Keystone Symposium: New Vaccine Candidates — and a Major New Player

BY PATRICIA KAHN

KEYSTONE, COLORADO—For six intense days at this well-known Rocky Mountains conference venue, researchers at the “AIDS Vaccines in the New Millennium” meeting (28 March-2 April) heard a broad range of presentations on HIV vaccine studies. Several talks extended the list of vaccine candidates that confer at least partial protection in monkeys (see Retrovirus Conference article, p. 3); these are summarized here. Other speakers discussed the role of mucosal immunity in vaccine protection or described strategies aimed at the still-elusive goal of inducing broadly neutralizing antibodies to HIV. Full meeting coverage will follow in our next issue.

**Merck’s DNA- and Adenovirus-based Vaccines**

In two much-anticipated talks, scientists from Merck Research Labs gave the first public presentation on the company’s current HIV vaccine program. Merck’s entry into the field is widely seen as an important boost, given how few large pharmaceutical companies are involved. (Another newcomer is GlaxoSmithKline; see Vaccine Briefs, p. 160.)

The Merck researchers described some of the extensive pre-clinical studies that led the company to move two types of vaccines—those based on naked DNA and on adenovirus vector—into Phase 1 trials. Both vaccines target cellular immune responses, although Merck is actively searching for approaches that stimulate strong neutralizing antibodies, according to Emilio Emnini, vice president of antiviral and vaccine research. Merck’s aim is to develop candidates both as preventive and therapeutic vaccines.

John Shiver, Director of Vaccine Research, began by showing comparative studies of single types of vaccines (i.e., no prime-boost combinations) in macaques. These were: plasmid DNA (5µg) in saline, alum or CRL1005 (anionic block co-polymer adjuvant); and MVA- (modified vaccinia Ankara) and adenovirus type 5 (Ad5)-based viral vectors, both used in the “highest achievable” doses. The Ad5 vector cannot replicate or integrate into the host genome, and its encoded proteins persist only for a “finite” time, accord-

INDIA BEGINS AIDS VACCINE WORK WITH IAVI

DEHLI—On Monday 19 March 2001, the Indian Ministry of Health and Family Welfare announced the launch of a partnership with IAVI to develop AIDS vaccines suitable for India. The agreement, which also includes the Indian Council for Medical Research (ICMR), provides a framework for joint projects in vaccine design, buildup of capacity for clinical trials and transfer of appropriate vaccine manufacturing technology to India. Plans also call for education and advocacy projects around HIV vaccines, and for facilitating community involvement in these activities.

The first program in the partnership will develop a vaccine that incorporates multiple HIV genes (env, pol, gag, rev, nef and tat) from an Indian isolate of subtype C into an MVA viral vector. Under a separate agreement announced on 20 March, this vaccine will be designed and engineered at Therion Biologics, a biotech company in Cambridge, Massachusetts, with visiting Indian scientist Sekhar Chakrabarti of the National Institute of Cholera and Enteric Diseases in Calcutta. Therion will also produce pilot vaccine lots for Phase 1 clinical testing in India.

The collaboration between India and IAVI grew out of a working group formed in January 2000 under the auspices of the ICMR, headed by N.K. Ganguly. It includes scientists from several ICMR-associated institutes and from IAVI.

Researchers led by Ramesh Paranjape at the National AIDS Research Institute (NARD) in Pune
GlaxoSmithKline Developing HIV Protein Vaccine

The newly merged company, GlaxoSmithKline, publicly announced its entry into the HIV vaccine field at the inaugural investor meeting on 22 February 2001. President and General Manager of Biologics, Jean Stéphenne, provided participants with a brief overview of the vaccine concept and a glimpse at preliminary, "proof-of-concept" data from a primate challenge study. The vaccine is a cocktail of purified recombinant HIV proteins in a novel adjuvant developed by the former SmithKline Beecham. Stéphenne showed preservation of CD4 counts and control of viral load in four vaccinated animals, but not in four unvaccinated controls, one year after a pathogenic virus challenge. He also reported that the National Institutes of Health’s HIV Vaccine Trials Network (HVTN) plans to initiate Phase I human trials of this vaccine before the end of the year. According to the HVTN website (www.scharp.org/hvtn/protocol), the proteins currently slated for inclusion in the vaccine are Nef, Tat and gp120. GlaxoSmithKline is also planning a therapeutic trial of the vaccine in HIV-infected people, to begin in early 2002.

Maxygen Partners With IAVI in Vaccine Effort

Maxygen, Inc. has announced plans for a collaboration with IAVI aimed at developing novel HIV vaccines. The project will exploit Maxygen’s Molecular-Breeding™ technology to rapidly screen variants of HIV envelope proteins for the ability to generate neutralizing antibodies in small animal models. Under the agreement, Maxygen will receive at least three years of HIV vaccine research and development funding from DBLV LLC, an entity established and financially supported by the Rockefeller Foundation. Maxygen has committed to provide IAVI with a royalty-free license to ensure that any successful product reaches all those who need it regardless of ability to pay.

Changes at Aventis-Pasteur

Alf Lindberg, who since 1995 has served as Vice President for Research & Development at Aventis Pasteur, left his post at the end of March. Lindberg will be replaced by Michel De Wilde, the former Deputy Executive Vice President for R&D. Prior to joining Aventis Pasteur, De Wilde was Director and Vice-President of SmithKline Beecham.

Also departing Aventis Pasteur is Pierre Caudrelier, the head of clinical development of the HIV product line. Caudrelier’s former post will now be held jointly by DNA vaccine expert Sanjay Gurunathan, previously a Senior Fellow in Robert Seder’s lab at the National Institute of Allergy and Infectious Diseases (NIAID), and Dominique Blanc.

Bioqual Wins NIH Contract for Animal AIDS Vaccine Studies

Bioqual Inc. has won a seven-year, US$8.2 million contract from the National Institutes of Health to provide nonhuman primates for animal studies of immunodeficiency viruses and potential AIDS vaccines. Under the contract, which was awarded through the National Institute of Allergy and Infectious Diseases (NIAID), Bioqual will house and maintain primates for NIAID’s new Vaccine Research Center (VRC) in Bethesda. The VRC was launched in 1999 and receives joint funding from NIAID and the National Cancer Institute (NCI).

The new contract comes on the heels of two other government awards received by Bioqual last year: $4.6 million from NCI in December for “Nonhuman Primate Models of AIDS: Prophylactic and Therapeutic Studies” and $10.6 million from NIAID the previous January for “Care and Housing of AIDS Research Animals.”

GlaxoSmithKline’s One Shot Deal

In a move the company hopes will contribute to international public health, GlaxoSmithKline (GSK) Biologicals is distributing a disposable syringe that is difficult to use more than once. The new product, known as an Auto-Disable (A-D) Syringe, is equipped with a plunger that locks into place once fully depressed. The design is intended to minimize the risk of transmitting blood-borne pathogens from one vaccine recipient to the next, by preventing re-use of the syringe.

GSK announced on 15 March that the A-D syringe will be supplied with all vials of their liquid pediatric vaccines delivered to “developing countries and emerging markets.” The company cites World Health Organization (WHO) and UNICEF recommendations that A-D syringes be used preferentially for both routine and mass immunizations. A recent WHO analysis estimated that disease transmission via syringe re-use causes 1.3 million deaths annually. The agency hopes to achieve a global shift to A-D syringe use by the year 2003.

VaxGen Receives NIH Grant to Develop Clade C Vaccine

VaxGen has received a Small Business Innovation Research (SBIR) grant from the US National Institutes of Health for the development of a vaccine against HIV from African clade C isolates. VaxGen’s recombinant envelope protein vaccine, AIDSVAX™, is in Phase III human testing in the US, Canada, Europe and Thailand. Currently there are two formulations of AIDSVAX™, one targeted to clade B and a second against clades B and E. The first phase of the SBIR grant provides VaxGen US$150,000 for the collection and characterization of clade C HIV isolates. The second phase of the grant, dependent on successful completion of this initial work and now in preparation, is a $950,000 award for development of a trivalent AIDSVAX® combining envelope proteins from clades B, C and E. The interim efficacy analysis of VaxGen’s ongoing US/European Phase III clinical trial is scheduled for November, 2001.
Over 3,000 people attended the 8th Conference on Retroviruses and Opportunistic Infections in Chicago (4-8 February 2001), the largest annual HIV science meeting. While virology and antiretroviral therapy generally dominated the agenda at past conferences, this year’s event continued the recent trend towards a more prominent role for immunology and vaccines.

Many presentations in Chicago added to the growing body of data indicating that broad, vaccine-induced cellular immune responses to SIV/SHIV can at least partially protect monkeys against pathogenic challenge viruses. The overall sense was therefore one of increasing conviction that vaccines which can control viral replication and prevent or delay disease are possible.

Yet, although several researchers described ongoing work to develop vaccines analogous to these SHIVs and SIVs in humans, few new types of candidates have entered Phase I clinical testing yet, pointing to the still-significant gap between primate promise and human trials.

Protection and Memory with a DNA/MVA Vaccine

One highlight of the meeting was Harriet Robinson’s presentation on a prime-boost approach that induced impressive protection. Carried out at the Yerkes Regional Primate Research Center/Emory University in Atlanta, the work has since been published (Science 292: 69-74, 2001). The research was published a month after the conference and made a significant media splash.

A key feature of the study was the long (seven month) lag between immunization and challenge, a contrast to the usual strategy of challenging the animals shortly after the last vaccine boost, when immune responses peak. Robinson’s study design ensured that vaccine-induced T-cell responses had matured into immunological memory, and therefore tested the critical question of whether resting T-cells can mediate protection against HIV. It also used a mucosal (rather than intravenous) challenge, mimicking the main route of HIV transmission in humans, and a two-component vaccine encoding multiple SIV/HIV proteins that induced broad immune responses.

The components are a DNA plasmid carrying eight viral genes (SIV gag, pol, env, vpr and vpr and HIV-1 env, tat and rev) and a recombinant MVA (modified vaccinia virus Ankara) vector with HIV-1 env and SIV gag and pol, made by Bernard Moss at the US National Institutes for Allergy and Infectious Diseases (NIAID; Bethesda). Rhesus macaques were divided into four groups of six animals each and received the DNA vaccine at weeks 0 and 8, either intradermally (i.d.) or intramuscularly (i.m.), followed by a combined i.d./i.m. boost with MVA at week 24. Four animals (two mock-immunized and two naive) served as controls.

The vaccine elicited both CD4 and CD8 T-cell responses, detected during the peak response (one week after the MVA boost) as well as in the memory phase (six months later). These responses were measured with ELISPOT assays for Gag-specific interferon-gamma production, carried out with the Centers for Disease Control (Atlanta). As expected, the post-boost peak in Gag-specific T-cells was followed by a 5 to 20-fold drop in the memory phase.

Seven months after the boost, animals were given a highly pathogenic intrarectal (i.r.) challenge (SHIV 89.6P) and then monitored closely. After a transient spike of viremia, the geometric mean viral load levels in all vaccinated groups declined to 1,000 copies or less, while CD4 counts were preserved. Thereafter, only one vaccinated macaque (from the low-dose i.m. group) showed intermittent viral load increases to >100,000 copies and a drop in CD4 counts. Clinically, all immunized animals remained completely healthy. In contrast, four non-vaccinated controls showed the expected high virus levels (averaging two million copies eight weeks after challenge) and a rapid crash in CD4 counts; all died within 32 weeks.

Analyzing the data for possible immune correlates of protection, Robinson reported a highly significant (p<0.001) inverse association between peak pre-challenge SIV-specific T-cell responses (as measured by ELISPOT) and viral load five weeks post-challenge. This correlation echoes data reported by Norman Letvin and colleagues, who demonstrated an inverse relationship between vaccine-induced CD8 CTL responses (measured by tetramer staining) and post-challenge viral load (Science 290: 496-492, 2000). However, Robinson may be the first to correlate both CD4 and CD8 T-cell responses with post-challenge virologic outcome.

Plans call for moving this vaccine approach into human trials through the HIV Vaccine Trials Network (HVTN) in early 2002. The test vaccine construct is based on HIV-1 subtype B and will include the gag, pol, rev, tat and env genes in the DNA prime and gag, pol and env in the MVA boost. In a parallel project, Robinson is working with NIAID and CDC on extending the approach to HIV-1 subtype A vaccines.

VSV-based Vaccines in Monkeys

Over the past several years, John Rose and colleagues from Yale University (New Haven) have been developing a vaccine vector based on attenuated vesicular stomatitis virus (VSV; see J. Virol. 74: 10903, 2000). VSV can be pathogenic in farm animals but rarely causes disease in humans. Exposure to VSV is unusual in most human populations, although Rose mentioned that antibodies to VSV are found in up to 94% of residents in some tropical areas of the
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Americas where VSV infection of cattle is common. At the conference, Rose presented results of the first primate SIV challenge experiments, done with Preston Mazx of the Tulane Regional Primate Research Center and Aaron Diamond AIDS Research Center (NY).

Animals received three immunizations at two month intervals, each time using a different variant of the VSV vector (to circumvent the neutralizing antibodies that develop to the viral envelope after each vaccination round). The three variants, called "glycoprotein exchange vectors," encode different VSV envelopes but otherwise carry identical vector sequences, along with the HIV envelope (89) and SIV gag genes. Either two or six months after the last boost, five vaccinated macaques and six controls (given vector without HIV/SIV genes) were challenged i.v. with SHIV89.6P.

In vaccinated animals, CD4 counts initially declined but showed a trend back towards normal levels within four months, and all of them remained clinically healthy. (Viral load data was not yet complete). Control monkeys showed high viral loads and a rapid loss of peripheral CD4 T-cells, although two animals gradually recovered CD4 counts and partially controlled their infections. Detailed analyses of SIV-specific T-cell responses are pending. Doug Nixon (Gladstone Institute, San Francisco) conducted preliminary ELISPOT assays on two vaccinees and found Env- and Gag-specific responses that increased after each boost.

Combining these results with an earlier pilot study of two immunized animals (on a different schedule) and two control macaques, Rose concluded that 7/7 vaccinated macaques remained clinically healthy, showed relative preservation of CD4 counts, and maintained low or undetectable viral loads. In contrast, 4/8 control macaques developed symptoms of simian AIDS by 140 days post-challenge, 2/8 have high viral loads, low CD4 counts and are expected to develop symptomatic disease. The remaining 2/8 controls are slowly recovering CD4 cells and controlling viral load. Wyeth Ayers is planning to work with Rose's group on the development of VSV vectors for HIV vaccines.

**Challenge Studies with VEE-based vaccines**

Bob Johnston (University of North Carolina) presented new data from challenge studies on monkeys continued on 14
For the second time, the IAVI Report profiles two individuals who have made important contributions to the AIDS vaccine field. In recognition of the thousands of people who have participated in HIV vaccine trials over more than a decade, this time we are featuring a volunteer from Africa's first AIDS vaccine trial and another from the heart of Amsterdam. Here they talk about their experience as trial participants and what it has meant in their lives.

From Trial Volunteer to AIDS Advocate: A Ugandan Soldier Makes Vaccines His Mission

BY EMILY BASS

For Paul Wetauka, life as a soldier in the Ugandan army comes with a sense of duty. It also comes with a sense of fear—not of dying in battle, but of acquiring HIV. "The most endangered people in our society are the army," says the stocky, soft-spoken 32-year-old. "We are the army of the people," he explains, referring to soldiers' casual sexual contacts. "We move door to door and we get friendship."

Unlike the majority of Ugandans, Wetauka has not lost any family members to the virus. But he has watched many colleagues die: the latest available data estimated seroprevalence among the military at 27% (UNAIDS, 1996), even as prevalence is decreasing in some parts of the general population.

In 1990, the Joint Clinical Research Center (JCRC) in Kampala offered Wetauka a way to confront his fears. The Ugandan research institute approached him and his fellow soldiers as part of its early preparation for the Phase I trial of the ALVAC HIV-canarypox vaccine, the first African HIV vaccine trial. The project appealed to Wetauka's patriotism. "The JCRC came and said, 'What vaccines are in America may not be useful in Uganda,' he recalls. "We will get involved in getting a vaccine for Uganda or eastern Africa.' I was convinced."

Confident and well-groomed, Wetauka is fluent in Swahili, English and Kiswahili, the language of his mountainous home district, Mbale. His relative wealth, education and stable employment set him apart from the majority of Ugandans. These advantages also made it easier for Wetauka to take part in the trial—both his immediate employers and his national leader supported the time commitments participation required.

Ugandan president Yoweri Museveni's early and effective efforts to make AIDS a national issue had helped sow the seeds for Wetauka's interest. From the late 1980's on, Museveni has spoken widely about HIV transmission and prevention. As a member of the President's protection unit, Wetauka had first-hand experience of Museveni's openness about HIV. "Every Ugandan became concerned about AIDS," says Wetauka, of the president's campaign. "No big person in government had ever come out about HIV."

Wetauka agreed to have his blood drawn and to join vaccine preparedness efforts, which included two years of focus group meetings. In these meetings, potential trial participants learned about everything from informed consent, placebos and ways to measure vaccine efficacy, to safer sex and modes of HIV transmission. The process whetted Wetauka's appetite—and tested his patience. "We were there for two years of just focus groups," he exclaims. "We said, 'Please give us the vaccine. We are ready.'"

The lengthy preparations gave the JCRC and its head, Peter Mugyenyi, time to set up the infrastructure needed for a vaccine trial and to determine that a largely military cohort could be used. "The advantage of the military is that it is an organized group," says Mugyenyi. "The disadvantage is that, by nature of the work, they move around. In spite of that, we were able to follow them." In addition, says JCRC counselor Christine Akola, it was relatively easy for soldiers to accept the idea of

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testing an unknown vaccine. "These are men who have already agreed to die for their country," she explains.

They were also the men on the front lines of the Ugandan AIDS crisis. Radio reports brought news of the explosive numbers of infections in the Rakai district, and in the barracks, Wetaoka's bosses started to die from the disease. By the time the JCRC called its meeting with the army, he and his fellow soldiers were all too familiar with the illness called "slim." It took the JCRC some years to get to us," he says. "We were already aware."

When it was finally time to select trial participants, Wetaoka agreed to be tested for HIV again. Waiting to find out whether he had been chosen was nerve-wracking. Wetaoka believed that a rejection would mean he was ineligible because he had become infected. When he got the news that he would be one of the 40 participants, it was a tremendous relief. "I was very happy, because that was not the end of me," he says. "When they used me, I knew that I was safe." With the relief came a heavy sense of responsibility. "I never wanted to let the JCRC down by having them announce to me one day that I have HIV," he says. "For those two years of the vaccine trial, I abstained from sex or used a condom."

Wetaoka's unflagging commitment drew the attention of the JCRC staff, who asked him to join the trial's community advisory board (CAB), a group that included religious leaders, HIV-positive activists and community leaders. Wetaoka was again ready and willing. At its meetings, invited speakers discussed a wide range of issues, such as the different standards of care possible in rural and urban settings. The experience gave Wetaoka an even closer look at the challenges of AIDS research in Uganda.

In spite of his newly-acquired scientific knowledge, Wetaoka's worries got the better of him: after the first injection, he wondered whether the vaccine would cause cancer, blindness or hair loss. But as the study continued, these anxieties waned. It helped to learn that no serious side effects emerged for Wetaoka or any of his fellow volunteers. Akola says that volunteers came with a myriad of medical problems not related to the vaccine study; the trial staff's willingness to treat these unrelated symptoms—and, frequently, to see the families of volunteers as well—was time-consuming, but helped cement trust and confidence.

While individual volunteers paid close attention to possible side effects, the AIHAC trial drew intense media attention, both locally and internationally. As a groundbreaking effort, it was subjected to a number of critical questions: Why were so few women enrolled? Why was the vaccine based on HIV clade B when clades A and D predominate in Uganda? If the vaccine eventually proved to be effective, would it be available to Ugandans?

Wetaoka says that he never felt the effects of this media attention. For him, the scrutiny came in a differ-

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A Gay Dutch Man Confronts AIDS Through Vaccine Trial Participation

BY CRAIG McCLURE

Just a few steps from the canals and bustle of the Weesperplein, a tranquil office at Amsterdam's Municipal Health Service serves as home to Europe's only site in the world's first Phase III HIV vaccine trial. Together with over 5000 gay men and 300 at-risk heterosexual women at 60 other sites in the US, Puerto Rico and Canada, its 120 volunteers are rolling up their sleeves to help determine whether AIDSVAX, VaxGen's subtype B-based gp120 vaccine, can reduce sexual transmission of HIV.

One of them is Henk Cazemier, a gay man in his late thirties. For many years Cazemier has witnessed the devastating impact of AIDS on the gay community in Amsterdam—and felt its impact on his own self-esteem. "When I came out many years ago in a small city in Holland, I became involved in the local gay organization, the COC, helping other people who were coming out and organizing social events," he recounts. "At that time we all thought AIDS was just an American thing. Then it became something that only happened in Amsterdam, and only to the 'bad' gay men. I guess I just tried to ignore it and thought it had nothing to do with me. I had a lot of denial."

Cazemier's decision to participate in the trial was rooted in these early experiences and by a subsequent period of personal difficulties, beginning with a wave of budget cutbacks at the library where he worked as a head librarian. "I became a librarian so that I could help children learn to read, but suddenly my life was all about stress and worrying about the people who worked for me losing their jobs," he said. At the same time he was growing more afraid of AIDS. "I had never been tested, but some people I knew had begun to get sick. I decided it was time to get tested and to stop being afraid. Being afraid of AIDS was like going back into the closet again."

Suffering from depression, Cazemier left his job and moved to Amsterdam. Soon after, he tested negative for HIV. "When I found out I was negative I was both surprised and happy. I wanted to get involved with AIDS in some way and thought about becoming a 'buddy' (a caregiver for someone with AIDS) through the COC. But I was still recovering from depression, so I was advised to do other volunteer work instead."

One outlet was helping to organize the Gay
Games, an international sporting competition for lesbians and gay men, held in Amsterdam in 1998. He also joined the Amsterdam Gay Men’s Chorus, where he met many new friends—some with HIV or AIDS. Then came the decision to go back to school and study ecological management—key steps in getting his life back in gear.

That’s when he found his chance to do something about AIDS. “It was just after this, in 1999, that I saw a poster for the vaccine study in one of the bars,” he recalled. “There were posters all over the gay scene, with photos of different beautiful men and the caption, ‘If you want to be a hero, here’s the way to do it.’” Henk found the caption a bit ridiculous and “too American,” but decided to phone the number on the poster for more information.

“After my first interview with Dieuwke Ram [the trial site coordinator] I was pretty sure I wanted to volunteer,” Cazemier continues. “But I went away and read a lot of information before I made my decision. I was confident that the vaccine seemed safe—that was very important. A lot of people didn’t think the vaccine would work, but that wasn’t really so important to me. What I wanted was to contribute something. I didn’t want to just ignore AIDS anymore.”

His greatest concern about joining the trial was the possible reactions from his partner and his parents. “When I told my partner about joining the trial, he was concerned about the safety. I gave him a lot to read and we talked and he was fine. My mother was worried at first, but for a different reason. She thought that if I was in the trial I must be having sex with lots of people.” Fortunately, both his partner and his parents are now supportive of his participation.

The material VaxGen provides to study volunteers specifically warns them of possible repercussions from disclosing their participation; in particular, they are advised not to inform employers or insurers, who might react negatively to the implication that volunteers are people at high risk for HIV. “I was surprised about how much the information focused on secrecy,” Cazemier said.

Henk’s experience of the trial thus far has been wholly positive. In fact, he enjoys his visits to the clinic. He feels at home with Dieuwke Ram and the other nurse working at the site, Nel Albrecht. “Dieuwke has become my first long-term relationship with a woman!” he laughs. “At first I thought it was strange to talk about my sex life with two women, but they have

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made me feel very comfortable." He even points to a troubled time last year when his clinical depression recurred and the two were far more supportive than his doctor.

Ram confirms the trial staff's strong emphasis on creating the right atmosphere, which originated in a history of engagement with the community and has permeated the recruitment process down to details such as scheduling enough time for clinic visits so that each participant has complete privacy. "We have a good cooperation with the gay community in Amsterdam," she says. "In the early stages of the trial we consulted all the major community organizations, who helped us with outreach and recruitment and advised us on how best to build support for the trial. Dr. Roel Coutinho [director of the Municipal Health Service and principal investigator of the trial site] also has a long-standing, positive reputation with the gay community here in Amsterdam."

Cazenier is confident that a lot will be learned from the study, however it turns out, and views his participation simply as a way to contribute to ending the AIDS epidemic. Even though antiretrovirals are helping people with HIV in Holland live longer and healthier lives, he worries that people are still getting infected—and that less attention is paid to the issue. "We used to have big campaigns in the media in Holland, billboards and television, urging people to wear condoms. Now there is very little. People here are forgetting about the risks. And more and more people around the world are getting infected, in Africa especially."

Participation in the trial requires a significant commitment, including a total of 17 clinic visits over three years, regular risk reduction counseling, HIV testing, sexual behavior questionnaires, the regular drawing of numerous tubes of blood and, of course, the 6-monthly injections of placebo or vaccine. But Cazenier says they are "not too much of a burden."

"Being in the study reminds me of what a luxury it is to live here in Holland," he adds. "I'm just giving back a small amount. It's just a little thing, but it's something." As for the "vaccine hero" label of this article, he finds it slightly silly. "I don't feel like a hero," he says. "The trial is just one of the things I do in my life."

Craig McClure is a Canadian vaccine advocate and IAVI consultant. He is also a participant in the VaxGen trial, volunteering at the Toronto site.

FROM THE EDITOR:

In our last issue, founding editor David Gold wrote to you that he has moved on to other work at IAVI and that I have succeeded him as editor of the IAVI Report. Now it's my turn to let you know what's new at the newsletter and what course we plan to steer.

There are many changes afoot, beginning with an obvious one: our new look. We asked graphics consultant Stephen de Francesco for a more open design that would make the Report easier and more inviting to read, and we hope the new layout achieves that.

We also have two new staff writers, both of whom have been freelance contributors to the Report in recent months. Richard Jefferys joined IAVI in February after six years at the AIDS Treatment Data Network co-editing their newsletter and helping to run their community education and treatment advocacy activities. While there he became increasingly interested in immune control of HIV, leading him to begin thinking and writing about vaccines. Richard's experience on the AIDS therapy front and his deep commitment to the fight against AIDS are already valuable assets to the Report. The same holds true for Emily Bass, who will join us half-time beginning in May. Alongside her many articles on AIDS—mostly for HIV Plus magazine and amfAR's Treatment Insider—Emily spent four months travelling in Africa and reporting on AIDS issues in the wake of last summer's conference in Durban.

A top priority for the three of us is to continue expanding our international coverage—for example, with articles like the recent "On the Ground" series about HIV field research sites, to be continued next time with a report from Haiti. We also have several "special focus" issues in the works, one on some key scientific areas and another on Asian countries involved in AIDS vaccine work.

Another innovation in the works is a bi-monthly IAVI Report Online, which will be sent to subscribers electronically beginning this summer. The online Report, to be coordinated by Richard Jefferys, will contain summaries of newly-published research articles from the scientific literature (with abstracts and links to full-text journal articles, where permitted by the publishers), as well as short news items on policy and other issues relevant to HIV vaccines.

Perhaps our key challenge—one we're more than glad to grapple with—will be to keep up with the rapid growth in the field. After years on the sidelines, AIDS vaccines are finally getting more recognition at the political, scientific and grass roots levels. Several new types of AIDS vaccines are poised to enter the pipeline within the next few years, and there is far more basic research fueling this development. What's more, a growing number of countries and individuals from many different walks of life are joining in the push for an AIDS vaccine.

That change is mirrored in the growth of the Report, which now reaches a subscriber list of nearly 10,000 people in 140 countries and amazingly diverse settings—from state-of-the-art laboratories at major research centers to the offices of policy makers and journalists around the world to rural AIDS care centers without electricity or running water.

But some things will not change. When David Gold started the IAVI Report back in 1996, he envisioned a focused, dedicated source of information on HIV vaccines that could serve a vital role in building a global AIDS vaccine movement. Five years later, we are still committed to this vision of the Report. And that means doing more of the same: covering the meetings, events, decisions, people and trends in the expanding global enterprise devoted to making AIDS vaccines that reach all who need them, wherever they live.

Let us hear from you. Tell us if we're writing what you want to read or if you have news we might want to include.

Patricia Kahn
"The Wayward Search for an AIDS Vaccine"

Jon Cohen is a science journalist who began following the AIDS vaccine field in 1989 while working as a general reporter. A year later he started contributing to Science magazine, and since then, in his continuing role there as Contributing Correspondent, has written dozens of articles on the progress and difficulties along the road to an AIDS vaccine. In January 2001, Norton published his provocative book, "Shots in the Dark: The Wayward Search for an AIDS Vaccine," a chronicle and critique of the 15-plus year effort. He spoke with IAVI Report Editor Patricia Kahn at the recent Retrovirus Conference.

You've followed the AIDS vaccine field longer and closer than just about any other journalist. What drew you into it?

I come from an era when Jonas Salk was a hero. Even though my generation wasn't afraid of polio, I knew the lore.

When I was an undergraduate in San Diego, Salk worked close by. I convinced him to let me write about his work and the controversy with the polio vaccine, which was still going on. We made a deal. I knew he hated journalists, for the most part, so I told him, 'I don't want to publish anything. I just want the exercise of writing about you.' He agreed. I wrote about him, and that was that.

In 1989, I was an editor and reporter at City Paper in Washington, D.C., writing about anything and everything. I read in the Washington Post that Joe Gibbs at NIH was collaborating with Jonas Salk on an AIDS vaccine. So I went to see Gibbs.

I came back and told my editor, I don't know if there's a story here. It's basically Salk being Salk—challenging the world once again with the same sorts of ideas and doing interesting things. My editor goes, whoa, whoa—what do you mean it's not a story?

So I did a search and looked at every article that had been written about AIDS vaccines. There wasn't a single one that had a point of view or critically analyzed the field. They were all about either the latest scientific pronouncement at a conference or in a journal, or they were about the hope meter—it's up, it's down, it's going to happen next year, it's never going to happen. Or they were catalogues: here's what everyone's doing.

I thought this was bizarre. Salk was approaching this wildly differently from everybody else, with his classic sort of empiricism—just push forward as quickly as you can, don't worry yourself to death about mechanism—which reflected what I had written about him and the polio vaccine.

I looked at this and thought, it's history repeating itself. I went back to the editor and said, I've got to do this. So I did a big point-of-view story, in 1989. That article led to a book contract.

But about four chapters into it, Bob Gallo said something which made me realize that this year didn't mean anything to AIDS researchers. The idea was a writer's conceit. After that, I couldn't go on.

So I asked the publisher if I could shift gears and write a book about the first vaccine to come to market. I thought, if I'm lucky, that will take me five years, if I'm unlucky, ten years. The idea was to watch the field over time. 'Process' stories like this are terrific, because you can show all the nuances of trying to accomplish something. There's always conflict. It's always difficult to do anything that matters.

Then I started to write for Science, so I was really able to follow the field closely.

Why did you decide to come out with the book now, even though there isn't an AIDS vaccine coming to market?

When Bill Clinton made his famous declaration in 1997 about the goal for an AIDS vaccine, it crystallized in my mind how the whole effort seemed badly off track. Clinton, who was making a big difference in AIDS—he even once proposed a Manhattan Project for AIDS—had really not provided the leadership he could have.

It harked all the way back to polio, and FDR [former US president Franklin D. Roosevelt]. There the president made a difference. He started the March of Dimes, put his law partner in charge and really directed and targeted research. Clinton just didn't get it.

I see a real problem with an illusion of leadership and progress, in AIDS research and in many other fields. Where an idea is put forward, the critics come out with all the reasons why the idea doesn't mean anything. The proponents defend it, but often the idea just disappears—even though it's never been fully tested, never really ruled in or ruled out.

You can argue that if the idea is right, the proponents will ultimately win the war and all is fine. Well, I don't see things that way. I think a lot of good ideas get stopped dead in their tracks, or end

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up running in place, with the same two or three people advocating for them. Critical experiments that need to happen, don’t happen—because funding doesn’t come forward or because people get sick of being advocates.

Anyway, after hearing Clinton’s speech, I decided that I have a book in me now. I’ve catalogued all these problems and I want to put it out there: here are all these promising leads that have gone nowhere; creative ideas that haven’t been put to use; research gaps and legal liability problems that aren’t being addressed, and market problems that aren’t being solved. I wanted to put it all in one place and then offer my own idea of a solution. And I really wanted the book to acknowledge that if this were polio, we would have a vaccine today.

HIV is a tough bug. That’s a given. The science is the biggest obstacle. There would be a vaccine if it were easy. Okay, fine. I’ve been attacked on this level many times. It’s tiresome to me—the scientists who say to me, Jon, you underestimate how difficult it is. No, I don’t. All I’m saying is, given this difficult problem, are researchers as organized as they could be? Is the field taking advantage of everything that it could? And I think the answer is no.

**Where do you see the fundamental failures?**

One is that industry hasn’t pushed forward here. You have two, three, four pharmaceuticals that are serious, but their interests wax and wane. Aventis Pasteur has been there all along and has tried hard to progress. But according to the NIAID-NIH plan at the BARC meeting in 1994, canarypox was supposed to be in efficacy trials in 1996. Well, that hasn’t happened. Merck is back into the game now. I think everybody is cheered about that. But that is only one big pharma. The biotechs come and go; they’re always cash-strapped, and they always are in danger of selling hope rather than a product.

Another failure is that the monkey model isn’t being effectively exploited. Again and again, leading researchers have called for organizing a large-scale, comparative trial of all the best vaccine ideas in monkeys, and setting it up in such a way that new vaccines which come along can be plugged into that protocol—or several protocols, even.

But if you go downstairs to one of the vaccine sessions at this conference, you’ll get dizzy, because somebody is using SIV89.6 as the challenge strain, and somebody is using E660, and somebody is challenging after one year, and someone else with two boosts after three months—it’s mind-boggling. There is no uniformity, no ability to make sense of how one vaccine stacks up against another.

What ultimately happens, in my mind, is that vaccine experiments almost become propaganda tools. If people like the data, they say, look how great my vaccine is—it worked in monkeys. And if they don’t like the data, they say, it’s not humans, it’s only a monkey model.

**What about the approach of doing human and monkey studies in parallel?**

That’s fine; things should proceed that way. But in addition to what’s going on, I’d like to see a monkey study that creates a public database around the testing of 50 or 100 of the best ideas using, for example, three different challenge strains, three different protocols. Then choose the five or six vaccines that look better than anything else and move them into humans, assuming their safety profiles are okay.

What I’m saying is, let’s move things forward based on the gamble that the monkey model means something. Granted, it’s a gamble. But let’s not play footsie with the model, let’s really move things quickly into clinical trials, with the only rationale being that they work better in monkeys than other vaccines do, and the only real concern being safety. Let’s stop using immunogenicity as the gatekeeper. To me, that delays things. We see that with Aventis Pasteur and canarypox. We saw it with Genentech and Chiron and gp120 vaccines.

Study immune response, absolutely. Great to know that stuff. But you don’t have to know that to make a working vaccine. As much as there are advocates for CTLs, for sexy new antibodies that will be uncovered when you take the dress off over here, nobody really knows the immune correlates of protection. So let’s stop pretending that we do.

It’s great that there are people who believe in CTLs, or in neutralizing antibodies. They should run with it; carry their vaccine forward.

But there’s a clock ticking. We’ve known for years that a 60% efficacious vaccine introduced today will prevent more infections and disease ten years down the road than a 50% effective vaccine introduced five years later. So there’s a great reward in coming up today with a mediocre vaccine that is crudely understood.

**You also said that many scientific ideas are prematurely dropped.**

That’s another one of the big failures. There is no real gap-filling committee; no group of people who meet regularly and ask, what are all the promising leads? Who’s doing what, and how can we speed these things along? This type of committee is something I propose in my book.

Instead, the field is ruled by grants and investigator-initiated science, which is the culture of American science. It’s a great culture; it pushes forward knowledge and creativity. But it doesn’t embrace risky ideas. And it doesn’t follow up on itself very well. It’s sort of a free-for-all, without
real leadership.

Targeted programs have something to offer. You could have smart people sit around a table—including someone with a checkbook—saying, the group over there has something going, so let’s ask them to e-mail us how much money they need to speed things along, and why. The next day, we can decide whether we’re going to send them the money. That’s it. No formal grant proposal. Or maybe they come and present in front of the board and explain why. Once they get some money, the committee could find two or three other smart groups that want to do a similar thing and light a fire under the first group. Create some competition in the way that many companies do, by starting two or three projects in-house to spur things forward.

What type of organization should this be?
The way I envision it, it can’t be government money. Government doesn’t have the freedom to move money around like that. Partly to be provocative, I call it a March of Dollars in the book. I think we have a lot to learn from the March of Dimes model.

It’s an organization that wouldn’t be wildly different from IAVI, but would have some different approaches. IAVI is approaching the problem with a certain approach—a blueprint—that makes sense. But it’s not the only one.

The new organization would raise money from philanthropists. Bill Gates is not the only person out there who has a lot of money and cares about public health. It would also draw on the public, which IAVI hasn’t tapped into. The public all around the world would like to contribute, and there is a way to involve them—just as the March of Dimes did.

I’m not sure it needs a scientist or doctor at the top. The March of Dimes model says to have a lawyer, someone with clout and influence around the world—as Basil O’Connor had. In my book, again to be provocative, I suggest Randall Robinson [the lawyer who heads TransAfrica, an organization that helped pressure the US government to adopt sanctions against South Africa in the apartheid era].

What are some specific examples of research gaps or missed opportunities?
There is an incredible fascination with new technology, to the detriment of old technology. We have not had the definitive experiment which says that whole-killed approaches are worthless. I’d like to see them in a comparative monkey study. We have never seen the whole-killed experiment cleanly done, with monkey-grown SIV to make the vaccine and a monkey-grown SIV challenge.

We do not have definitive information saying that the Th1/Th2 concept (describing the inter-relationships among T-cell subclasses) is worthless. I don’t know that low-dose vaccination is a worthless idea; I see lots of hints that it might be a useful idea. I’d like to see that experiment done cleanly.

The same with attenuated vaccines. Why is Ron Desrosiers out there alone? It’s a great idea to exploit. Desrosiers has also come up with some new ideas. I think deleting V1 and V2 [regions of the envelope protein] deserves attention.

Another neglected area is cellular proteins. We know from Jim Scott’s early work that cellular proteins by themselves are protective. Gene Shearer argues that we can do alloanmunization, at least in countries where they don’t have organ transplantation. Why haven’t we seen that move forward in a comparative fashion?

Those are several ideas that I think deserve more attention. I could keep going.

What are some of the positive signs you see now?
I’m excited by the notion that the field is now much more diverse. When I started following it, nearly all the approaches were based on envelope alone. The few other approaches were either dying on the vine or were mocked. I like the fact that there is so much creativity happening right now, and that different targets are getting serious attention.

The world has also changed since the book was finalized. I think the field still has major problems—and the problems seem to keep changing. But I see some of the things discussed in my book getting better.

There’s a lot of optimism in the field.
Yes. Having Merck and Glaxo in the game is exciting. Overall, things have gone up a notch. The field has matured.

But it’s important to recognize that we’ve moved the goalposts. We went from aiming for sterilizing immunity to delay of disease. And when we look at old, “failed” data using today’s criteria, we see that some of those older vaccines didn’t actually fail—there’s data from ten years ago showing some real protection.

Another thing to realize is that a lot of the excitement today is about mechanism—it’s about the ability to explain why monkeys are controlling viral load.

How do you now see the prospects for a vaccine?
There’s more serious attention being paid to the problem than ever before. I think a vaccine is eminently doable, and I think there will be a bevy of efficacy trials starting in the next five years. I would hope that, within the next ten years, we at least have conclusive data that something doesn’t work, and maybe have a vaccine ready to go out to the world. I think that’s possible.

There is not going to be the answer. There was a mistake on the cover of my book early on—fortunately, it was corrected. The subtitle said, “The Wayward Search for the AIDS Vaccine.” I got very concerned—that was never my subtitle, and it’s not my point. There isn’t going to be the AIDS vaccine; it’s an AIDS vaccine. I think there will be many ways to beat HIV with vaccines.
IAVI TRANSITIONS

On 1 August 2001, Dr. Patricia Fast will join IAVI as Director of Medical Affairs, with responsibility for IAVI's HIV vaccine clinical work. Fast brings a wealth of experience in immunology, AIDS vaccine development and clinical trials. In the late 80's and mid-90's she held a series of positions at the Division of AIDS (National Institute of Allergy and Infectious Diseases, NIH) up to Associate Director for Vaccines and Prevention, where she oversaw development of HIV vaccines in animal models and worked on preparing and running HIV vaccine trials, coordinating NIAID's AIDS vaccine efforts with other national and international programs, and communication with scientific and lay audiences. Prior to NIH she worked at Upjohn, UCLA, Wellcome Research Laboratories, and the National Cancer Institute.

Fast, a board-certified pediatrician and Ph.D. in immunology, is currently Associate Director for Clinical Research at Aviron, Inc., a biopharmaceutical company working on vaccines against flu and cytomegalovirus. She has also served on several national and international vaccine advisory committees, and was recently appointed to the US National Vaccine Advisory Committee.

Across the Atlantic, Frans Van den Boom will become IAVI's first European Programme Director as of 15 April 2001. In this role, he will oversee IAVI's efforts to build additional support for AIDS vaccine work in Europe and serve as liaison between IAVI's New York headquarters and European governments and partner organizations. He will be based in Amsterdam (at the Dutch AIDS Fund).

Van den Boom, a social scientist, is currently Deputy Director of the Netherlands Red Cross, where he develops and manages the organization's programs, international policies and 300-person staff. He previously held positions at the Blood Transfusion Council, the National Committee on Chronic Diseases, and the Netherlands Institute of Mental Health.

IAVI's Founding Vice President for Public Affairs/Communications, Victor Zonana, has been named Vice President for Communications and Public Affairs at the Global Fund for Children's Vaccines. For the past three years, Zonana oversaw IAVI's efforts to build global awareness about AIDS vaccines and its own vaccine development and policy programs. He also led IAVI's US Public Policy and Advocacy Team and was an editorial advisor to the IAVI Report. Zonana previously served as Deputy Assistant Secretary for Public Affairs at the US Department of Health and Human Services and as a reporter for The Wall Street Journal and The Los Angeles Times.

The Global Fund for Children's Vaccines, initially capitalized with a US$750 million grant from the Bill & Melinda Gates Foundation, works with its Global Alliance for Vaccines and Immunization partners to immunize children against vaccine-preventable diseases in the world's 74 poorest countries. About 3 million people die each year for lack of existing vaccines. The Global Fund is led by Jacques-François Martin, who is also a member of IAVI's Board of Directors. A search is underway to fill Zonana's position at IAVI.

The AIDS Pandemic in India

India's foray into AIDS vaccine development comes amid growing evidence of HIV's increasing foothold in the country and its frightening implications for this nation of one billion people—about one-sixth of the world's population.

Official estimates are that 3.7 million people in India are infected with HIV (although the actual number is thought to be higher), second worldwide only to South Africa (UNAIDS, December 2000). This still corresponds to a low overall prevalence of <1%, which is far below the double-digit prevalence rates seen in much of Africa.

But high risk groups show a vastly different picture, and HIV is clearly making inroads into the general population. For example, there are an estimated four million commercial sex workers in India, and HIV prevalence among those in Mumbai is about 70%. Among MSM (men who have sex with men) seeking health care at a Mumbai-based sentinel surveillance site, about 25% are HIV-positive, and similar prevalence rates are being described for tuberculosis patients. Intravenous drug users, truckers and migrant laborers constitute other highly affected groups, and who in turn often infect their wives. Portending a significant worsening of the situation, several metropolitan areas now report prevalence rates above 2% in women attending antenatal clinics, as well as a corresponding rise in the number of infected infants and children.

Prevention efforts have lagged behind, and they face an array of enormous obstacles: widespread poverty, a 70% illiteracy rate, the low status of women, deep-seeded taboos about discussing sexuality, and the tremendous stigma of homosexuality.

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have begun work on the MVA project and are characterizing HIV isolates from several recent seroconverters in the region, where NARI will conduct clinical trials of the candidate vaccine. Chakrabarti is now cloning and sequencing genes from one of the isolates and will bring the subcloned sequences to Therion for vaccine construction. The MVA vaccine is expected to enter Phase I testing in about two years.

At a later stage, Therion will transfer the technology to an Indian vaccine manufacturer, which will produce lots for eventual Phase II/III clinical trials. India has a large vaccine production capacity that manufactures nearly a dozen different childhood vaccines for use throughout the developing world.
ing to Emini. All vaccines contained an SIV gag gene (optimized for human codon usage) but no env, and were administered intramuscularly either three or four times (for viral vectors or DNA, respectively) over 32 weeks to three animals each. Three months after the last immunization, all vaccinated animals and six controls were challenged i.v. with a high dose (50 m.i.d.) of SIVmac251.

Prior to challenge, animals given Ad-5 showed the highest immune responses, followed closely by DNA/CRL1005 adjuvant (as measured by intracellular cytokine staining and ELISPOT for interferon-gamma production, and by tetramer binding). Responses to DNA in saline or alum were much lower, showing a clear advantage of adjuvanting the DNA with CRL1005. CD4 T-cell responses were also induced in most vaccines and tended to predominate in the DNA group, while Ad5 vaccines had significantly higher CD8 cell responses.

At 180 days post-challenge, all vaccinated animals were alive and apparently healthy (although infected), compared with 4/6 controls that had died and a fifth that was symptomatic. However, there were clear differences among the groups. The Ad5-vaccinated monkeys showed the best clinical course: CD4 counts remained high, peak viremia was blunted by 1.5 logs and viral load gradually brought under control. Animals given DNA/CRL1005 adjuvant were next best, followed by MVA, both initially showing CD4 cell decline but then recovering and successfully controlling viral load. DNA in alum/MPL or saline led to the biggest drop in CD4 cells and the least control of viremia.

Speaking two days later, Emilio Emini presented immunogenicity data from DNA or Ad5 vaccines with HIV gag (rather than SIV). Animals given DNA/CRL1005 adjuvant and boosted with Ad5 showed a 5-10-fold increase in the number of HIV-specific T-cells (by ELISPOT) after the boost. That generally reflected both CD4 and CD8 T-cell responses, again with CD8 T-cells predominating after the Ad5 boost (even in animals with predominantly CD4 responses following the prime). Unadjuvanted DNA was a less effective prime, with boosted monkeys showing little increase by ELISPOT.

Emini also addressed the issue of pre-existing immunity to adenovirus, a key to the feasibility of using this vector as a vaccine since about 10% of the U.S. population has significant neutralizing antibodies to adenovirus and another 40% shows lower levels, according to Emini. In these studies, 6 monkeys were pre-exposed to adenovirus and immunized later with a high dose (10⁸ particles) of Ad5-gag. While the presence of neutralizing antibodies reduced the strength of the T-cell responses several fold, it did not eliminate them, leading Emini to express optimism that pre-existing immunity could be overcome, especially with a DNA prime/Ad5 boost strategy. As support, he cited the effectiveness of the Ad5-gag boost described above (which used 10⁷ particles), suggesting that a 10⁷ particle dose, even if neutralized by 99%, should still induce good responses.

Lastly, Emini reported that PBMCs from about two dozen people infected with HIV subtype B gave “comparable” responses (in ELISPOT assays) against peptides from Gag and Nef consensus sequences of subtypes B and C, lending some support to the feasibility of broadly reactive vaccines (at least those targeting cellular immunity). He also said that nef and pol will be added to Merck’s vaccines alongside gag, based on the company’s data showing that a high proportion of HIV-infected people with good immune function and low but detectable viral load respond to these proteins.

The company has several Phase 1 studies underway. A DNA-gag trial in uninfected people began last year, and one in infected people a few weeks ago. Ad5-gag studies recently started in uninfected volunteers and will soon begin in infected people. Depending on these results, some recipients of the DNA vaccine may be boosted with adenovirus.

Protection with Adeno-Associated Virus Vectors
Phil Johnson (Children’s Research Institute, Columbus, Ohio) showed macaque data from ongoing studies of an SIV vaccine based on vectors of adeno-associated virus (AAV), a single-stranded DNA virus common in the population. The vaccine is a mixture of 3 types of AAV particles that collectively carry SIV env, rev, gag, int and protease. In 8 immunized animals challenged i.v. with a high dose (5-50 m.i.d.) of SIV-B660, a strain that is difficult to protect against, viral peak was reduced by about 1.3 logs, setpoint by 1.7 logs, and viral load by 3 logs. Vaccinated animals were still healthy at 6 months, while 3/8 controls died. All vaccines showed both CTL responses and neutralizing antibodies, which persisted for at least 14 months. Viral load after challenge correlated with both neutralizing antibody titer on the day of challenge and peak levels of SIV-specific CD8 cells. In a prime-boost study of the AAV vaccine with SIV-DNA, peak viremia was blunted even more (by 2.5 logs).

Johnson also reported that the vector, which targets quiescent cells, shows extreme longevity (>17 months) in the muscle cells of immunized monkeys. Unlike the wild-type parental virus, it appears to persist in host cells as an episome rather than integrated into chromosomal DNA. Together with Targeted Genetics (Seattle), Johnson’s group is making a candidate C vaccine from South African isolates, under an IAV-sponsored collaboration.

Sabin Polio Vaccine as an HIV Vector
Shane Grotta (University of California at San Francisco) presented a macaque study of SIV vaccines made with vectors of the live Sabin vaccine, known to induce potent, lasting immunity when taken orally. Since the vector can fit only very small pieces of foreign DNA (500-700 bases), the researchers used two mixtures of 20 different recombinant viruses representing the entire SIV genome in overlapping fragments.

Seven animals were immunized intranasally four times and challenged vaginally nine weeks later with the highly virulent SIVmac251. While 12 unvaccinated controls became infected and 3 of 6 closely monitored controls died by week 40 (and the other three showed disease symptoms), two vaccinees had no detectable viremia; two were able to control viremia, and the

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remaining three became highly viremic, like controls. All 7 vaccinated monkeys maintained body weight, unlike the controls. The HIV-polio viruses are also being tested as boosts in a prime-boost regimen.

Crotty says that these viruses are 1,000-10,000 more infectious in humans than in macaques, and therefore monkeys may not be a good model for these vectors. His group, headed by Raul Andino, is working on human trials of the approach.

**Immune Responses in Oxford Phase I Trial**

In a talk on cellular immune responses to HIV, Andrew McMichael of Oxford University presented some preliminary results from the UK's ongoing HIV-DNA vaccine trial. (A second Phase I trial recently began in Nairobi.) The vaccine, designed by Oxford's Tomas Hanke and manufactured by Cobra Ltd., carries most of the gag gene (containing many known CD8 and some CD4 epitopes) from subtype A and a string of CTL epitopes from pol, nef and env. The 18 vaccinated volunteers received either 100ug or 500ug DNA (low doses chosen for initial human safety studies).

Four weeks after the second immunization, 9/18 volunteers showed positive responses in ELISPOT assays of CD8 cells expressing interferon-gamma (performed by Matilu Mwau). The data have not yet been unblinded in terms of the two dosage groups. Plans are to use this DNA vaccine with an MVA boost, which just entered separate Phase I trials in Oxford (see Vaccine Briefs, p.16).

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immunized with another viral vector-based vaccine, this one made with attenuated Venezuelan equine encephalitis virus (VEE; see IAVI Report, Nov-Dec 1999, p.8). The study included six animals vaccinated with a mixture of vector particles (encoding SIVgp160, gp140 or matrix/capsid proteins) at 0, 1, and 4 months, as well as six mock-vaccinated animals. One month after the last immunization, all animals were challenged i.r. with the pathogenic virus SIVE660.

Data at 23 weeks post-challenge show evidence of vaccine-mediated protection. Four of the six immunized macaques vigorously controlled SIV viremia (two below the 400 copy cut-off for the assay and two <2,000 copies), compared to just one mock-immunized animal in this low range. (The remaining five controls and two vaccinees had loads well over 100,000 copies at this timepoint). On average, CD4 counts were increased to 130% of baseline in vaccine recipients, while controls showed a decline to 40% of baseline. All animals were euthanized at 41 weeks and complete data analysis is pending.

The VEE-based vaccine is being developed for human clinical trials by an IAVI-sponsored partnership with South African scientists and AlphaVax (Durham), with trials planned through the HTVN's vaccine trial site in Durban. The trial will test vaccines containing gag, env and pol genes from a South African clade C HIV isolate.

**Immune Control in PEP-Treated Monkeys**

Jeff Lifson (National Cancer Institute, Frederick, MD) gave an update on his studies of post-exposure anti-retroviral therapy in macaques. Previous work has shown that the majority of monkeys treated with a short course of the drug PMPA within 24 hours of exposure to pathogenic SIVE660 do not seroconvert, and they exhibit sustained control of viremia after PMPA is withdrawn (see J. Virol 74: 2584, 2000). These animals also show enhanced SIV-specific proliferative responses compared to untreated or suboptimally treated controls. Of three macaques rechallenged with SIVE660 six weeks after PMPA termination, two seroconverted but then controlled virus to undetectable levels, while a third showed a transient increase in SIV-specific T-cell proliferation but no viremia or antibody response.

Lifson's group has now extended these studies by challenging the macaques with a heterologous pathogenic strain (SIVmac239). He reported that the animals all became infected with the challenge virus but strongly controlled viral replication. Depletion of CD8 cells by monoclonal antibody (mAb) led to a rapid increase in viral loads (up to 5 logs), with some animals showing replication of both the original E660 and the challenge mac239 viruses. As the effects of the mAb wore off (and CD8 cells returned), control of viremia was regained. This restriction of viral replication occurred without measurable neutralizing antibody and at cellular response levels (measured by lymphoproliferation and ELISPOTs for CD8 T-cell derived interferon-gamma production) that Lifson describes as "unremarkable." These encouraging findings suggest that, with a sufficient head start (potentially through vaccination), the immune system may be able to mediate prolonged containment of SIV and, by extension, HIV.

**Antibody Booster**

Ron Desrosiers (Harvard Medical School, Cambridge) reviewed his team's work on attenuated SIVs created by mutating the V1/V2 region of the envelope gene (see IAVI Report, Sept.-Nov. 2000). The attenuated variants cause only transient viremia and no apparent disease in infected macaques, and they are highly sensitive to neutralization by antibodies. Upon challenge with a pathogenic strain, these animals appeared completely protected from secondary infection.

Desrosiers believes that neutralizing antibodies are largely responsible for the attenuated course of infection with these mutants. In support of this view, he said that depletion of B-cells (which produce antibodies) in six animals led to increased replication of the attenuated virus. Desrosiers is now collaborating with Larry Arthur and Jeff Lifson at the National Cancer Institute (Frederick, MD) on whole-killed derivatives of these viruses for testing as potential vaccines.

**Towards a "World Clade" Vaccine**

While vaccine designers usually focus on a specific HIV subtype and/or population, a few researchers are taking the opposite approach: designing vaccines to work as broadly as possible across the many different HIV subtypes and strains. Anne De Groot (Brown University and EpiVax, Providence) began by developing two bioinformatics tools: Conservatrix, which identifies potential CTL epitopes that are highly conserved across clades; and EpiMatrix (available at http://thtv.biomed.brown.edu), a predictor of which epitopes are most likely to be immunogenic and to bind the common HLA molecules (the highly diverse molecules that help display epitopes to T-cells). De Groot then tests peptides selected in this way against CTLs from healthy, HIV-infected individuals. Of more than 100 peptides tested so far, 71%
have turned out to bind the predicted MHC molecule. Her team is now analyzing DNA plasmid carrying the chosen epitopes by transfecting them into dendritic cells and testing whether they stimulate CTLs from HLA-matched, HIV-infected people.

**Identifying Epitopes**

Kariya Yossum from Bette Korber's group (Los Alamos National Laboratory, CA) described a finding that might assist in CTL epitope prediction. After observing that CTL epitopes tend to cluster in certain regions of HIV proteins, she began looking at where HIV proteins—which must be choped into small peptides for presentation to CTLs—are most likely to be cleaved. Drawing on hundreds of CTL epitopes from the Los Alamos HIV Genetic Sequence and Immunology Databases (hiv-web.lanl.gov), Yossum and coworkers found a clear correlation between the boundaries of published CTL epitopes and predicted protein cleavage sites in Nef and p17. Conversely, in variable regions of the virus with few known epitopes, Yossum found that protein sequences are cleaved differently, or not at all. This suggests that strategies for identifying useful CTL epitopes should consider where viral proteins are likely to be cleaved, and conversely, that HIV regions resistant to cleavage and/or MHC binding might be omitted from vaccine constructs without reducing immunogenicity.

**Exposed Seronegatives**

Three posters featured data on seronegative individuals with documented exposures to HIV (ESNs). Ann Dunn (CDC, Atlanta) and colleagues looked for evidence of “resistance factors” in a cohort of ESN women married to HIV-infected men from Chang Mai (Thailand), by comparing them to wives who became infected. Factors that did not differ between the ESN and infected women included HIV class 1 alleles, CCR5 delta 32 genotype and the presence of SLPI (a HIV-inhibiting protein) in the cervico-vaginal lavage.

Looking at samples from the husband, investigators found no differences between the groups in seminal viral load, defects in HIV accessory genes or co-receptor use by the viral isolates. More than half the ESNs and all the infected women showed HIV-specific T-cell responses (measured by ELISPOT). The proportion of women with T-cells specific for Gag, Nef and Pol was similar in both groups, but only 10% of ESNs had detectable responses to Env, compared to 83% of the infected women.

Two collaborations involving Sarah Rowland-Jones' group (Institute of Molecular Medicine, Oxford) studied a cohort of eight serodiscordant couples (with one HIV-infected and one uninfected partner). One poster was presented by Ruth Branganza (Imperial College School of Medicine at St. Mary's, London), the second by Susana Pinheiro from the Oxford team. All eight ESN partners had CD8 T-cell responses to HIV. The researchers sometimes saw responses to whole HIV proteins but not to known epitopes from that same protein, implying the existence of still-unidentified CTL epitopes. In one person, an expansion of CD8 T-cell responses was detected after a documented exposure (due to condom breakage) that did not lead to overt HIV infection. In five ESNs analyzed for CD4 T-cell responses, three showed HIV-specific activity directed against gag p24 and p16 in ELISPOT assays.

Two years ago at the Retrovirus conference, Zhu and coworkers (Fred Hutchinson Cancer Research Center, Seattle) presented evidence of extremely low-level, cryptic HIV infections (0.1-0.01 copies of DNA per million T-cells) in a cohort of ESN individuals. To search further for evidence of such low-level infection, Zhu has now studied macaques selected from previous vaccine challenge studies. None of the monkeys displayed detectable SIV on standard tests, but they all showed evidence of low levels of SIV DNA (average of 4.5 copies per million peripheral blood mononuclear cells). Zhu's work raises the question of whether other ESNs harbor latent HIV infections that are undetectable using standard methods.

**CTL Responses to HIV Regulatory Proteins**

Bruce Walker's team at Harvard University (Cambridge) presented two posters on the epitope specificities of HIV-specific CD8 T-cells. The first study, led by Marilyn Addo, examined whether infected individuals show CTL responses to the HIV regulatory proteins Tat and Rev. This study was subsequently published in PNAS 98: 1781, 2001. Although these two proteins are small, Addo detected multiple CTL epitopes in each by performing ELISPOT assays with overlapping peptides spanning their entirety. Of 57 people studied, 19% showed CTL responses to Tat and 35% to Rev. The second study, led by Marcus Altfeld, used similar techniques to detect responses to two other regulatory proteins, Vif and Vpr. In 50 infected individuals, 33% demonstrated Vif-specific and 46% Vpr-specific CTL activity. Both posters stressed that these regulatory proteins are expressed early in the viral life cycle and may therefore be useful vaccine antigens.

**Visualizing Immune Activation**

David Schwartz (Johns Hopkins, Baltimore) presented a novel poster on using positron emission tomography (PET) scans to visualize lymphocyte activation, both in HIV-infected people and in vaccines. Study participants were infused with a radiolabeled glucose analog (118FDG) and then PET-scanned. This technique has been used to identify solid lymphomas and lymphocyte activation in SIV-infected macaques (see Schurko et al, PNAS 92: 1425-1430). In people infected with HIV, the degree of lymph node activation detected by PET was correlated with viral load. This correlation was seen both in recently infected individuals and in those infected for more than 10 years. Schwartz also applied the technique to seven uninfected recipients of a canarypox vaccine (Alvacc VCP205 or vCP1452). Interestingly, the only two individuals to show evidence of activation (5 days after the fourth immunization) were also the only ones with detectable CTL responses to the vaccine.
Estimating Need and Demand for an HIV Vaccine

A new effort is underway to assess the potential worldwide demand for HIV vaccines. The project is a collaboration between IAVI’s Access Project, the World Health Organization (WHO) Health Technology & Pharmaceuticals Cluster and the WHO/UNAIDS HIV Vaccine Initiative. Input is being invited from the European Union, the World Bank, the vaccine industry and countries representing a spectrum of possible vaccine needs. The project will look at scenarios involving three hypothetical vaccines with different rates of efficacy and will conduct focus group meetings in four countries covering a broad range of HIV incidences. The study will also make country-specific policy recommendations appropriate to each vaccine scenario, taking into account the many factors likely to influence vaccine acceptance and distribution. The ultimate goal is to compile results from the four representative countries into global estimates of the need and demand for each hypothetical HIV vaccine. Results from the project should be available by mid-September, 2001.

MVA Vaccine Trial Begins in Oxford

On 14 March 2001, researchers from the UK’s Medical Research Council (MRC) Human Immunology Unit at Oxford University (UK) announced the first inoculations of five volunteers in their small (6-person) Phase I trial of an HIV vaccine based on the MVA (modified vaccinia virus Ankara) vector. Despite showing promise in animal models for several years, HIV-MVA vaccines have not yet been studied in humans. The MVA trial is part of an ongoing IAVI-sponsored vaccine development partnership involving the MRC and the University of Nairobi in Kenya. The aim is to develop a prime-boost vaccine regimen (based on an East African HIV subtype A strain) using naked DNA as a prime and MVA as a boost. Human trials of the DNA component began at Oxford in August 2000 and in Nairobi in March 2001. A Nairobi MVA trial is expected to begin later this year, followed by combined DNA/MVA studies in both the UK and Kenya.

Phase II Vaccine Trial Starts in Haiti

On 27 March, Haiti announced the first immunizations of volunteers in an international Phase II vaccine trial. The study, funded by the US National Institutes of Health (NIH), is testing a combination of Aventis-Pasteur’s ALVAC canarypox vaccine and Vaxgen’s recombinant gp120 product AIDSVAX®. One trial site in Brazil and another in Trinidad/Tobago are also participating, with 40 low-risk individuals to be enrolled at each site. The trial should yield data on the effects of differing genetic backgrounds, nutritional states and infectious disease burdens on immune responses to these vaccines.

The Haitian study is being conducted by the Haiti Study Group on Opportunistic Infections & Kaposi’s Sarcoma (GESKIO), a non-governmental organization with a long history of conducting AIDS research. A full description of GESKIO’s work and Haiti’s AIDS vaccine effort will appear in the next issue of the IAVI Report.

Salim Karim Heads to Natal

Salim S. Abdool Karim, a well-known South African HIV vaccine scientist and vocal vaccine advocate, will be leaving his current posts as of 1 July 2001 to take on the role of Vice-Principal and Deputy Vice-Chancellor for Research and Development at the University of Natal. Abdool Karim is Director of HIV Prevention and Vaccine Research at the Medical Research Council (MRC) in Durban, South Africa, and principal investigator at the Hlabisa site of the HIV Vaccine Trials Network (HVTN). He is also Professor of Clinical Public Health at Columbia University in New York. AIDS scientists around the world know him as chair of the scientific program committee at last summer’s Durban International AIDS Conference (July 2000). As yet there is no decision on who will replace him at the MRC.