshalling worldwide support for the Vaccine Purchase Fund. "We are committed to encouraging real private sector investment in HIV vaccines and to guaranteeing that all the countries of the world have full access to any HIV vaccines that are developed," says Berkley. "We must begin working now to ensure that the huge disparity that we see in terms of access to HIV treatments never happens with HIV vaccines." ◆

PALM SPRINGS MEETING
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epitope enhances CTL response in mice. He also reported that his lab has tested a broad range of different cytokines that were inserted into the adjuvant. Of the different cytokines tested, GM-CSF seemed to generate the most potent immune responses and GM-CSF and interleukin-12 appear to act synergistically in inducing CTL response. In addition, Berzofsky's team found that intrarectal immunization of the peptide vaccines protected mice against challenge with a vaccinia construct expressing HIV *env*, while the subcutaneous injection of the vaccines did not protect the mice against challenge. The NCI is currently testing two different HIV peptide vaccines in HIV-infected individuals. ◆

UGANDA VACCINE TRIAL *continued from page 1*

The ALVAC vCP205 candidate vaccine is a recombinant canarypox virus with HIV genes inserted in the vector (see *IAVI Report*, vol.2,no.1). It is based on a clade B strain of HIV. While the A and D clades of HIV predominate in Uganda, preliminary data suggests that some of those vaccinated with a clade B canarypox vaccine could have immune responses against some clade A and D HIV isolates. Pasteur Merieux Connaught is also developing ALVAC vaccines based on HIV subtypes found in Uganda (see page 8).

A total of 40 participants will be enrolled in the trial. Of these, 20 will receive the vCP205 vaccine, 10 will receive the ALVAC-rabies vCP65 (an active control) and 10 will receive a placebo. The trial is designed to examine the nature of the immune responses generated by the candidate vaccine and not whether the vaccine protects against HIV. It will be coordinated by the Joint Clinical Research Centre (JCRC), an HIV research facility created in 1992 by a collaboration of MUK and the Ministries of Health and Defense. JCRC is one of the most advanced HIV research lab facilities in all of Africa. A state of the art cellular immunology laboratory has been established at the lab to measure cytotoxic T lymphocytes (CTLs) in the trial.

Screening and enrollment of volunteers for establishment of transformed B-cell lines began last year. The establishment of viable cell lines is an essential step for the evaluation of CTL responses in vaccine recipients. Cell lines have been successfully established for about 70 volunteers, according to David Hom of CWRU. There are two additional rounds of informed consent and screening before volunteers will actually be enrolled in the trial.

Why has it taken so long to start a trial in Uganda of a vaccine that has been demonstrated to be safe in almost 1,000 volunteers in the U.S. and France?

One major setback was the death of Catherine Othieno, the Ugandan immunologist responsible for the CTL lab at the JCRC. Othieno was tragically killed in an auto accident in Kampala in March.

Bureaucratic red tape, challenges in setting up the CTL lab and a multi-faceted approval process contributed to delays in the trial.

"Catherine's death set us back a bit," says Mugerwa. Because of the accident, the trial is likely to begin without a Ugandan researcher to oversee the in-country measurements. A team of U.S. researchers from the lab of Bruce Walker at the Massachusetts General Hospital will remain on-site to conduct the CTL assays for the trial while another Ugandan researcher is identified to replace Othieno. Laboratory oversite for the trial is being provided by Haynes Sheppard of the U.S. HIVNET Central Lab at the California Department of Health in Berkeley, in collaboration with Kent Weinhold of the AVEG Central Immunology Lab at Duke University.

Mugerwa notes that the process of educating the Ugandan community about the trial has taken longer than anticipated. Bureaucratic red tape, technological challenges in setting up the CTL lab and a multi-faceted approval process have also contributed to the delays. "But this is the first trial of its kind in Africa," he observed. "And we needed to make sure that everything was set up properly and done right. When you do things for the first time, there are always delays."

The Uganda government, according to both Mugerwe and Hom, has been very supportive of the trial.

And Mugerwe believes that the long preparatory process will make future HIV vaccine trials in Uganda much easier. "We have gotten over the hurdles and that is no small accomplishment. We are very optimistic about testing other candidate HIV vaccines in Uganda." ◆

THE INTERNATIONAL AIDS VACCINE INITIATIVE

The International AIDS Vaccine Initiative (IAVI) is a global initiative founded in 1996 to ensure the development of safe, effective, accessible preventive HIV vaccines for use throughout the world. For further information on IAVI or HIV vaccine development, visit our website at http://www.iavi.org or e-mail us at: info@iavi.org.

IAVI REPORT

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You have to realize that, within each vaccine company, HIV vaccine programs compete with other vaccine programs for R&D resources. There are many factors that go into the equation. One reason we have worked so hard in this area has been the strong humanitarian tradition that exists at Pasteur Mérieux Connaught and our belief that a vaccine is ultimately the only way to end the worldwide HIV epidemic.

IAVI REPORT: IAVI has talked to the World Bank, and the G-8 nations, about creating an HIV Vaccine Purchase Fund, which would help provide funding and financing for poorer countries to purchase HIV vaccines.
KLEIN: That would be very useful because I don't think that most countries will be able to afford an HIV vaccine for their entire population.

IAVI REPORT: There are concerns that the use of post-exposure prophylaxis or early treatment in HIV vaccine efficacy trials could make it difficult or impossible to learn whether a vaccine prevents infection or disease.
KLEIN: These issues at the center of our preoccupations and we are discussing them not only among ourselves, but also with government agencies. PMC is committed, as always, to running totally ethical trials that can also give us the answers we need to move forward.

IAVI REPORT: Last year, U.S. President Bill Clinton set a goal of developing an HIV vaccine within ten years. Do you think we're going to be able to meet that?
KLEIN: I think it's a real challenge. It's very stimulating to have stretched objectives. Maybe, in ten years, we will know whether a candidate vaccine is partially efficacious. That would be a great step forward.

IAVI REPORT: If there's one thing that could help speed things up, what would it be?
KLEIN: The first thing is to strengthen collaborations between scientists from academia and industry and join research efforts in a concerted manner. As important is the political willingness of governments, particularly the wealthy ones, to help finance these research and development efforts. This is essential if we want to have an HIV vaccine in ten years. ◆

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viral load in the volunteers' blood during the test. According to IAPAC representatives, therapy with a cocktail of the four drugs would begin immediately if any volunteers showed any signs of disease.

Plenary Talk by David Baltimore

In his plenary talk, David Baltimore described the lack of knowledge about the immune system as the major obstacle towards rapid development of an AIDS vaccine. He noted that "there are three ways you can imagine a vaccine working – through antibodies, CTLs and the third is something else. And no one knows what this mechanism is." However, Baltimore expressed optimism that new knowledge about immune mechanisms may lead to the creation of different vaccine approaches.

The head of the AVRC voiced skepticism about the ability of previously developed gp120 envelope vaccines to protect against HIV. He noted that the vaccines induce high levels of antibodies but that these antibodies do not neutralize wild-type strains of HIV. *[Editor' note: The relationship of these "wild-type" HIV strains to HIV that circulates in body has yet to be definitvely understood.]*

Baltimore also noted that many vaccines are able to protect animals against non-pathogenic HIV or SIV challenge viruses. "But as the challenge virus becomes more pathogenic, far fewer vaccines protect." However, he cautioned that "we may be we asking too much to expect a candidate vaccine to protect against pathogenic, intravenous challenge." The AVRC head stated that human testing has to be an integral part of the vaccine research effort.

When asked whether U.S President Bill Clinton's goal of developing an HIV vaccine by 2007 could be met, the AVRC head replied that "Only if we have really good candidate vaccines. But overall," he added, "it is unlikely that we will have a vaccine that we know is safe and effective in ten years." In response to another question, Baltimore stated that he wouldn't personally take a live attenuated HIV vaccine at this time. ◆

IAVI in Geneva

IAVI will be conducting a number of informational meetings and briefings during the XII International Conference on AIDS, which will be held in Geneva on 28 June - 3 July 1998. These include:

- **IAVI Press Reception and Briefing**
  Sunday, 28 June 1998
  Noga Hilton Hotel,
  Food and drink at 7:00 pm
  followed by a short briefing
- **Focus: Toward Global AIDS Vaccine Action**
  Tuesday, June 30
  7:30 - 9:30 pm, Room I
  Refreshments will be served.
- **Meeting of Call To Action Organizations**
  Wednesday, July 1
  7:30 - 9:30 pm
  Palexpo Room E
  Light dinner served.

In addition, there will be a number of other vaccine-related sessions including:

- **Plenary Session on HIV Vaccines, presented by Hans Wigzell**
  Monday 29 June 1998,
  9:00-10:30 am
- **Bridging Session on HIV Vaccines, chaired by Seth Berkley and Jose Esparza**
  Tuesday, 30 June 1998,
  3:00-4:45 pm

For additional information, contact IAVI's website at www.iavi.org.