



IAVI Report

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AIDS Vaccine 04

International conference in Lausanne provided an update on recent scientific progress

by **Richard Jefferys**

Overlooking the glittering waters of Lake Geneva and surrounded by the stunning snow-capped peaks of the Alps, the third international conference on AIDS vaccines took place from 30 August to 2 September, 2004 in Lausanne, Switzerland. The mountainous terrain offered an appropriate metaphor for the long climb that lies ahead for researchers attempting to develop an effective AIDS vaccine. In keeping with this theme, many discussions at the conference revolved around the idea that—like mountaineers scaling a precarious rock face—scientists may need to link more closely if they are to reach their lofty goal.

In terms of AIDS vaccine science, the meeting featured several reports highlighting incremental but significant progress in developing strategies for inducing broadly neutralizing antibodies against HIV, suggesting that this perennial obstacle in vaccine development might eventually be overcome. Less encouragingly, the utility of modified vaccinia Ankara (MVA) vectors and current DNA vaccine constructs came into question with the unveiling of disappointing immunogenicity data from trials involving a DNA prime/MVA boost approach developed by scientists from Oxford University in collaboration with IAVI and the Kenyan AIDS Vaccine Initiative (KAVI). Given that many AIDS vaccine candidates are currently based on these platforms, this unwelcome news added to the general sense that the vaccine pipeline might not be as robust as it had once appeared and efforts to identify additional novel vector strategies need to be stepped up. A related issue is the problem of circumventing pre-existing immunity to certain vectors, such as adenovirus, which also received considerable attention in Lausanne. Among scientists focused on human immunology, the potential limitations of the most commonly used assay for evaluating vaccine-induced T cell responses—the interferon (IFN)- γ ELISPOT—were widely discussed, and a broad consensus reached that assays evaluating additional T cell functions should be standardized and validated for use in future vaccine trials.

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Perspective

HIV prevention: Rethinking the risk of transmission

by **Myron S. Cohen, MD**

Great hope has been pinned on biological solutions to HIV prevention including vaccines, topical microbicide agents, male circumcision, and novel use of antiretroviral therapy. The key to the successful development of these interventions is a complete understanding of the transmission of HIV. The purpose of this communication is to examine new ideas about the sexual transmission of HIV.

What defines HIV transmission?

The routes for transmission of HIV have been well established (Table 1)¹. Heterosexual vaginal intercourse is of greatest overall importance to the epidemic. However, anal intercourse may also play an important and underestimated role, even in heterosexual couples.

Epidemiological studies designed to determine the transmission of HIV per risk

event are both enlightening and confusing. Mother-to-child and needle-stick transmission have been defined with great accuracy (Table 1), because in these situations the status of the index case is known and the precise event(s) responsible for HIV acquisition risk can be defined.

Studies of the sexual transmission of HIV have included the use of three methods: cross-sectional collection of data with

modeling, prospective longitudinal cohorts of high-risk subjects, and cohorts of discordant couples. The results collected are confounded by a substantial number of problems that lead to considerable inaccuracy and great heterogeneity of transmission probability (Table 2). The aggregate of these limitations has led to widely varying estimates of risk and the somewhat misguided dogma that HIV transmission is inefficient.

However, HIV transmission risk is greatly amplified during brief, discrete periods of time. Studies of amplified transmission may provide a more realistic view of HIV risk and the biological forces that a vaccine or other interventions must confront.

Can HIV transmission be amplified?

The probability of HIV transmission depends on the infectiousness of the index case and the susceptibility of the exposed subject². Considerable progress has been made regarding infectiousness, with far less progress in understanding susceptibility.

Infectiousness is determined by the concentration of HIV during exposure and viral phenotypic factors. The inoculum required for HIV transmission has been estimated from a series of studies. The largest and most compelling work was conducted in the Rakai district of Uganda, where HIV transmission was directly related to the blood HIV concentration³. Transmission was not observed when the blood viral burden was less than 3,500 copies/ml, whereas about 50% of transmission events were observed in subjects with the greatest blood viral burden.

Neither this nor other transmission studies examined the genital tract viral burden directly, which can differ from that in the blood. Nor is it clear whether HIV is transmitted by cell-free or cell-associated virus. While the cell-free virus in genital secretions has been carefully measured⁴, the number of genital tract cells infected has been far more difficult to quantitate. In addition, HIV RNA copies in genital secretions include a large but unknown number of defective viral particles which may not actually be infectious.

Another way to measure the potential effect(s) of the inoculum on HIV transmission is to study examples of amplified and reduced HIV transmission. Reduced HIV transmission has been associated with usage of antiretroviral (ARV) drugs in some (but not all) studies (reviewed in ref. 5). For example, in an early study of discordant couples significant reduction of HIV transmission among men who were taking AZT was reported⁶. More recently, several population-based studies have suggested that widespread usage of ARV therapy in a community or country can lead to reduced inci-

TABLE 1: ROUTES OF EXPOSURE AND HIV

INFECTION ROUTE	RISK OF INFECTION
SEXUAL TRANSMISSION	
Female-to-male transmission	1:700 to 1:3,000
Male-to-female transmission	1:200 to 1:2,000
Male-to-male transmission	1:10 to 1:1,600
Fellatio	0 to 6%
PARENTERAL TRANSMISSION	
Transfusion of infected blood	95:100
Needle sharing	1:150
Needle stick	1:200
Needle stick /AZT PEP	1:10,000
TRANSMISSION FROM MOTHER TO INFANT	
Without AZT treatment	1:4
With AZT treatment	Less than 1:10

Adapted from Royce, Sena, Cates and Cohen, *NEJM* 336, 1072 (1997)

dence of HIV (reviewed in ref. 5).

Conditions associated with increased excretion of HIV are of considerable importance for biological prevention strategies. Viral burden in blood is greatest during the first weeks of infection⁷, consistent with modeling and epidemiology studies that suggest extremely efficient transmission of HIV from subjects with acute infection⁷⁻⁹. New analysis of the Rakai data demonstrates that nearly half of the HIV transmission events observed could be ascribed to the earliest time(s) of infection⁸. Accordingly, describing HIV transmission risk in terms of intercourse events over extended periods of time is an oversimplification, and HIV transmission should probably be considered in phases, the most important of which may well be

TABLE 2: ROUTES OF EXPOSURE AND HIV: LIMITATIONS OF ESTIMATIONS OF SEXUAL TRANSMISSION

- Complex sexual behaviors with potential concomitant exposure of several different mucosal sites; anal intercourse in heterosexual couples may be fairly common.
- Sexual history provided by study subjects limited by their memory. All studies depend on the reports of study subjects about the quantity and quality of sex, signs and symptoms of sexually transmitted diseases, use of medications, etc. Sexual diaries have proven cumbersome and sometimes inaccurate.
- Lack of knowledge of the HIV status of the sexual partners (except in the case of discordant couples).
- Unrecognized or undetected factors that can amplify transmission, especially STDs. Many STDs are asymptomatic yet might still increase the risk of HIV acquisition (2).
- Long periods of follow-up between visits of people at risk, confounding accurate interpretation of risk. HIV-uninfected subjects probably suffer only very brief periods of high risk, but if they are studied infrequently a large number of low risk sexual encounters are included for consideration, reducing the calculated probability of HIV transmission.

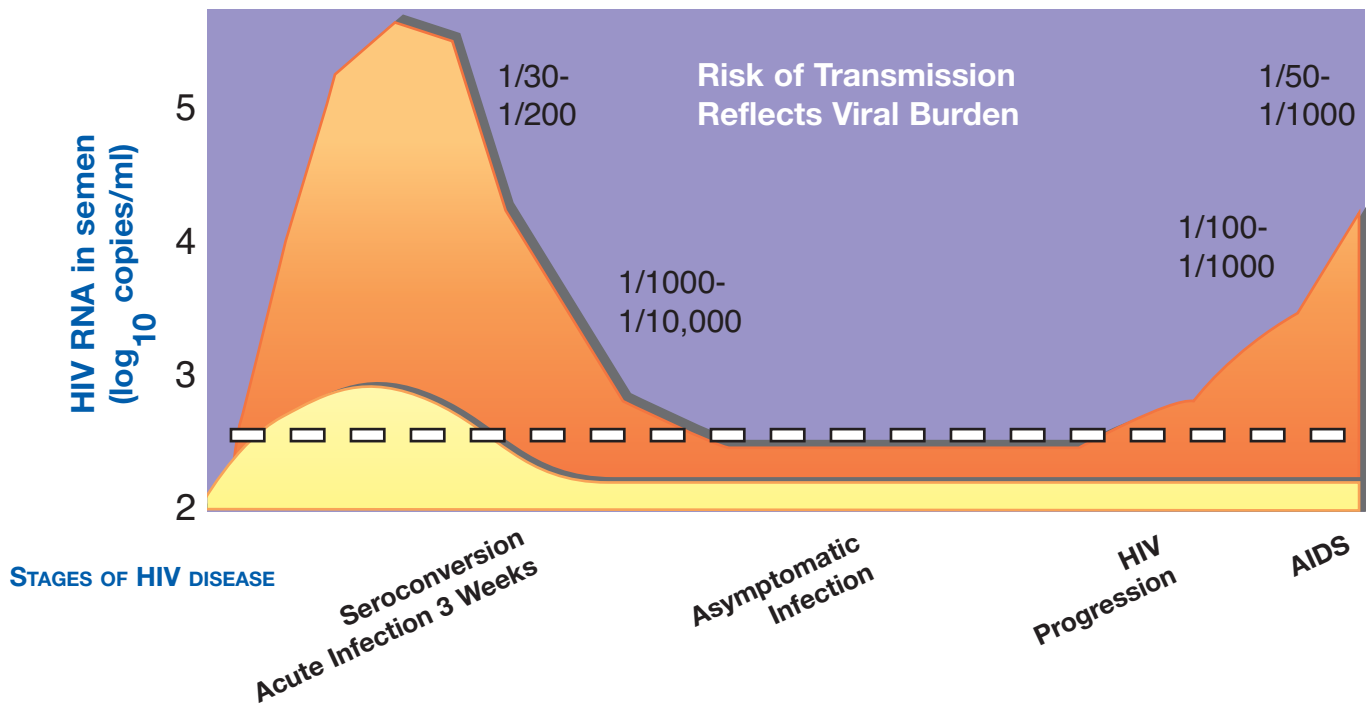


Figure 1: Sexual transmission of HIV. The relative risk of transmission over the course of the disease, as a function of viral load in semen. Orange indicates HIV RNA copies per ml semen, yellow indicates the reduced viral burden expected from host defenses evoked by a vaccine or early use of antiviral therapy. The dashed line offers a theoretical viral burden threshold below which HIV transmission will not occur. Numbers provided at the different stages of disease represent the probability of HIV transmission/episode of heterosexual intercourse. Adapted from ref. 9.

acute infection (Figure 1, adapted from reference 9).

What is the role of other STDs?

Classical STDs likely play a central role in the HIV epidemic^{2, 4, 10-12}. Mucosal STDs (gonorrhea, Chlamydia, trichomonas) and genital ulcer pathogens (herpes simplex virus [HSV], syphilis, chancroid) affect both infectiousness and susceptibility. STDs clearly facilitate HIV transmission¹⁰ because: i) STDs cause mucosal breaks and increase tissue inflammation and the number of receptive cells; ii) inflammation associated with STDs increase the concentration of HIV in genital secretions; iii) STDs and HIV are frequently co-transmitted. Several recent studies¹³⁻¹⁵ have led to detection of a substantial number of subjects with acute HIV infection in STD clinics, supporting the idea of co-transmission of a classical STD and HIV. In a detailed study of HSV and HIV in India, incident HIV and HSV were closely correlated¹⁴. Since many STDs are rapidly and efficiently transmitted they may set the stage for concomitant acquisition of HIV from virus harbored in the mucosa or during subsequent episodes of intercourse.

What must host defenses confront during exposure to HIV?

The HIV RNA concentration in semen under different conditions has been quite well defined. Subjects in the US and Europe with acute HIV clade B infection can be expected to have 1 - 2 x 10⁵ copies/ml semen¹⁶, whereas subjects in Africa with HIV clade C infection appear to have substantially more HIV in this secretion during acute infection (unpublished observations from Malawi). The copy number of HIV in the female genital tract is not easily defined, in part because the sample collection procedures may be traumatic

and often involve dilution with collection buffer. Menstrual cycle stage and oral contraceptive methods also influence the results.

Male patients with established HIV who acquire an inflammatory STD may excrete >1 x 10⁵ copies HIV/ml, or 6-8 times more than at baseline¹², and substantial increases of HIV in female genital secretions have been observed in women with STDs^{2, 4}. HIV excretion stimulated by inflammation can on occasion overwhelm the suppressive effects of antiviral therapy¹⁷.

Does viral phenotype of genital tract isolates affect HIV transmission?

Only a small number of investigators have compared the genotype or phenotype of HIV in secretions harvested concomitantly from genital secretions and blood^{4, 18}. The predominance of CCR5-tropic (R5) HIV in primary infection suggests either a selective advantage of R5 HIV in transmission, selective viral expansion after infection, or escape from host defenses. In general, HIV recovered from blood and genital secretions demonstrates homology, whether detected by heteroduplex mobility assay, heteroduplex tracking assay, envelope sequence analysis or antiviral resistance markers. However, some differences surface in the face of inflammation¹⁸. There are few data related to viral factors that might affect transmission efficiency, but one recent report suggests that HIV strains with unique envelope properties that confer increased susceptibility to neutralizing antibodies may be preferentially transmitted¹⁹. In addition, uneven distribution of resistance mutations in untreated patients with early HIV infection suggests the possibility that viral fitness could affect transmission efficiency²⁰.

Men and women may differ in the number of unique variants recovered after HIV transmission. It appears that heterosexual men


acquire a single variant, whereas heterosexual women acquire multiple variants^{21,22}. Gay men may also have one or many variants, suggesting that a large surface area (i.e., the vagina and the rectum) may permit more access for simultaneous discrete transmission events²².

What about susceptibility?

Nearly all studies related to HIV transmission have focused on the individual who has become HIV infected, and factor(s) that allowed or promoted infection^{10,11}. These factors include (but are not limited to) host sexual behaviors, host anatomy (e.g., circumcision), host genetics, genital tract flora including acute or chronic STD infections, ill-defined innate or acquired immune defenses, cytokines, and drugs that affect mucosal biology (e.g., oral contraceptives). However, determining the relative importance of each of these factors has been virtually impossible. Unfortunately, animal models are not sufficiently similar to humans to provide definitive conclusions. Rather, clinical trials in humans will likely prove most instructive. For example, if an ongoing clinical trial of cervical barriers supported by the Gates foundation demonstrates that these devices prevent HIV infec-

tion, the central role of the endocervix will be clarified.

Conclusions

Improved understanding of HIV transmission has resulted from a large number of studies of the epidemiology and biology of HIV transmission. Sexual transmission of HIV is clearly not homogeneous. Successful interventions (whether behavioral or biological) should anticipate and be directed toward the time(s) of greatest transmission risk (Figure 1). Given our current difficulty in recognizing and treating people with acute HIV infection this is a substantial challenge, but one that must be met if we are to control the HIV epidemic⁹. In addition, biological interventions should be developed to deal with the real conditions of transmission: high viral burden, the potential for viral diversity, and substantial STD-mediated inflammation. 

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Progress in neutralizing antibody research

Still the most significant problem facing AIDS vaccine researchers is HIV's notorious ability to avoid neutralization by antibodies, a critical component of the protection afforded by licensed vaccines for other diseases. However, a small number of monoclonal antibodies (MAbs) have been isolated over the years that do show some neutralization of primary HIV isolates from multiple different clades (see *J. Virol* **78**, 13232, 2004 for a recent report on the activity of these MAbs). In Lausanne, several presentations focused on efforts to better understand the mechanisms by which the most effective MAbs work, in order to develop vaccines that can induce neutralizing antibodies capable of preventing HIV infection.

A considerable body of research has focused on a neutralizing antibody epitope called ELDKWA in gp41, the transmembrane component of the Env glycoprotein. This epitope is targeted by one of the more broadly neutralizing MAbs identified to date, christened 2F5. However, attempts to use the ELDKWA epitope to elicit similar neutralizing antibodies have so far met with failure, perhaps because the epitope needs to be presented in its correct three-dimensional conformation in which it occurs naturally as part of gp41 (see below, Richard Wyatt's presentation). A presentation by Gail Ferstandig Arnold from Rutgers University described a novel approach to try to present the epitope in its correct conformation by randomizing the amino acids surrounding it. Arnold and colleagues have inserted the ELDKWA epitope into rhinovirus as a vaccine vector after introducing amino acid substitutions on either side where the epitope joins the rhinovirus sequence. These amino acid substitutions were introduced in the hope of hitting on a combination that forms the right framework around the ELDKWA epitope so that it is presented in its authentic conformation. A combinatorial library of variants was engineered and then screened using an elegant competitive immunoselection strategy. This involved immobilizing MAb 2F5 in an assay dish, adding the naked ELDKWA peptide to bind to the antibody, and then washing the recombinant rhinovirus vectors over it; the rhinovirus recombinants presenting the ELDKWA epitope in the most natural conformation would competitively bind to MAb 2F5, displacing the naked ELDKWA peptide, and these bound recombinants could then be col-

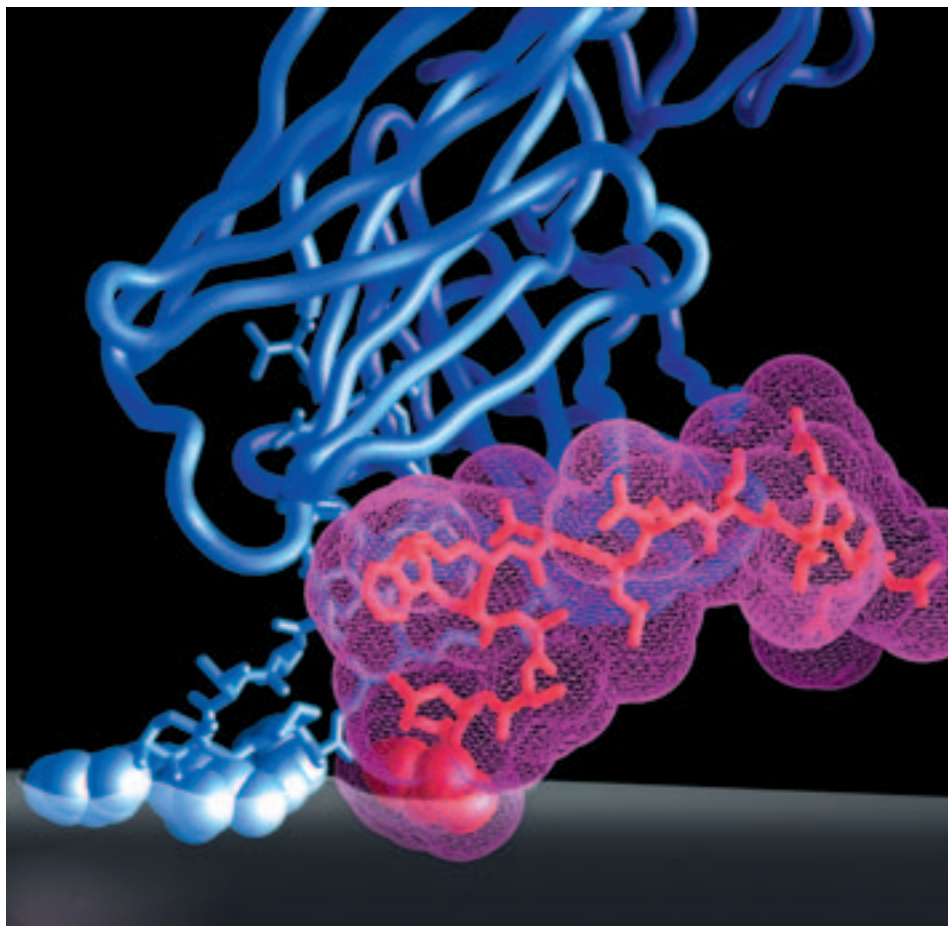


Figure 1. The crystal structure of a broadly neutralizing anti-HIV antibody in complex with its epitope on Env. The monoclonal antibody, 2F5 (blue), is shown in complex with its epitope (red) in the membrane proximal region of the HIV-1 envelope ectodomain. The indole side chain (red spheres) of the final residue of the epitope is coplanar with a hydrophobic region (blue spheres) on 2F5. This region probably interacts with the viral membrane (depicted as opaque plane at bottom of picture) to substantially enhance antibody affinity for HIV.

Image courtesy of P Kwong and R Wyatt and the American Society for Microbiology

lected and enriched by tissue culture passage.

Arnold selected 21 ELDKWA-encoding rhinovirus recombinants for immunogenicity studies in guinea pigs. Sera from animals immunized with either the rhinovirus recombinants alone or the recombinant plus a booster using ELDKWA-based peptides were tested in Virologic's luciferase-based HIV neutralization assay. Neutralizing activity against some primary HIV isolates was demonstrated, including viruses from clades A, B, D, and E (now known as CRF01_AE), the first report of antisera elicited by any ELDKWA-based immunogen that can neutralize HIV primary isolates *in vitro*.

Arnold's research team are now planning immunogenicity studies in non-human primates and efforts are underway to determine the three-dimensional structure of the most promising constructs. Arnold suggested that

rhinovirus, which causes the common cold, may have a number of advantages as a vector, including its mild pathogenicity, serological diversity (there are >100 serotypes), robust immunogenicity (both systemically and mucosally) and the well-characterized nature of its structure and immunogenic sites. Arnold also noted that rhinovirus acts as a multivalent carrier for encoded epitopes, expressing around 60 copies per virion, and, because rhinovirus cannot grow in guinea pigs, Arnold is optimistic that the approach will be more immunogenic in a permissive species.

During the conference late-breaker session, Richard Wyatt from the Vaccine Research Center (VRC) at the US National Institutes of Health (NIH) debuted the three dimensional structure of MAb 2F5 (see Figure 1). Wyatt and colleagues, including Peter Kwong, have succeeded in producing a

three-dimensional crystal structure of 2F5 in complex with its complete epitope in the membrane-proximal region of gp41 (which includes the ELDKWA sequence). This structure has revealed that 2F5 recognizes its epitope in a fully extended conformation rather than the helical structure that it adopts in the fusion-competent conformation (which, in its entirety, is referred to as the 6-helix bundle), suggesting that the mode of action of 2F5 might be to bind to the pre-fusogenic state of gp41, “hang on to” this extended conformation and thereby interfere with the fusion process. The structural analysis also revealed that the 2F5-bound surface of the epitope is charged and that the non-bound surface is occluded, possibly by the viral membrane or some other hydrophobic elements of the Env spike. This interpretation is consistent with binding data that was presented demonstrating that 2F5 binds to its epitope with a relatively higher affinity in the presence of lipid. The group hopes that this structural and biochemical analysis will provide important new clues to enable them to present the gp41 epitope in a stabilized conformation, occlude the distal surface, and provide lipid to generate an immunogen that will better elicit 2F5-like neutralizing antibodies.

In a summary talk on envelope-based antigen design, VRC’s director Gary Nabel cited the ongoing work of Wyatt and Kwong and also made a number of general points about this area of research. Firstly, Nabel stressed that investigators need to compare liganded and unliganded conformations of the envelope antigens under study in order to look for differences in their ability to induce neutralizing antibodies. Nabel also noted that the conformation of potential immunogens can be stabilized (e.g., by disulfide linkages) and argued that researchers should confirm that their candidate envelope constructs retain their ability to bind to CD4. Another potential approach to enhancing envelope immunogenicity mentioned by Nabel involves testing the effects of mutations in the stem of the V3 loop, part of gp120 involved in CD4 binding.

Nabel closed his talk by revealing that VRC has constructed chimeric immunogens based on transposing the V3 loop from clade C HIV into a backbone based on a combination of clade A and B viruses. Preliminary results suggest that this construct may have an improved ability to induce neutralizing antibodies.

Vaccine vectors: Problems and promise

Immunogenicity data was presented from several Phase I and Phase I/II trials of a DNA prime/MVA boost approach developed by Andrew McMichael and Tom Hanke from Oxford University. Development of this vaccine was supported by IAVI and the trials were conducted in the UK, Kenya (in collaboration with KAVI) and Uganda. The results proved disappointing; only 10-25% of volunteers developed cytotoxic T-lymphocyte responses to the HIV Gag protein contained in the vaccine (which encodes the clade A *gag* gene fused to a string of 25 partially overlapping CTL epitopes from *gag*, *pol*, *nef* and *env*; see *Vaccine Briefs*, page 27). As a consequence of these data, IAVI announced that ongoing studies will be completed and additional immune responses evaluated but “unless there are new immune response data that are dramatically different, IAVI will not develop the candidates further, and will focus on its other research and development projects.”

In another presentation McMichael hypothesized that the problem may have related to the poor immunogenicity of the DNA construct, and that MVA appears to be poor at priming T-cell responses but could still have a role as a boost. In support of his argument he offered a glimpse at unpublished data from a trial using the MVA construct as a therapeutic vaccine in individuals on HAART; in this setting detectable CTL responses did appear to be induced in a higher percentage of participants, which McMichael suggested was likely the result of boosting pre-existing responses that were primed by natural infection. An issue often raised about MVA as a vector is the large size of the genome and the possibility that immune responses may be directed more toward the multitude of MVA proteins rather than the HIV antigens it encodes. A study presented recently by Bavarian Nordic, who make an MVA vector encoding the Nef protein, found that while 8/8 HIV-negative trial participants developed T cell responses to MVA, only 3 showed evidence of a response to Nef (and these responses were borderline, ranging from 9-49 spot-forming cells [SFC] in IFN- γ ELISPOT). Analysis of MVA-specific T cell responses in the IAVI trials is ongoing.

Although it remains unclear to what extent the poor immunogenicity of the Oxford MVA vector is specific to the particular construct rather than the platform as a whole,

researchers are looking at ways to enhance the utility and immunogenicity of MVA vectors generally. David Garber and colleagues from the Emory Vaccine Center at Emory University, Atlanta have constructed an MVA vector that has the uracil-DNA-glycosylase O gene deleted; this deletion prevents progression from the early to late phases of the poxvirus replication cycle and thereby restricts the expression of antigenically complex MVA proteins that may interfere with the generation of immune responses against the inserted HIV (or other) antigens. The deletion is also associated with increased apoptosis of infected cells, potentially increasing priming of T cell responses via uptake of the apoptotic cells by antigen-presenting cells.

Garber described macaque experiments in which groups of four animals each were immunized with the modified MVA encoding the HIV Gag protein (MVA Δ *udg-gag*), the parental MVA or a vector based on measles virus (both also encoding Gag). Six weeks after a single immunization Gag-specific T-cell responses were analyzed using IFN- γ ELISPOT. The three macaques immunized with MVA Δ *udg-gag* displayed an average of 376 SFC/million PBMC (peripheral blood mononuclear cells) compared to 26 SFC in animals immunized with the parental MVA vector (a statistically significant difference, $p=0.034$). While the results are preliminary, Garber suggested that MVA Δ *udg* is likely to be a better priming vaccine than currently used MVA vectors. Confirmatory studies in a larger group of macaques are ongoing and Garber also plans to compare the efficacy of the modified and parental MVA constructs as a boost subsequent to vaccination with a measles virus vector.

Two presentations reported on the potential of recombinant measles virus (rMeV) as an AIDS vaccine vector. Hussein Naim from the University of Zurich cited a number of potential advantages to this platform, including that it is easy to manufacture and in widespread use as a vaccine already, long-lived protective immunity is induced after single dose, MeV replicates in the same compartments as HIV (macrophages, dendritic cells and T cells), and a relatively large 5kb of DNA can be inserted.

But a major stumbling block, in all but infants, is pre-existing immunity to measles virus. However, previous studies in macaques (*J. Virol.* **78**, 146, 2004) and Naim’s work in mice suggest that rMeV vectors may remain

Table 1. Interim results post-prime (Week 8) with trivalent MRK-Ad5 in participants with anti-Ad5 titers <1:200

VP/dose	Gag	Pol	Nef	≥2 antigens
3 x 10 ⁹	48% (10/21) 171 SFC	38% (8/21) 270 SFC	48% (10/21) 103 SFC	48% (10/21)
3 x 10 ¹⁰	59% (13/22) 166 SFC	45% (10/22) 301 SFC	50% (11/22) 134 SFC	55% (12/22)
1 x 10 ¹¹	71% (12/17) 289 SFC	53% (9/17) 406 SFC	47% (8/17) 261 SFC	53% (9/17)

Vaccinations (VP = viral particles) at weeks 0, 4, (priming series) & 26 (boost). Responder defined as ELISPOT ≥55 spot-forming cells per million PBMC and ≥4-fold over background; summaries are based on 15-mer peptides. Results shown as the percentage of T-cell responders (absolute numbers), and the geometric mean spot-forming cells (SFC) in IFN-γ ELISPOT. These data were presented at AIDS Vaccine 04 and have not yet been peer-review published.

immunogenic despite the presence of high levels of MeV-specific antibodies. Naim reported the construction of an rMeV vector that can encode multiple genes at three insert sites, and pointed out that expression levels of the encoded gene are site-dependent. In mice, rMeV vectors encoding either Env alone or Env plus Gag elicited both antibody and T cell responses. Naim noted that responses to Env were enhanced in mice that received the constructs containing both Env and Gag and he hypothesized that this might be due to the formation of virus-like particles. In concluding his presentation, Naim emphasized his feeling that rMeV is a promising platform for developing an inexpensive AIDS vaccine that could be utilized by both adults and children.

Frédéric Tangy from the Institut Pasteur followed Naim's talk with a description of his group's efforts to develop a MeV-based AIDS vaccine. He stressed that low cost, the fact that it could easily be produced at large-scale in many countries and the existence of distribution systems for extant MeV vaccines are all important parts of the rationale for pursuing this approach. Tangy's construct is based on the Schwarz strain of MeV that is included in licensed vaccines, including MMR. For macaque challenge experiments, a MeV vector encoding the Gag, Env, Tat, and Nef proteins from SHIV89.6P was constructed. The construct induced T-cell and antibody responses to both MeV and HIV proteins in macaques and led to control of a SHIV89.6P viral challenge in 6/8 animals. While the relevance of this particular challenge system to human HIV infection has been a subject of debate, Tangy concluded that as a proof-of-concept it supports the further development of MeV vaccine vectors. However, in questions after the presentation Stanley Plotkin (consultant to Aventis-Pasteur) also raised the important point of persistence of MeV, a phe-

nomenon that, along with the widespread pre-existing immunity, might well dampen enthusiasm towards this as a potential vector.

Another vector widely discussed at the meeting was adenovirus. These viruses naturally cause severe colds in humans and circulate in several distinct serotypes, the most common being type 5 (Ad5), which Merck has developed into a vector for their lead AIDS vaccine candidate (soon due to enter a Phase II "proof of concept" efficacy trial—see *LAVI Report*, 8(2), 2004). Merck's initial studies involved an Ad5 vector encoding Gag alone (Ad5-gag), but at the conference Robin Isaacs (Director of Clinical Vaccine Research at Merck) presented the first preliminary data on the new "trivalent" construct that encodes Gag, Pol and Nef (see Table 2). Based on these results, Merck has decided that the trivalent Ad5 vector will be tested in the Phase II trial.

A major problem facing Ad5 as a vaccine vector is pre-existing anti-Ad5 immunity. Previous studies in the US have indicated that about a third of the population has anti-Ad5 antibody titers greater than 1:200, levels which have been shown to compromise substantially the immunogenicity of recombinant Ad5 vector-based vaccines for HIV. In Lausanne, Dan Barouch and colleagues from Beth Israel Deaconess Medical Center and Harvard Medical School in collaboration with Crucell Holland BV reported that in South Africa, Zambia, and Botswana, more than 90% of tested individuals had pre-existing anti-Ad5 antibodies, with median titers over 1:1000. In comparison, pre-existing antibody responses to the less common adenovirus serotypes Ad11 and Ad35 were seen in only 20-35% of individuals, in whom titers were generally less than 1:100, suggesting that these serotypes may be worth pursuing as vaccine vectors for developing countries.

Barouch also conducted immunogenicity studies in mice and showed that Ad35 and Ad11 encoding SIV Gag elicited 10-fold higher Gag-specific T cell responses than did the Ad5 vector in animals with anti-Ad5 antibodies at levels comparable to humans. He also evaluated several prime-boost vaccine regimens (including Ad5/Ad5, Ad35/Ad5, Ad11/Ad5, and Ad35/Ad11) and demonstrated that the Ad35/Ad11 regimen was more immunogenic than any combination that included Ad5 in the presence of pre-existing anti-Ad5 antibodies.

Merck has explored a different strategy for overcoming the problem of pre-existing immunity to Ad5. In macaque experiments, priming with Ad5-gag followed by boosting with Aventis-Pasteur's canarypox vector (ALVAC) also encoding Gag led to significantly improved immunogenicity. Unfortunately, Robin Isaacs reported at the conference that these macaque results were not mirrored in a Phase I trial in humans: individuals primed with Ad5 and boosted with ALVAC displayed levels of HIV-specific T cells that were statistically indistinguishable from those seen in participants primed with Ad5 and boosted with Ad5.

Adeno-associated virus

Adeno-Associated Virus (AAV) is a novel vector approach developed by Phil Johnson from the Children's Research Institute in Columbus, Ohio, with support originally from NIH and more recently IAVI. AAV is a parvovirus that is dependent on adenovirus for replication; the vector has been further modified so that it is completely replication-incompetent. An attractive feature of AAV is its prolonged persistence as an episome within cells, which may facilitate the induction of robust and long-lived immune responses to the immunogen after just a single dose. In

Lausanne, Alan Schultz from IAVI offered the first look at immunogenicity data from a study in which rhesus macaques were vaccinated with an AAV serotype 2 vector encoding SIV Gag. Among 24 animals receiving the construct there was a clear dose-dependent T cell response to the Gag protein, reaching 500 SFC/million PBMC (by IFN- γ ELISPOT) in macaques that received the highest dose. These robust Gag-specific T-cell responses were also accompanied by high and persistent titers of Gag-specific antibodies averaging 1:1600 (and as high as 1:8000 mean peak titer). A Phase I safety study of the AAV vector in humans is ongoing.

Targeting toll-like receptors

A current hot topic in immunology revolves around toll-like receptors (TLRs). This family of molecules (eleven have been reported so far, simply numbered TLR1 through TLR11) was discovered relatively recently and they appear to play a critical role in allowing immune system cells to sense “danger signals” within potential pathogens and then initiate an immune response. Basic research on TLRs suggests that they may have the potential to be targeted with adjuvant in order to enhance the immune response to vaccines. Bob Seder from the NIH presented results from a macaque study that tested the immunogenicity of a single vaccination with the HIV Gag protein emulsified in the adjuvant Montanide ISA 51 (an oil-based compound with similarities to the standard Freund’s adjuvant) in the presence or absence of synthetic TLR7/8 agonists, TLR8 agonists, or a TLR9 ligand, CpG oligodeoxynucleotide (ODN). Two weeks post-immunization all animals that received the TLR agonists had significantly greater Gag-specific T cell responses (assessed by IFN- γ ELISPOT) than the macaques that did not (five animals per group). Six weeks post-immunization, levels of both IFN- γ and interleukin (IL)-2-producing HIV-specific T cells remained significantly higher in the animals immunized with CpG ODN or TLR7/8 agonists compared to those not given a TLR ligand. TLR8 is present on the myeloid dendritic cell subset while TLRs 7 and 9 are present on plasmacytoid dendritic cells (PDCs), and Seder suggested targeting PDCs may be important and that further studies of TLR-based adjuvant modalities are warranted.

Cellular immunology

Several presentations reinforced a recent

shift toward evaluating additional markers of antigen-specific T-cell function beyond just IFN- γ . At the closing ceremony, Clive Gray (National Institute for Communicable Diseases, South Africa) reviewed these data and noted the consensus among researchers that intracellular cytokine staining (ICS) for IL-2 production should be included when evaluating T cell-based vaccines; efforts to standardize ICS assays for this purpose are underway. An example of the potential importance of IL-2-based ICS was provided by Helen Horton from the University of Washington. Horton showed that, in a Phase I study of GlaxoSmithKline’s Nef-Tat-gp120 protein vaccine, the majority of vaccine-induced CD4⁺ T cells produced IL-2, not IFN- γ , and would therefore have been missed with the standard ELISPOT assay. Clive Gray also cited additional techniques that may contribute to a more comprehensive evaluation of vaccine-induced T-cell responses, such as flow cytometry-based monitoring of antigen-specific proliferation utilizing staining with the dye CFSE (every time a T cell divides half the CFSE dye is lost, so cells with proliferative capacity can be quantified based on their loss of CFSE) and multi-parameter tests that assess the ability of T cells to produce multiple cytokines and chemokines simultaneously.

Conclusion

The final presentations in Lausanne reflected upon the impact of the European and US political landscapes in shaping AIDS vaccine research efforts. Michel Kazatchkine, head of the French Agence Nationale de Recherches sur le Sida (ANRS) discussed the current European Union situation, noting that while representatives of the EU are generally morally supportive of AIDS vaccine research, this is not mirrored by appropriate fiscal support. Currently European scientists lack any kind of coordinated lobbying mechanism to advocate for funding at the EU level and instead there is a patchwork of efforts highly dependent on the munificence of individual governments, coordinated wherever possible by the relatively youthful European Vaccine Effort against HIV/AIDS (EuroVac). Kazatchkine advocated strongly that this problem needs to be addressed if the EU is to develop a more credible, coordinated and well-financed AIDS vaccine research program.

Bart Haynes, chair of the AIDS Vaccine Research Working Group which advises the US government’s Division of AIDS (DAIDS) on vaccine-related issues, noted that the situ-

ation in the US is somewhat better due to a significantly higher level of research funding support both from government and independent entities like the Bill & Melinda Gates Foundation. Haynes cited the Partnership for AIDS Vaccine Evaluation (PAVE), a DAIDS-sponsored initiative chaired by Peggy Johnston that is making progress in coordinating research across the differing government bodies (including the National Institutes of Health, the NIH Vaccine Research Center, DAIDS, HIV Vaccine Trials Network, Centers for Disease Control and Prevention, Walter Reed Army Institute of Research, Henry M. Jackson Foundation, Food & Drug Administration, Adult & Pediatric AIDS Clinical Trials Groups) and also external groups such as IAVI, Merck and the Canadian Network for Vaccines & Immunotherapeutics (CANVAC).

PAVE’s ongoing work includes developing the laboratory support necessary for evaluating vaccine immunogenicity in efficacy trials (including standardization of immunological assays), building capacity for clinical trials by standardizing site development and investigator training tools, and harmonizing research protocols to better facilitate cross-trial comparisons and data sharing. Haynes articulated his hope that PAVE will enhance the ability of the US to participate productively in the global collaborative effort being proposed under the Global HIV Vaccine Enterprise, with the ultimate outcome being the speedier development of an effective AIDS vaccine.

In the final period of questions and comments from conference attendees, Larry Corey (head of the HIV Vaccine Trials Network) noted that the type of coordinated research being advocated under these various mechanisms will require something of a cultural shift among scientists used to a more independent approach—in Corey’s pithy words: “everyone likes to collaborate but no one likes to be coordinated.” Peggy Johnston, Director of Vaccine Research at DAIDS, emphasized the need to focus on training new investigators, stating: “this [HIV/AIDS] may well be a problem that will be handed to the next generation.” Only time will tell whether the new commitment to global coordination discussed in Lausanne can prevent Johnston’s dire scenario from coming to pass. ■

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

Bangkok 2004:

A glimpse of the future at the dawn of the “scale-up” effort

by Emily Bass

At times, the 15th International AIDS Conference in Bangkok (11-16 July 2004) crackled with the paradigm-shifting outrage and activism that enlivened the Durban meeting in 2000; at other moments it was steeped in the Barcelona 2002 gathering's sober acknowledgement that, two decades into the epidemic, the response is too little, too late in far too many parts of the world. Ultimately, however, the Bangkok gathering—the first of its kind since substantial support for antiretroviral (ARV) programs began to come from the Global Fund to Fight AIDS Tuberculosis and Malaria, the US Presidential Emergency Fund for AIDS Relief, the World Bank and other sources—moved beyond these past meetings and provided a first glimpse of both the inspiring possibilities and the significant shortcomings of the nascent efforts now underway to “scale-up” ARV treatment in the world's resource-poor countries.

Clinicians from resource-poor countries like Haiti, Zambia and Uganda reported that even small-scale ARV programs are having a dramatic impact on individual health, as well as HIV-related stigma and voluntary counseling and testing. As Haitian AIDS clinician Jean Pape showed slides of rudimentary clinics dispensing ARVs and program vehicles navigating flooded roads, it was possible to imagine that the ambitions expressed in Durban—treatment for all who need it—might someday be realized.

However these moments of uplift were outweighed by dire predictions from global leaders and activists alike that the existing targets for treatment will not be met without additional investments of human and financial resources and political will. Jim Kim, AIDS director at the World Health Organization, warned that the organization would not meet its “3 by 5” program goal of treating 3 million people in resource-poor countries by 2005 without increased funding and human resources. With just 13 months until the end of 2005, there are still only an estimated 440,000 people from resource-poor countries receiving ARVs. “By these measures of human life, the ones that really matter, we have failed. And we have failed miserably to do enough in the precious time that has passed since Barcelona,” said Kim. A UNAIDS report released at the conference predicted by 2005 a 50% shortfall of US\$6 billion of resources required for prevention, care and treatment services if funding continues at existing levels.

There was also alarming news on the prevention front. Released at the conference, the most recent UNAIDS report estimates that there were a record 5 million new HIV infections in 2003. The

report also emphasizes the increasingly devastating impact that the virus is having on women, who are disproportionately infected in virtually every corner of the world. Unfortunately the meeting will also be remembered for the instances when unanimity on the urgent need to improve existing prevention options was nearly sidelined by debates over the relative contributions to prevention outcomes of the various components of the “ABC” strategy—abstinence, being faithful and using condoms. Many researchers, including South African scientist Quarraisha Abdool Karim, sought to counteract the ideological tenor of these debates with hard evidence. In her plenary address, Abdool Karim reminded the audience that “the promotion of male condoms is effective in both

research and real world settings and it can play a critical role in prevention of heterosexual transmission in developing countries—this has been clearly demonstrated in both Thailand and Uganda.”

On a more positive note, there were no signs that prevention would be sidelined in the context of treatment scale-up. Instead, many speakers emphasized the complementarity of these two aspects of a comprehensive response to the epidemic. “Without a greatly expanded prevention effort, treatment is simply not sustainable,” said Peter Piot, Executive Director of UNAIDS, during the conference's closing ceremony.

Recognizing the role of injection drug use in spreading the epidemic in Thailand and neighboring countries like Vietnam, Laos and Burma, many speakers called for expanded access to syringe exchange and drug replacement programs. Others described novel trials that will measure the effect of “structural” interventions such as income generation on vulnerability to HIV, asking, for example, whether HIV incidence is lower among women who

have their own financial resources.

There was also considerable enthusiasm for novel approaches which will be tested in efficacy trials in the near future, including five candidate microbicides, pre-exposure chemoprophylaxis using tenofovir and herpes simplex virus type 2 prophylaxis. On the conference's final day, prevention track rapporteur John Kaldor (National Centre in HIV Epidemiology and Clinical Research, Australia) commented that, while none of this research was news at Bangkok, there was still a mood of “relief” among people committed to prevention “even if we have the collective feeling of having to hold our breath for a couple of years until the results start coming through.” Microbicides, in particular, were hailed as a crucial



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Peter Piot



The development of an HIV vaccine represents one of the most difficult challenges that modern biomedical science is confronting

Jose Esparza



area of research that might help reduce women's vulnerability to infection (see *IAVI Report*, 8(2), 2004).

On the vaccines front, there were updates on recruitment and retention strategies from the multiple trials that started in 2003 (see page 11) as well as studies looking at the willingness to participate in vaccine trials, vaccine demand and other issues in different populations (see page 13). On the scientific front, there were reports on viral diversity and superinfection (see page 18) and updates on vaccine science and HIV immunology (see page 22).

AIDS vaccines: A marathon amidst a sprint

While at times the conference highlighted the interdependence of prevention and treatment, at others it sharpened the distinction between immediate responses to the epidemic, such as treatment programs, and longer term research endeavors like the development of an effective AIDS vaccine or microbicide. The sprint to scale-up is happening at a time of scaled-back expectations for the AIDS vaccine effort and at Bangkok the immediacy of the scale-up goals at times made vaccine timelines seem all the more distant.

"The development of an HIV vaccine represents one of the most difficult challenges that modern biomedical science is confronting," said Jose Esparza, Senior Advisor on HIV Vaccines at the Bill & Melinda Gates Foundation in his plenary speech on vaccines. His comments echoed the assessment of IAVI's *2004 Scientific Blueprint* for AIDS vaccine research that was released at the conference, which stated that "the progress of the past few years is outweighed by critical scientific, operational and resource challenges." The Blueprint also noted that the field will not know whether the current vaccine strategies being tested will provide any benefit until "late 2007 at the earliest."

The general sense that AIDS vaccines are a long-term prospect may have contributed to the relative silence on them outside of sessions specifically devoted to the topic. For the first time in several years AIDS vaccines were not mentioned by any speakers during the opening ceremony. Likewise, much of the news coverage of AIDS vaccines during and after the conference emphasized the extended timeframes for identifying an effective candidate.

"I was a bit concerned that the press clips [on AIDS vaccines] focused mainly on the dif-

iculties and scientific challenges," said Esparza. "Many people took that to be pessimistic. It is also realistic."

"No major leadership event or speech should go without mentioning short- and long-term goals" for the global response to AIDS, said Seth Berkley, IAVI president and CEO. Berkley also voiced hopes that AIDS vaccine advocacy will be taken up by a wider range of groups, saying that "AIDS vaccine work needs to roll into the global AIDS community, because they are the ones who have to take the lead."

Many vaccine advocates feel that distinguishing between realism and pessimism may be the field's greatest challenge in coming years. "Realism will be a boon to the field if it takes the form of accurate estimates of the resources needed to finance the search for an AIDS vaccine, broad coordination among research groups, and widespread political support from governments and communities who have appropriate expectations of the pace of research and its likely short- and long-term outcomes," says Mitchell Warren, Executive Director of the AIDS Vaccine Advocacy Coalition. Such an outlook would include a sense of the magnitude of the challenge but stops short of pessimism, which runs the risk of dissuading donors from further investment, research groups from further collaboration, and communities and governments from further participation in the effort, Warren says.

One way to help draw this distinction is to develop more detailed estimates of the funds needed for the AIDS vaccine research effort, says Rob Hecht, head of Public Policy at IAVI, who attended a pre-conference satellite where resource-tracking estimates for vaccines and microbicides were discussed. "We need to be very conservative in our predictions about how long it is going to take to find an AIDS vaccine and how much it is going to cost," Hecht says, adding that another top priority is to develop improved estimates of the public health impact of an effective AIDS vaccine which "could be one of the most cost-effective interventions ever developed" even if the research process is as lengthy as scientists now predict it will be.

Just four years ago in Durban, AIDS vaccines were seen as a beacon of hope and treatments were deemed all but impossible; at a moment when the positions are reversed, treatment advocates are also emerging as important teammates in the AIDS vaccine

marathon. And in the midst of the sprint to scale-up, treatment activists like South Africa's Zackie Achmat emphasized that additional improved prevention strategies

will be needed to complement services for those already infected. "It is important to find an AIDS vaccine and I think it is doable, although it will not be easy—just as

it is not easy to do treatment. But it is important to do that too." ■

Reports from the frontlines:

AIDS vaccine trial site experiences from a busy year

by Emily Bass

Bangkok may be remembered as a meeting of scaled-back expectations and, in some quarters, outright pessimism regarding the short-term future of AIDS vaccine research. But in the bustling poster hall there was a more energized mood as many sites reported on their community outreach, recruitment and retention efforts in the record number of trials launched in 2003.

While these presentations didn't reveal any scientific breakthroughs, there was news of innovative enrollment strategies—many of which were tested for the first time—that will likely provide a foundation for future efforts. Many of these reports from the frontlines also gave a sense of the time-consuming and challenging process this can be, even when the trial is being conducted by an experienced team.

The HIV Vaccine Trials Unit at Chris Hani Baragwanath Hospital in Soweto, South Africa has so far launched two Phase I preventive AIDS vaccine trials. A poster (Abstract number ThPeC7436) detailed the results of its recruitment strategy, which uses the "Bara" voluntary counseling and testing (VCT) center and other community-based VCT centers to identify potential volunteers. This approach initiates recruitment at existing community VCT centers, rather than VCT sites that have been established specifically by or for a vaccine trial. HIV uninfected individuals were offered the opportunity to participate in vaccine discussion groups, which met on a monthly basis to provide information on a range of topics related to vaccine research and trial participation. When the trial was ready for enrollment, discussion group members were offered a chance to be screened for participation and were enrolled if found to be eligible.

In total, more than 3000 people were seen at the VCT center and 1581 individuals were found to be HIV uninfected. Of the 336 individuals who chose to enroll in the vaccine discussion groups, 194 completed the required number of group sessions and 32 were finally determined to be eligible for participation in the trial. Ultimately this approach yielded a 10:1 ratio of screening to enrollment, a typi-

cal ratio that is indicative of the effort and resources required for trial recruitment efforts, even at experienced sites. Principal investigator Efthya Vardas says that the "Bara" vaccine trials will in future work with VCT counselors to ensure that information about enrolling in vaccine trials is given to all HIV uninfected individuals during post-test counseling. On a positive note, the Soweto trials unit showed that it is still possible to enroll HIV uninfected individuals (who are at relatively low risk of contracting infection) from a community with very high rates of HIV infection—nearly 50 percent of the individuals screened for this study were already HIV infected.

Roughly 12 percent of individuals were not eligible for the trial because their blood tests for liver or kidney function fell outside of reference ranges. Other reasons for ineligibility included loss to follow-up (15%), chronic illnesses (9%) and participant decision to withdraw (7%).

Blood tests also accounted for the exclusion of a large proportion of individuals screened for the first vaccine trial in South Africa's neighbor, Botswana; 36 of 76 volunteers screened were ineligible due to results outside of reference ranges (ThPeC7450). These reference ranges are based on normal values in developed countries, and Site Director Tonya Villafana says that developing local reference ranges that reflect the values found in healthy persons under prevailing conditions in the country is a large undertaking that may soon be tackled by Botswana's Ministry of Health. "It is something that the country is interested in doing given the multiple research studies

being undertaken in healthy individuals, as well as the ongoing implementation of national programs for the treatment of HIV-infected people."

Another poster from the trial in Botswana described their media outreach strategy (ThPeC7429), which played a crucial role in trial recruitment. Of 192 potential volunteers who came forward, 48 reported the media as their primary source of information; Villafana says that 11 of the 14 volunteers who ultimately enrolled first learned



**news of
innovative
enrollment
strategies...
that will likely
provide a
foundation for
future efforts**



about the trial through the newspaper or media. The poster listed the key messages that the trial site had worked to convey through the media: An experimental AIDS vaccine cannot treat or cure HIV in people who are already infected; an experimental AIDS vaccine cannot cause HIV infection or AIDS; the experimental vaccine being tested in Botswana had been designed to work against several subtypes of HIV; Botswana has played a key role in HIV prevention research worldwide; and that community involvement was vital to the success of the trial.

Media was also the focus of a poster presented by IAVI and the Brazilian National AIDS Control Program (ThPeC7441). The investigators surveyed the quantity and quality of media coverage of AIDS vaccine research in the country. The project, which was complemented by a national journalist workshop, provided important leads to guide press outreach strategies in the country, identifying gaps and priority areas for future action.

A report from the Kenya AIDS Vaccine Initiative (KAVI)-IAVI trial site in Nairobi described yet another approach to enrolling volunteers (ThPeA6999). Following its first vaccine trial, during which it took six months to enroll 18 volunteers, the site developed a peer leader program. Individuals nominated by the communities earmarked for trial recruitment participated in training on HIV/AIDS, AIDS vaccine research and clinical trials that lasted two months, with follow-up for a further six months. These peer leaders then conducted outreach and education events in their communities, after which interested individuals could visit the KAVI clinic for one-on-one counseling and information. Recruitment rates increased to 14 volunteers per month—up from 3 per month prior to the initiation of the program.

These reports also suggest some important challenges facing the field. First and foremost there is the need to acknowledge that recruitment may proceed more slowly than anticipated. Perhaps the most dramatic example of this comes from the ongoing Phase III “Prime-Boost” trial in Thailand. Principal investigator Supachai Rerks-Ngarm gave an update (ThOrC1428) on enrollment for the study, which aims to recruit 16,000 volunteers in the provinces of Rayong and Chon Buri. All adult residents of these provinces are eligible to enroll in this community-based

cohort, which is using existing health facilities as the entry point for recruitment.


Recruitment began in late September 2003 and, as of June 2004, only 2571 volunteers had enrolled in the trial, a slower rate than originally anticipated. Enrollment will be extended by another year. “Even though we are enrolling slowly, we are confident that we will achieve enough volunteers after this extension,” said Rerks-Ngarm. [At time of press, 8,315 volunteers had been enrolled and had received at least one immunization.]

Recruiting women is a challenge at some sites, such as the Uganda Virus Research Institute-IAVI site in Entebbe, Uganda (ThPeA7006). The site’s Phase I trial enrolled 42 men and 8 women; during the recruitment process 7 women withdrew after giving informed consent, many citing their partners’ objections. Trial sites in Botswana and Kenya have also reported low numbers of women participants.

A related issue is the involvement of adolescents in vaccine trials. Across sub-Saharan Africa, adolescent girls are disproportionately infected compared to their male counterparts; in South Africa, for example, 25% of South African women are infected with HIV by the time they are 22 years old. To prevent HIV infections in this age group, once a vaccine has been proven safe and effective it will be important to vaccinate adolescents or, possibly, pre-pubescent girls who have not yet become sexually active. Most licensed vaccines for other diseases have been tested in children (after preliminary safety tests in adults) since they are the primary recipients of protective vaccinations, but AIDS vaccines are likely to be tested extensively in adult populations first. To be routinely used in children or adolescents, an AIDS vaccine efficacious in adults would have to be tested for safety in adolescents, and the immune response would have to be shown to be similar to that in adults and to last long enough so that it’s still strong years after vaccination, when they may be exposed to HIV infection. It may be necessary to enroll some adolescents in key efficacy trials if it is possible to identify a group at sufficiently high risk, obtain truly informed consent and to ensure that they are effectively counseled.

Another challenge to enrolling young people in trials of HIV prevention strategies is that many countries have varied or conflicting regulations regarding young people’s participation in trials. Ann Strode of South

Africa’s HIV/AIDS Vaccine Ethics Group presented a review of that country’s heterogeneous legal statutes regarding adolescents (WeOrC1249). South Africa is one example of a country where “children have limited but evolving legal capacity,” she reported. For example, young people can obtain contraceptives at age 14 without parental consent, the legal age of sexual consent is 16, and young women are allowed to terminate a pregnancy at any age. However, Strode noted, South Africa has “no independent age for [children to] consent to research.”

Strode recommended that countries develop national systems to help assist in recruitment of adolescents for HIV prevention trials and that research and human rights groups work together on advocacy for legal and ethical reform of age of consent laws. She also recommended further research on children’s ability to understand the risks and benefits of trial participation. 

Forecasting the future:

Studies at Bangkok explore upcoming issues in trials, delivery and demand

by Emily Bass

It is still uncertain precisely when an effective AIDS vaccine will be identified, but it's clear that the development process will require a series of trials in different populations as well as detailed plans for manufacturing and distributing a licensed product. While some of the hallway discussions in Bangkok focused on the uncertainties of the field's scientific endeavors, a series of posters and presentations focused attention on issues that need to be addressed, including different populations' willingness either to participate in clinical trials or to purchase licensed vaccines.

Willingness to participate

One comprehensive multisite cross-sectional study conducted by researchers at Johns Hopkins School of Public Health and international partners (Abstract no. ThPeC7440; go to www.iasociety.org to view abstracts) surveyed 3155 participants' attitudes towards hypothetical vaccines and trials. Survey participants came from a variety of settings, ranging from a US university campus to a sexually transmitted infection (STI) clinic in India to members of an injection drug users seroincidence cohort in Chiang Mai, Thailand.

The study found that willingness to participate (WTP) in an AIDS vaccine trial varied widely depending on the population surveyed, ranging from 31% among college students in Baltimore to 64% among STI clinic attendees in Pune, India. The study also analyzed responders' answers for predictors of WTP. Acceptance of a licensed AIDS vaccine was a predictor for five out of six sites, while belief in the success of an AIDS vaccine was only associated with WTP in one site. Negative predictors included fear of side-effects, belief that a partner would refuse sex, and the possibility that antibodies induced by a vaccine would result in a false-positive test for HIV infection.

Another study conducted by investigators at Emory University (ThPeC7431) looked at WTP among a predominantly black (63%), female (72%) population (n=220) of students from a small college in Georgia. The study was designed to probe participants' understanding of and beliefs about AIDS vaccine research, and it yielded some striking findings. More than a third of those interviewed believed that an AIDS vaccine exists but is withheld from the public, and almost half agreed with the statement "in a trial I may be injected with HIV." Overall, just 17% of those interviewed agreed with the statement "after a researcher told me about an HIV vaccine clinical trial, I would enroll in one." The study also found that African Americans and Asians were more likely than whites to feel that it was important to have people of the same ethnic group participating in the trial ($p = 0.001$) or on the research team ($p = 0.001$).

Concerns of people in the US were also the focus of several posters presented by Project VIBE, a research study that posed an elaborate set of scenarios involving hypothetical vaccines with different characteristics (e.g., cost, level of efficacy, potential for causing a falsely positive reading in an HIV test), as well as trials with different types of designs (e.g., greater or fewer numbers of immunizations, blood samplings, etc.). These scenarios were used to

explore attitudes about research participation, interest in licensed vaccines, and other issues through interviews and focus groups at sites around Los Angeles, including a gay and lesbian community center and health clinic, a needle exchange center, and a health clinic serving the city's Latino community.

Here, too, there was heterogeneity in the responses with individuals identifying a number of barriers to uptake of an effective vaccine (TuPeD5105), including the belief that doctors experiment on patients without telling them, prior experience of being refused medical coverage, and concerns about the stigma that might be associated with seeking such a vaccine. Other abstracts from Project VIBE looked at willingness to participate in vaccine trials (ThPeC7435) and women's concerns around uptake of a hypothetical effective vaccine (ThPeD5154).

Risk behavior/mitigation of vaccine protective effects

The benefits of an effective vaccine could be offset by other trends such as decreased condom use or increased numbers of sexual partners, which might emerge if people widely believed that an AIDS vaccine was a "magic bullet" against the virus. Since the first generation of AIDS vaccines is expected to have low or moderate levels of efficacy, many in the field feel that it's particularly important to consider how various shifts in risk behavior might offset the benefits of a vaccine with low-, moderate-, or even high-efficacy.

In Bangkok, Peter Vickerman (London School of Hygiene and Tropical Medicine) presented a new mathematical model that attempts to address some of these questions (ThOrC1430). The model allows researchers to examine how changes in rates of unprotected sex and/or numbers of sexual partners might affect vaccines that confer different degrees of protection. The study considered two types of preventive vaccine, one which reduces the susceptibility of uninfected individuals and a second that reduces the infectiousness of infected individuals. The model was also designed to account for increased levels of STIs in vaccinees who increased their sexual risk behavior.

In a sample calculation that assumed 50% condom use prior to the vaccine's introduction, Vickerman showed that with a vaccine that reduced the susceptibility of uninfected individuals with 40% efficacy, sexual risk must not increase by more than 70% for the vaccine to be beneficial. But if the efficacy of that vaccine is 80% then sexual risk can increase by over 300% without fully compromising the benefit.

Vickerman acknowledged the many limitations of mathematical models to predict real world outcomes but concluded that "vaccine introduction should be accompanied by increased efforts to promote reductions in risk behavior. If this is not done, and people increase their sexual risk, the benefits from a vaccination program will be reduced and transmission could increase."

Forecasting demand

A poster (ThPeC7445) by Martha Ainsworth (World Bank) and

continued on page 18 



Immunology and AIDS vaccines

Gary Nabel, MD, PhD, has long been a leader in the development of AIDS vaccines. Since 1999 he has been Director of the Vaccine Research Center (VRC) at the National Institutes of Health in Bethesda, Maryland. Prior to taking this position, he was director from 1997-1999 of the Center for Gene Therapy and a Howard Hughes Medical Institute investigator at the University of Michigan in Ann Arbor. Nabel also served as the co-director of the University of Michigan Center for Molecular Medicine from 1994-1999. He is a member of the Institute of Medicine of the National Academy of Sciences.

Nabel's diverse research interests range from applied immunology to fundamental molecular biology. His laboratory has developed gene transfer strategies that are being applied to the development of vaccines for and treatment of AIDS, Ebola virus, cancer, and other diseases. In addition, he has long been involved in studying NF- κ B gene regulation, with an emphasis on its relation to cell cycle progression, transformation, and viral infection by HIV.

Here, Nabel speaks with IAVI Report Editor Simon Noble about our current understanding of the scientific issues critical to developing an AIDS vaccine.

Originally the VRC had a wide remit to tackle multiple infectious diseases. What are your current broader plans for the VRC and how much emphasis is currently being put on vaccines against potential bioterror pathogens?

Our raison d'être is really to drive the effort to develop an effective HIV vaccine, whether that's by developing a candidate that we bring along or by helping to assess various candidates and find one that works the best, however we can help. Needless to say, what is often lost in our concerns about bio-defense is the fact that HIV today dwarfs the danger that most other bio-threats pose to us.

Having said that, when there are economies of scale and when there are platform technologies that will allow us to either understand emerging pathogens or bioterrorism threats, then we are interested, willing and able to help in those efforts.

What particular pathogens, aside from HIV, are you currently focusing on?

The short list for us is HIV, Ebola, West Nile, and SARS viruses, and more recently we've tried to help with the influenza virus problems. Those are the other VRC efforts. We just received word from the FDA that it's okay to proceed with our SARS DNA vaccine. That should start next month, provided the rest of the approvals go according to plan. So we're moving in several areas.

To switch to HIV more specifically, the VRC is closely involved in the efforts to standardize assays. Which assays and protocols do you see as the best way forward in that effort?

For cellular immunity, our major focus has been on flow-based technologies, flow cytometry, and particularly looking at intracellular cytokines, staining for a variety of different cytokines. We've also been performing the ELISPOT assay. Our experience so far is that flow cytometry appears to be more sensitive and a bit more informative about which cell subsets are responding. So that's our preferred assay. Despite the power of the flow-based technology, we still have a lot of work to do, as a field, to develop even more sophisticated assays that I hope will lead us to an immune correlate.

Do you think some of these assays are practical though for resource-poor settings?

You don't need to implement flow cytometry, at least of the kind that we would put into place to support licensure of a vaccine product, at every international site. But you do need to have the capability of storing and transporting the cells properly to where the analysis can be done. We have to recognize the importance of defining an immune correlate and taking whatever steps are necessary to do those analyses.

On that point, it's perhaps becoming increasingly evident that interferon (IFN)- γ might not be the only marker to focus on. What other markers do you think might be critical?

Mario Roederer and Rick Koup at the VRC have been looking at a variety of factors in different vaccine models; they have looked at TNF- α , MIP-1 α , IFN- γ , IL-2, a variety of cell surface markers, as part of our discovery research. We need to evaluate them all before we know which will be the best. I think RANTES, MIP-1 α , the chemokines generally, are of great interest, and a number of cytokines as well.

Do you think there is any functionality strongly linked to any of these markers that makes one perhaps more promising than another?

At this point I don't know. I'm intrigued that there may be innate immune mechanisms that might be involved in protecting against infection, like MIP-1 α , RANTES, or other chemokines, products of the natural inflammatory response that block infection. Is it possible to develop an acquired immune response that generates some of those protective chemokines in a more rapid fashion?

Although we're all convinced that cellular immunity is important in protecting against viral replication, we still don't really understand the mechanism by which that occurs. We often say that it's killer T cells, but is it killer T cells that are killing infected target cells or are they elaborating cytokines or are there other mechanisms? We still have a long way to go when it comes to understanding immune mechanisms and immune correlates of protection.


Which of the current viral vector candidates do you think are most promising, the ones that capture your enthusiasm?

In many ways the candidate platforms are still testing the waters. I guess we've put our money down on the things that we think work well; I'm impressed with how well adenoviral vaccines have worked in a variety of animal and human models, not only for HIV but also for Ebola virus and other pathogens. Compared to other vectors that have been tested, the adenoviruses are much more effective in eliciting cellular immunity and also induce humoral immunity, especially after DNA priming. They are very high on our list.

DNA, particularly as a prime for adenovirus, has done very well in animal models for both HIV and Ebola virus; if we can get

the DNA to work well in people, the platform technology is one that has great promise.

Together, those technologies will allow us to make a reasonable first-generation vaccine, and that should give us an opportunity to test the concept that cellular immunity can protect against HIV infection.



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Whether it be the Merck adenovirus vaccine or our vaccine, I think they will both be informative, with the difference between Merck's and our's being that we'll be testing DNA-adenovirus in a prime-boost combination and we have also included envelopes. So I think we'll learn very different things from these upcoming trials.

How is your multivalent DNA vaccine candidate proceeding?

We've actually completed Phase I for

our first-generation multivalent DNA vaccine, and we've completed enrollment for our multiclade adenovirus candidate. We decided to make the multiclade, multivalent vaccine after convening a meeting at the VRC where we invited our international colleagues from India, from different countries in Africa, from China, from the Caribbean; we put our heads together and asked "What's the best we can do to deal with the global problem in terms of a vaccine candidate?" and the concept of the multiclade ABC candidate arose from these discussions. We're very dedicated to it; it's our top priority. We're trying to move it into efficacy testing in different parts of the world, particularly the developing world, as fast as we can.

How do you see the future of DNA vaccines? Do you think they can make the grade?


We've been pleasantly surprised at how well DNA vaccines are performing in people. That's not to say that DNA alone will be the answer, I think it's likely to be a prime-boost combination. But having said that, we see pretty consistent cellular immune responses, we're even seeing antibodies with some consistency, not just for HIV but with other vaccine targets as well.

What we should remember about DNA is that, like all new technologies, it can and will evolve. The use of altered regulatory signals, the CMV enhancer with the HTLV-1 R region, for example, represents a technically simple improvement that enhances the immunogenicity of the vaccine significantly, and there will be other improvements in the future; there are technical aspects in terms of how you purify the DNA. We're still just scratching the surface of DNA adjuvants. If electroporation can be developed into a safe, clinically-acceptable delivery method, it could have utility. I look at DNA vaccine technology as an opportunity.


Moving from platforms to the actual immunogen, what do you think are the best approaches to rational design of immunogens?

The critical path to a highly effective vaccine runs through the HIV envelope. We are already able to induce cellular immune responses to the internal proteins, and we'd like to broaden that response by generating immunity to conserved regions of the envelope.

But to have a highly effective vaccine, the broadly neutralizing antibody remains the Holy Grail. The jury is still out in terms



it may also be helpful to re-visit the spectrum of neutralizing antibodies that one sees in nature... [and]...ask whether the universe of broadly neutralizing antibodies for HIV might be larger



of whether we can get there. I think that the most logical and productive way to proceed is the structure-based approach. Peter Kwong and Rich Wyatt at the VRC have spent years studying the envelope and the interactions with CD4. We're trying to modify the protein in such a way that it will be conformationally fixed yet expose determinants that we hope will elicit these antibodies. Peter's also working on new structures, including the V3 loop, getting the trimeric rather than the monomeric structure. Peter and Rich's recent paper on the 2F5/4E10 region is another important step forward (see *AIDS Vaccine 04*, page 5).

Steve Harrison has reported the un-liganded form of SIV at meetings and it will be an extremely important contribution to the field. We're conferring with Steve and trying to incorporate that information into vaccine design as well—there may be HIV structures that we can capture with rational design that are more representative of the un-liganded structure. Also, there are clade differences that we don't understand yet: Why in clade C do we see so little variability of the V3 loop, and yet there are regions just downstream of V3, and V4 of the clade B's, which are variable? Understanding the structural basis of these clade differences will be important.

It may also be helpful to re-visit the spectrum of neutralizing antibodies that one sees in nature. Many of the antibodies were originally selected by binding to gp120, as a pre-screen for neutralization. If you look at Antonio Lanzavecchia's work in SARS, he simply screened by neutralization in an infection assay and the monoclonals that he selected were ones that may not have been picked up if he pre-screened by binding to the SARS protein. They're very weak binders that recognize conformational determinants. So I don't see why we can't go back and ask whether the universe of broadly neutralizing antibodies for HIV might be larger than those that we have defined so far.

I'm also intrigued with the idea of using the genetic diversity of the virus in a positive way for vaccine design—there are clues in what the virus does and does not conserve. So both structural and genetic approaches are now our highest priorities.

How do you think immunodominance of epitopes can be addressed? Do you think the immune response should be directed towards subdominant epitopes?

That's a really interesting question. The more I look at the virus and how it manipu-

lates the immune system the more I'm convinced that the concepts of original antigenic sin and immunodominance are major scientific questions that we must resolve. So yes, it's a very important area of research. The virus has apparently used this phenomenon for its own gain.

How important do you think improved adjuvant approaches will be?

Adjuvants have always been an essential part of vaccines, and they will remain so. Finding ways to improve mucosal immune responses are important. It would be wonderful if we could design a chemical adjuvant that does what adenovirus does to the immune response, because adenovirus, particularly as a boost after DNA prime, generates a very strong cellular immune response and a potent antibody response. Current adjuvants largely improve antibody responses but don't do a lot for cellular immunity. We're understanding more and more about the toll receptor pathways and about the nature of the specific antigen-presenting cells, so I think there will be opportunities for adjuvant improvement.

You mentioned mucosal immunity, it does seem to be increasingly accepted that HIV is essentially an infection of the gut-associated lymphocytes (GALT). In light of some recent direct corroborating evidence in humans that there is massive depletion of CD4 cells in the gut throughout infection, should more attention be paid to mucosal immunity in general and GALT in particular?

I think it is very important to look at protecting the mucosal compartment. Going back to Ron Veazey's and Ron Desrosier's original paper and, as you've said, the recent papers from Danny Douek and from Marty Markowitz (see *Research Briefs*, page 25), it's clear that the virus takes off in this compartment. It introduces a whole series of questions: What's the best way to protect that compartment? Do you provide higher levels of immunity by generating mucosal immunity? If you do, what kind of immunity do you want? Is a good IgA response protective in the mucosa? Do you want mucosal cellular immune responses? Do you need both? Are you better off generating a good IgG response so that the virus never can make it to the mucosal site? It would help to have better tools to stimulate those mucosal immune responses more selectively and parse out the relevant issues.

More generally, do you think CD4 help will be critical in a vaccine-induced immune response? Everyone's focusing on cytotoxic T lymphocytes (CTLs) at the moment, but increasingly evidence points towards CD4 help in propping up any CTL response.

I've been impressed with the importance of CD4 cells, particularly memory cells. You're quite right to point out that their role in protective immunity is sometimes overlooked. Clearly, one won't be able to generate strong CTL responses without potent CD4 memory responses, and there are other contributions these cells might make. For example, they may elaborate cytokines, like MIP-1 β or RANTES. Getting more specificity on precisely what type of CD4 cells are needed would be even more helpful.

What are our best leads for inducing CD4s as part of a vaccine-induced immune response?

DNA vaccines induce CD4 cells pretty nicely and have some potential. In our VRC004 trial, the responses to the DNA vaccine were more consistent in CD4 cells, and they prime well in animal models for the adenovirus boost, which then brings a more balanced CD4:CD8 ratio. DNA vaccines seem to get around some of the questions that you raised earlier relative to epitope dominance. That's another reason to really drill into the DNA technology and not abandon it prematurely. And there are adjuvants that will induce a CD4 response; we just need to make sure they're Th1-like, not Th2-like. Some of the CpG adjuvants are interesting in that regard.

How do you think we can better proceed with the non-human primate (NHP) model, given the lack of successful protection data in that system? Do you think there's still research value in that model or should it be considered more as a filter when moving towards the clinic?

I wouldn't use the NHP challenge as a filter because we know now that any vaccine that induces a reasonable cellular immune response seems to protect against SHIVs (simian human immunodeficiency viruses). But I do think, as with DNA technology, there's enormous potential to understand and model the disease in the monkey. It's very important to look rigorously at more physiological models, for example the low-dose repetitive challenge model like the one David Watkins described last year. If we can work out those models so that they're consistent and more repre-

sentative of human infection we can better use them to test our candidate vaccines. Even if the animal groups need to be larger to do those studies it will be much more expeditious to address scientific questions in those animal models. The only other option is to do proof-of-concept trials [in humans] that take hundreds of millions of dollars to perform and years to complete.

The Merck Ad-5 [adenovirus serotype 5] vaccine trial has been widely touted as proof-of-concept that will test whether cell-mediated immunity can impart some efficacy as part of an AIDS vaccine. What should the field do if Merck Ad-5 doesn't work?

Keep working! There are a number of assumptions in that trial that are very specific, and if it were to fail the results will inform us about those assumptions. But that trial is powered to look primarily at differences in viral set-point and CD4 numbers, and it's not well-powered to look at acquisition [of infection]. So one possibility is that it might not have an effect on set-point or CD4 counts, and yet it might have an effect on acquisition because they involve very different parts of the infectious life cycle. Another caveat is that there is no envelope component in the Merck vaccine, and envelope offers the opportunity to expand the diversity of the immune response and to generate neutralizing antibody responses.

Another possibility is that we all guessed wrong. Maybe the cellular immune response is helpful but not the response that adenovirus induces. The plan that has been espoused by the HIV Vaccine Enterprise is one that I think we all can agree with conceptually, that we need more human studies, potentially ones that induce alternative immunologic responses. Each trial explores one part of the multi-dimensional immunologic space—if the trial fails, we can exclude that part of the space, but there's a lot of area to explore. We need to make sure that we cover new ground with each trial.

What should be the priorities for the field in the next five years?

I would say Env structure with an eye towards defining a target for broadly neutralizing antibody; definition of immune correlates, and the judicious use of the animal model to define them; advancement of the most promising candidates into proof-of-concept efficacy studies.

What are your hopes for the Global HIV Vaccine Enterprise?

Well, I hope that it provides a common ground for people to come together and become more organized. Hopefully it will allow us to proceed with the highest priority items, scientifically and clinically, and help us to address the most critical needs. Part of the challenge that lies before us is practical: How do we mobilize people for trials? How do we mobilize a diverse group of scientists? The Enterprise really provides a mechanism to catalyze an intellectual and practical response, and to bring much-needed resources to the field.

How can young investigators be best encouraged to enter into HIV research?

Young investigators are critical to the success of the field. If you accept the idea that this disease is not going to disappear overnight, we need to ensure that the right people are involved as the efficacy trials are done, whether it's in five years or in 20 years. Young investigators should recognize that there is no more important problem in biomedical research than this problem, for several reasons. Number one, the scientific questions that underlie it are fascinating and they will uncover basic biology relevant to immunology, virology, genetics and evolution. Secondly, by working on this problem you are contributing to an effort that will have perhaps the greatest impact on human health on this planet. It's important for us to get that message out; this is a unique opportunity to address the scientific challenges and to meet a public health imperative. In an age where scientific trainees are reading in the newspapers about the excitement of stem cell, neurobiology, or genetic research, it's important to understand that the energy and excitement in our field is perhaps even greater. We are in the midst of an incredible renaissance in the field of HIV vaccines. The science and technology that we can now apply to advance the field is unprecedented. ■

colleagues presented a demand forecasting study conducted in Uganda in 2001-2002, using similar techniques to a study conducted in Thailand (*Health Policy* 57, 111, 2001). Both studies communicated concepts of vaccine efficacy and partial effectiveness and then tested respondents' comprehension of these concepts prior to administering the questionnaire.

Participants were asked about willingness to spend from US\$2.86 to US\$286 on a vaccine of either 50% (n=812) or 95% (n = 803) efficacy (defined as protecting that percentage of people against infection). Controlling for household assets, efficacy and price, the study found that higher education level (upper secondary and university) correlated with willingness to purchase a vaccine. One of the striking findings in this study—and in the Thai research—was that demand for 50% and 95% effective vaccines was roughly equivalent.

The researchers also collected data on respondents' risk factors for HIV infection in order to make rough estimates of the effect that a low- or high-efficacy vaccine would have on incidence. Twenty-five percent of men and 10% of women reported a non-spousal sexual partner in the last year, and

only 51% of these individuals reported using condoms with these partners. This relatively low rate of condom use led the authors to conclude that a vaccine of low efficacy would have a limited impact on new infections in the absence of strong education and outreach campaigns for other prevention strategies.

Preparing for “the day after efficacy”

On 12 July, a satellite symposium (www.day-after-efficacy.org/symposium.html) reviewed issues that will arise once an effective vaccine candidate has been identified. Helen Rees (University of Witwatersrand, South Africa) emphasized the need to strengthen regulatory capacity in developing countries and reviewed some of the steps that the Global HIV Vaccine Enterprise might take to help facilitate this process, including sponsoring a workshop of regulators, trial sponsors and key industry partners to discuss risk-benefit evaluation and regulatory decision making as they relate to AIDS vaccine candidates. This would allow the various stakeholders to discuss how an effective candidate with a particular safety and efficacy profile might be viewed in developing versus developed countries.

Viroj Tangcharoensathien (Thai Ministry of Public Health) reviewed a study which explored potential groups to be targeted in Thai vaccination campaigns, categorizing them in terms of the numbers of infections that would be averted and the cost of delivering the vaccine to each group. Sex workers, prisoners, conscripts and male STI patients were all described as populations that could be reached at low cost with high numbers of infections averted (based on 2002 prevalence data), whereas civil servants, police, pregnant women and military personnel could be reached at low cost but with low numbers of infections averted. Tangcharoensathien pointed out that voluntary counselling and testing is a crucial entry point for vaccine studies, and these services should be included in program cost estimates.

Other issues addressed at the symposium included the need to further explore how to deliver vaccines to adolescents and young people so that they are protected before sexual debut, and how to develop pricing structures and purchasing plans that ensure an effective vaccine is available in hard-hit resource-poor settings as soon as it is licensed. ■

Viral diversity, a growing feature of the HIV pandemic

by Sheri Fink

The International AIDS Conference in Bangkok added new layers of subtlety to the understanding of HIV genetic diversity and the pace of its evolution. The picture that emerged was that of an increasingly complex molecular epidemic changing at an even more rapid pace than previously appreciated. At this scientifically challenging time for the vaccine research field, the data presented provided new insights—and raised new questions—about how viral diversity and genetic mutability will impact vaccine design.

Investigators have tracked HIV diversity and geographic distribution, detecting a high prevalence of co-infections in some populations and increasingly identifying unique and circulating recombinants. A consensus in Bangkok was that better tools are needed to pinpoint the causes and effects of viral diversity within communities and individuals, and to better assess the impact of newly-acknowledged phenomena such as fluctuating populations of unique recombinant forms within individuals.

Co-infection can occur either when an uninfected individual is infected simultaneously with more than one genetic form of HIV or when an individual with an established HIV infection becomes infected with a new viral strain; the latter is known as “superinfection”. In either type of dual infection, the various viruses can recombine, leading to the development of unique recombinant forms (URFs) within a single individual, some of which could theoretically be transmitted to many other individuals, becoming circulating recombinant forms (CRFs).

“We need a lot more surveillance of populations, particularly newly-infected people, and with full-length sequencing,” said Chris Beyrer of Johns Hopkins University. “It’s not enough to... do serotyping with a few segments of the genome.”

Understanding co-infection

Some of the most interesting data came from Francine McCutchan

of the Henry M. Jackson Foundation and her colleagues, who have spent many years defining intersubtype HIV-1 genetic diversity on several continents in preparation for vaccine trials. In Bangkok they presented data from the HIV-1 superinfection study (HSIS) that focuses on a cohort of high-risk (initial seroprevalence 68%) female bar-workers in Mbeya, Tanzania, where subtypes A, C and D co-circulate. Investigators hypothesized that some of the women would be infected with more than one viral subtype.

To detect intersubtype recombinants, McCutchan's group developed a multi-region hybridization assay (MHA) (*AIDS* **16**, 2055, 2002) using real-time PCR to screen DNA samples across five genomic regions (short regions of *gag*, *pol*, *vpu*, and *env*) with probes specific for clades A, C and D; this specific assay is named MHAacd, and the group has developed similar genotyping screens for use in other geographic regions like South America and Asia. These "high throughput" assays offer a way to identify intersubtype recombinants more rapidly and less expensively than with full genome sequencing, the gold standard technique, and also have the power to identify dual infections.


In the Tanzania study, peripheral blood mononuclear cells (PBMCs) collected from a random sample of 98 HIV-infected women from the high-risk cohort were tested with the MHAacd assay every three months for a period of four to eight visits. Seventy five percent of the women had an identical HIV genotype every time they were screened, with a single probe hybridizing to each genome region, suggesting infection with a single HIV subtype—38% had only subtype C infection, 16% only subtype A, 3% only subtype D, and 43% a variety of single recombinant forms. However, 25% of the women appeared to be infected with more than one HIV strain, evident from either dual probe reactivity in a given genome region or a shift in subtype over time. Those with dual infections had A+C (37%), A+D (15%), C+D (4%), or A+C+D (44%) genotypes, which were interpreted as representing infection with more than one subtype and/or recombinant strain.

Focusing on women with evidence of dual infection, the research group has used cloning and partial sequencing to more fully characterize HIV strains and to quantify the relative proportion of these strains over time. McCutchan described four cases in which different genetic forms of HIV fluctuated dra-

matically over time in PBMCs from women infected with more than one subtype, based on evidence from at least 20 *gag* clones, with different genetic forms apparent, then absent, then reappearing. "People who are co-infected are the source of many, many recombinant forms that presumably they can pass along. And they're continuously generated in those people over time" concluded McCutchan. As for the fluctuation of certain recombinants, she said "you can postulate of course that the immune response is suppressing some strains... there can also be a kind of chance element; strains lying around in memory T cells [may re-emerge] when those cells become reactivated by encountering their antigen."

There are also new efforts to try to better understand the characteristics that make certain genetic forms of HIV more likely to be transmitted. In a recent small study (*Science*, **303**, 2019, 2004), Eric Hunter (now at Emory University, Atlanta) and colleagues found that individuals newly infected with HIV had viruses whose envelope neutralizing epitopes appeared to be more exposed, rendering them more neutralization sensitive than most of the viral forms present in their infecting sexual partners. The implication of this work may be that there is some particular characteristic of a transmitted virus that enables it to establish a new infection.


Possibly related to such a phenomenon, issues of comparative viral fitness were also discussed at Bangkok. Eric Arts of Case Western University and colleagues at Antwerp's Institute of Tropical Medicine and the Cleveland Clinic (Abstract number MoOrA1012) reported infecting PBMCs with pairs of viruses and allowing them to compete. In all the researchers compared 11 group M isolates (subtypes A, B, C, D and CRFO1), 5 group O isolates, and 6 HIV-2 isolates obtained late in disease progression. These *in vitro* results, some of which were reported in 2003 (*J. Virol.* **77**, 1021, 2003; *AIDS* **17**, 780, 2003) showed that relative fitness in the competition assay—group M > HIV-2 > O—matched relative worldwide prevalence. But the researchers found that within group M the least fit subtype was C, a major pandemic strain now responsible for roughly 50% of worldwide infections. Although data from an *in vitro* system must be extrapolated to real world scenarios with caution, the investigators are now testing the hypothesis that "lower replicative capacity...




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Francine McCutchan





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Todd Allen

leads to slower disease progression, longer times for possible exposures and transmission events," and thus a higher "transmission fitness."

Another factor—immune pressure on an individual and population level—also influences which viruses circulate. At a Bangkok symposium on HIV vaccine design, Simon Mallal of Murdoch University, Australia, argued that vaccine design should take into account not only viral diversity but also the HLA (human leukocyte antigen) diversity of a population (ThSy274); his work has shown that HLA, a genetically-determined component of each individual's immune response, exerts selective pressure on HIV in a manner analogous to antiretrovirals. During chronic infection, people with certain alleles of the 1300 HLA types develop HIV escape mutants; for example, HLAB*5701 and 5801 are strongly associated with the Gag T242N mutation. Mallal has been using information about HLA and the epitopes that stimulate the strongest protective immune response to try to design vaccine immunogens that will avoid falling prey to virus escape mutations.

Superinfection

McCutchan's presentation of the HSIS study also touched on an issue that was a buzzword at the Barcelona AIDS conference two years ago—superinfection. In one case of superinfection from the HSIS Tanzania cohort, a patient who was HIV uninfected at the beginning of the study was found to have seroconverted at three months and had evidence of infection with a second strain at nine months. "Because we had serial samples, we were able to make strain-specific nested PCR and go back to the earliest samples and show by our most sensitive technique the second strain wasn't present either in plasma or PBMCs... Nested PCR is supposed to detect anything. The evidence [for superinfection] is very strong and very convincing."

Still, McCutchan stresses that moving beyond case studies is crucial. "Understanding dual infection in high-risk populations is really a key theme that's coming to the forefront. Our own work in East Africa is part of the picture, but this kind of work needs to be repeated in high-risk populations, to identify how easy it is to get infected with a second strain and what the consequences are." McCutchan and her collaborators in Germany are studying the

immunological profiles of the Tanzania cohort. "We have to be married up with strong immunology to determine what the CTL and the neutralizing antibody responses are in individuals who get co-infected versus those who do not in a population with similar risk."

Several other presentations addressed the superinfection issue but key questions remain unanswered; how common is superinfection as opposed to simultaneous co-infection, and what implications, if any, does the phenomenon have for vaccine-induced immune responses?

Davey Smith of the University of California at San Diego offered three case descriptions of superinfection occurring an estimated five to thirteen months after initial HIV infection (TuOrB1140). After infection with a second HIV strain each patient experienced an increase in viral load and a decrease in CD4⁺ T-cell counts, suggesting to Smith that superinfection can "negatively impact individual clinical course."

Todd Allen of Massachusetts General Hospital co-chaired the superinfection forum, which also featured a presentation by Tuofu Zhu of University of Washington on long-term HIV-exposed seronegative individuals who ultimately seroconverted. "They had HIV positive partners and weren't infected by them, but by someone with a more distantly related [virus]," said Allen. "It's pretty good suggestive evidence that there may be some component to an immune response that is protective in these exposed uninfected individuals...[The researchers] used the virus as a tool to measure whether or not there may be some truth to the idea that a component of the cellular immune response protected."

Across the globe, an increasingly diverse pandemic

The consensus at Bangkok, with reports based on near full-length genome sequencing coming from disparate locales like west Africa, the Myanmar/China border region, Thailand, and South America, was that recombination is on a marked upswing, and not only because better tools are available to assess it. Chris Beyrer co-chaired one of the oral presentation panels: "It was very striking... viral diversity is increasing about as quickly as people are able to measure it."

On a population level, groups from around the world reported finding new HIV recombinant forms in increasing prevalence, partic-

ularly among highly-exposed populations or in areas considered epicenters of the HIV pandemic. Some groups applied sequencing and phylogenetic analyses to preserved serum samples, finding evidence of recombinants stretching back for decades. For example, samples collected in Kinshasa in 1984 and 1986 were analyzed by Marcia Kalish and colleagues at the US Centers for Disease Control, the National Institutes for Health, and Project SIDA in Kinshasa (ThOrC1362), in a recently published study (*Emerg. Infect. Dis.* **10**, 1227, 2004). Analyzing just 10% of the HIV genome yielded evidence for at least 37% intersubtype recombinant viruses, “likely a low estimate,” according to Kalish. “Virtually all the HIV-1 subtypes [with high intrasubtype diversity] and unclassifiable gene regions were identified.” In contrast to these central African samples, HIV samples collected in 1986 from Burkina Faso in west Africa showed much lower diversity, where almost 95% of circulating strains comprised two recombinant forms, CRF02 and CRF06, with a low intra-CRF genetic diversity, suggesting that the HIV epidemic was introduced there at a later time by a limited number of founder viruses.

Several investigators used the real-time generation of new forms to identify dynamic epidemics and to connect and relate epidemics in different geographical locations. There was a strong emphasis in Bangkok on characterizing emerging and established epidemics in the region. Here and also in other regions, molecular tools are painting an alarming portrait of border-crossing epidemics driven by injection drug use and sex trafficking—activities that many governments have been slow to address.

Sequencing viral genomes in seven countries across South America, Jean Carr of the US Military HIV Research Program and South American colleagues found a significant proportion of diverse BF recombinant viruses in an emerging epidemic among female commercial sex workers in the Southern Cone of the continent (ThOrC1365). Yutaka Takebe and colleagues from Japan’s National Institute of Infectious Diseases (ThOrC1364) studied viral strains from injection drug users in Central Myanmar and western Yunnan, China, finding these areas to be “geographical hotspots of extensive recombination.” Fine mapping of recombination breakpoints

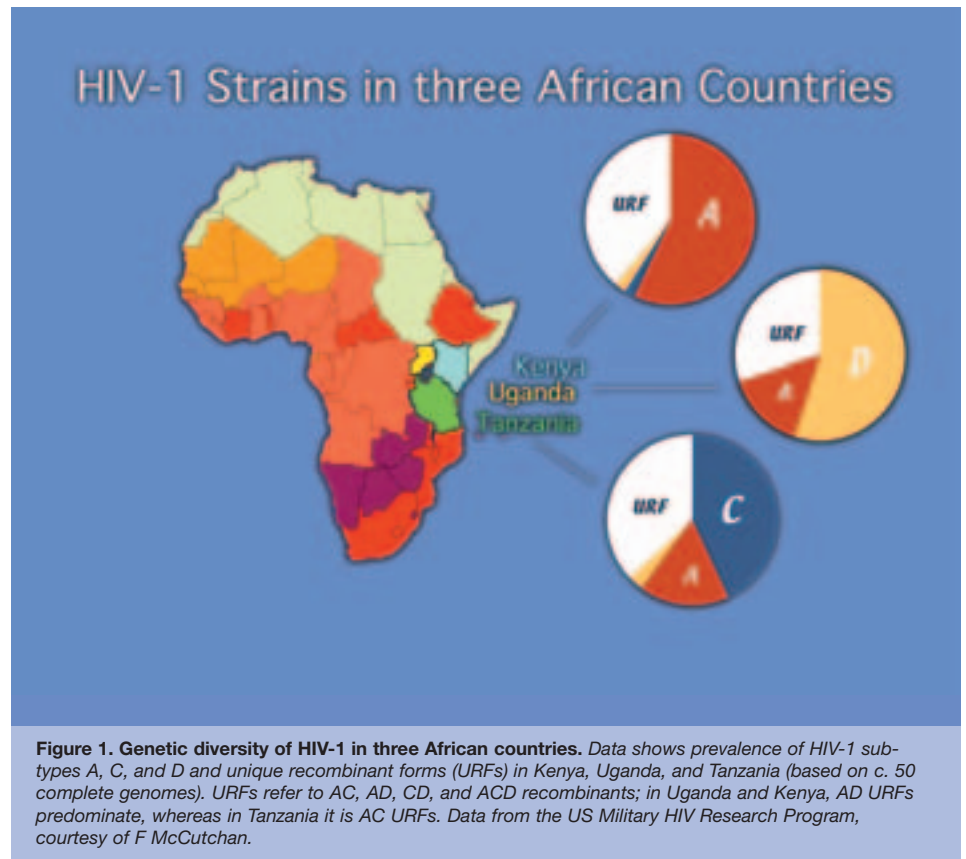


Figure 1. Genetic diversity of HIV-1 in three African countries. Data shows prevalence of HIV-1 subtypes A, C, and D and unique recombinant forms (URFs) in Kenya, Uganda, and Tanzania (based on c. 50 complete genomes). URFs refer to AC, AD, CD, and ACD recombinants; in Uganda and Kenya, AD URFs predominate, whereas in Tanzania it is AC URFs. Data from the US Military HIV Research Program, courtesy of F McCutchan.

revealed that some Myanmar URFs shared precise breakpoints with CRFs circulating in Yunnan, China, suggesting a link in the epidemics and the possibility of common ancestors. “Connecting the epidemics in Burma and China is news,” says McCutchan. Takebe drew lessons for vaccine researchers. In an email, he wrote: “Our study suggests that the mixing of different lineages of HIV-1 strains in highly exposed populations and in the social networks... could quickly lead to the evolution of new forms of recombinants [which could], for instance, acquire new sets of ‘escape’ mutations in viral epitopes by shuffling different parts of the genome and complicate the development of effective HIV vaccines.”

Looking to the future

Presenters seemed to agree that increasingly there is even more viral diversity than previously appreciated. “On at least three continents there is good evidence of recombination between subtypes, generating both CRFs but also a host of novel one-off recombinants really found only in individual patients [URFs],” says Beyrer. “What’s driving [this increased diversity] and where it’s going is hard to say.”

It remains for researchers to sort out the

implications of HIV diversity for vaccine design. “What may be more worrisome is not that recombination is happening and circulating recombinant forms are generated, but rather the increasing level of these novel recombinants [URFs], reaching levels of 30 or 40% of new infections, suggesting a tremendous amount of viral diversity particularly in east Africa,” says Beyrer. “It gets worrisome if you agree that what we need is a vaccine developed not on a single variant but a consensus sequence. That gets tough if there are multiple recombinants and there really isn’t a consensus sequence. One approach is to look for ancestral sequences.” This approach has been promoted by researchers such as Beatrice Hahn and Bette Korber. Other ideas are to consider using multi-epitope constructs or multivalent vaccines.

The high level of HIV diversity is a fact, and one that experts are viewing as a huge challenge to vaccine development but also in some respects as an opportunity. Techniques to study the molecular diversity of HIV, such as the McCutchan group’s multi-region hybridization assays, have been developed specifically to aid researchers preparing for vaccine trials. “This is an opening field, at its birth,” she says.

Even superinfection may offer more than negative implications for the vaccine research field. “It presents an excellent opportunity to try to address the ability of existing immune responses to cross-react against the virus,” says Allen. “A lot of the initial negativity over the superinfection studies was that people were starting to extrapolate this to vaccine design. But it wasn’t a fair parallel to draw because these patients are immunocompromised so we don’t know what accounts for the lack of

protection. Our understanding of superinfection is still quite limited, but it does provide a unique opportunity to look at the ability of primed T cells to cross-protect *ex vivo*. How well are these T cells able to recognize a second clade B or C virus?”

McCutchan echoes the idea that vaccine researchers should use dual infection to their advantage. “It’s the only *in vivo* system I know of to determine whether the immune responses generated by natural infection have the breadth of protection that

we’re looking for in a vaccine,” she says. “It’s an opportunity. I’m going to stay tuned. I hope you will, too.”

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

Vaccine science in Bangkok

by Richard Jefferys

Over the past several years, there has been a sense that both the number and overall quality of the basic science presentations at the International AIDS Conference has declined. As Robert Steinbrook wrote in a post-conference review in the *New England Journal of Medicine*, “it is no longer the cutting-edge scientific meeting that it once was.” The throng of attendees that braved the sweltering humidity of Bangkok in July faced an additional problem: the US Department of Health & Human Services limited the number of US government-sponsored researchers to just fifty, resulting in withdrawn abstracts, speaker changes and blank walls where scientific poster presentations were intended to hang. Nevertheless, there were still some interesting, thought-provoking presentations on vaccine-related science.

Enhancing DNA vaccines

After an initial wave of excitement generated by studies in small animal models, DNA vaccines have hit something of a wall due to poor immunogenicity results in humans and researchers are now exploring strategies to overcome this problem. H Li from Yi-Ming Shao’s group at the National Center for AIDS/STD Control and Prevention in China described their recent work in this area. Li began by noting that DNA vaccines have to gain access to the nucleus so that the DNA can be transcribed and translated into protein antigens that can then trigger an immune response. There are two important barriers the DNA vaccine must pass through: the outer plasma membrane and the nuclear envelope. Li reviewed published work demonstrating that a short (72 base-pair) sequence of DNA from simian virus 40 (SV40) encodes a signal that promotes nuclear import (*Exp. Cell. Res.* **233**, 713, 1999). Li and colleagues decided to exploit this finding by incorporating the SV40 sequence into a DNA vaccine construct encoding the Gag protein from HIV. They then conducted studies in mice to directly compare the immunogenicity of the original and modified DNA constructs. Animals were immunized at weeks 0, 2, 4 and 6 and responses to the HIV Gag protein evaluated at week 8.

Li showed that both antibody titers and CD8⁺ T cell responses,

measured by both ELISPOT and intracellular cytokine staining (ICS) for interferon (IFN)- γ production, were approximately two-fold higher in mice that received the DNA vaccine containing the SV40 sequence compared to those that received the original construct. While it certainly cannot be assumed that this apparent enhancement of immunogenicity in mice will be mirrored in humans, Li and colleagues are sufficiently encouraged by the data to move the construct into human testing.

A collaborative effort between the research groups of George Pavlakis and Barbara Felber at the US National Cancer Institute is also evaluating novel methods for improving DNA vaccines. Pavlakis presented new data from studies of DNA vaccines that encode the simian immunodeficiency virus (SIV) proteins Gag and Env fused to genes with potential adjuvant effects. One set of constructs (the researchers have created one DNA vaccine for each protein) fuses the SIV protein to the chemokine monocyte chemoattractant protein-3 (MCP-3), to try to improve the secretion of the proteins from cells that take up the DNA vaccine; MCP-3 is able to exit cells particularly easily, using a route called the secretory pathway. The second set of constructs fuses the SIV protein to a fragment of β -catenin which chaperones the proteins out of the cell via a different route called the proteasomal degradation pathway.

Pavlakis briefly reviewed a preventive vaccine study in juvenile macaques that was presented at the Keystone conference earlier this year. Four groups of four animals each were assigned to receive DNA constructs containing: (a) Gag and Env alone; (b) Gag and Env alone + Gag and Env fused to β -catenin; (c) Gag and Env alone + Gag and Env fused to β -catenin + Gag and Env fused to MCP-3; (d) no immunization (controls).

Immunizations were given at weeks 0, 4, 12, 24 and 48, followed by a challenge with the highly pathogenic SIVmac251 at week 54. Surprisingly, given the virulence of the challenge virus and the fact that only DNA vaccines were used, animals in group (c) had viral loads that were statistically significantly lower than controls during both the acute and chronic phases of the infection. Pavlakis next unveiled results from two new studies involving the combination of

both fusion constructs. The first was conducted in collaboration with Marta Marthas (University of California at Davis) using a macaque model of breastfeeding transmission (see *IAVI Report* 5(8), 2001, p4 for background on this model). Neonatal macaques were immunized at weeks 0, 2 & 3 and challenged orally with SIVmac251 (in milk, twice a day for 5 days) beginning at week 4. Six of eight vaccinated animals and seven of eight non-vaccinated controls became infected (a non-significant difference), but the post-infection viral loads in vaccinated animals were significantly lower compared to controls ($p=0.029$).

Lastly, Pavlakis offered a glimpse of some intriguing results from a study aimed at exploring the therapeutic potential of the DNA vaccines. Thirty one macaques that had been infected with SIVmac251 for 15-70 weeks were treated with combination antiretrovirals (ARVs; ddI, d4T & PMPA) for approximately 20 weeks. Fifteen animals were immunized with the DNA constructs at weeks 8, 12 & 16 of the on-therapy period. At week 20, ARVs were stopped in all macaques. In the group that did not receive vaccines, viral loads quickly returned to baseline levels. Among the immunized group, however, five animals were able to control SIV viremia to below the levels of detection after an initial rebound. Six additional macaques displayed viral loads that were 1-2 logs below baseline values. Viral loads in the remaining vaccinated animals were indistinguishable from controls. While interesting, there are some important caveats to the results. Due to high costs and a shortage of macaques, animals were enrolled as they became available from other studies. Furthermore, a subgroup of both the vaccinees and controls had received DNA immunizations prior to infection with SIVmac251 (although separate analyses suggested that this did not bias the overall outcome). The data does at least suggest that further studies of the approach may be warranted. In a follow-up interview, Pavlakis stated that "we hope these results will stimulate interest in human trials using DNA vaccination, which appears to be very safe and—unlike some vector approaches—can be administered many times." Pavlakis acknowledged that recent disappointments have cast a pall over the DNA approach, but argued that "it's too early to abandon DNA vaccines for AIDS." The two NCI groups currently lack support for GMP manufacturing, but they hope to advance some of these projects through a collaborative agreement with Wyeth Vaccines. In the immediate future, further macaque studies are planned in collaboration with David Weiner at the University of Pennsylvania.

Phase I vaccine studies

Bangkok saw the presentation of the first human immunogenicity data from two Phase I studies of new AIDS vaccine candidates. Guiseppe Pantaleo of the Laboratory of AIDS Immunopathogenesis, Lausanne and the EuroVacc consortium

described results from a trial of a NYVAC vector, an attenuated vaccinia virus, encoding a Gag-Pol-Nef polyprotein and the Env protein from a Chinese clade C virus. Twenty-four individuals with a low risk of HIV infection (11 women and 13 men) were recruited at sites in London, UK and Lausanne, Switzerland. Immunizations were given intramuscularly at weeks 0 & 4; four participants were given placebo while the remaining 20 received NYVAC. Pantaleo reported that there were no serious adverse events. CD8⁺ T-cell responses were evaluated at week 6 using IFN- γ ELISPOT and the cut-off for a positive response was defined using the same criteria employed by Merck in their recent vaccine studies: >55 spot-forming cells (SFC) per million PBMCs (peripheral blood mononuclear cells) and at least 4-fold greater than the background number of SFC; assays with a background of >50 SFC/million PBMC were considered invalid. Approximately 45% of participants displayed a positive ELISPOT response, mainly targeted at the Env protein. Responses to


Gag and Nef were less common, and no Pol-specific CD8⁺ T cells were detected. Follow-up is ongoing, with further immunogenicity evaluations planned for the week 8, 24 and 48 timepoints to assess the degree of long term T-cell memory induced by the vaccine. EuroVacc is conducting similar studies with additional vectors (including MVA) containing the same insert in order to compare immunogenicity and thus prioritize which candidates will be advanced into Phase II testing.

Representatives from the German company Bavarian Nordic presented preliminary data from an ongoing study of their MVA vector encoding the Nef protein in HIV uninfected volunteers (a therapeutic study was published last year; *Vaccine* 22, 21, 2003). Fourteen individuals are enrolled, and immunogenicity data was available for eight of them. Immunizations were given subcutaneously at weeks 0, 4 & 16. T-cell responses were evaluated by IFN- γ ELISPOT using overlapping pep-


tides, short peptides corresponding to optimal epitopes, the Nef protein and the MVA vector. All eight participants evaluated so far have developed MVA-specific T cell responses, with a median frequency of 122 SFC/million PBMC at week 6. Six participants have been followed out to week 18; median MVA-specific responses were 177 SFC/million PBMC at this timepoint. In contrast, however, only 3/6 showed any evidence of Nef-specific T-cell responses and at a very low borderline level of 17 SFC/million PBMC (range 7-49). These data, while preliminary, suggest that responses to MVA-based vaccines may skew toward the vector itself rather than the encoded HIV antigen. Studies using MVA vectors encoding a broader array of HIV antigens should help discern whether this is a generalized problem or specific to the Nef protein.

Immunology

The vast majority of AIDS vaccine candidates currently in human trials aim to induce HIV-specific CD4⁺ and CD8⁺ T cell responses.



***It's too
early to
abandon
DNA
vaccines
for AIDS***



George Pavlakis

The degree to which such responses may benefit an uninfected individual upon subsequent exposure to HIV remains a critically important question, which can only be answered definitively by efficacy trials of T cell-based vaccines. In the meantime, however, investigators continue to try and understand the role of T cells in controlling HIV replication in infected individuals. In Bangkok, a session on immunology featured reviews of current knowledge regarding HIV-specific T cell responses. Guiseppe Pantaleo addressed CD4⁺ T cells and Juliana McElrath (University of Washington) discussed CD8⁺ T cells.

Pantaleo began his talk by reviewing previously presented work by Alexandre Harari from his group regarding the potential importance of interleukin (IL)-2-producing HIV-specific CD4⁺ T cells in controlling viremia (see *IWI Report*, 8(1), 2004). Pantaleo then described a new study that used ICS to analyze the functional profile of tetanus toxoid (TT)-specific CD4⁺ T cells in individuals with a history of prior TT immunization, both before and after receipt of a TT booster. The take-home message of Pantaleo's presentation was that the cytokine profile of TT-specific CD4⁺ T cells changes in response to stimulation with the TT booster. Prior to boosting, TT-specific CD4⁺ T cells primarily produced IL-2, indicative of a long-term memory population. In the weeks immediately after boosting, the TT-specific CD4⁺ T-cell population expanded and comprised a mixed population of cells secreting either IL-2 alone, IL-2 + IFN- γ or IFN- γ alone. Pantaleo noted that the population secreting IFN- γ alone were likely to be short-lived effector cells generated from the long term memory CD4⁺ T cells that were present prior to boosting. In support of this hypothesis, he showed that by day 60 after the booster, the TT-specific CD4⁺ T cell population had contracted and was again almost entirely made up of cells that secreted IL-2 alone. Some researchers have argued that the functional profile of responding T cells differs based on the particular pathogen that the cells are targeting; Pantaleo argued that the profile largely depends on how recently the T cells have been stimulated by antigen. For analysis of vaccine-induced CD4⁺ T cell responses, Pantaleo suggested that it will be critical to quantify not just IFN- γ production but also IL-2 since this cytokine appears to be a sig-


nature of long term immunological memory (shortly after the conference, Pantaleo and Rick Koup from the US Vaccine Research Center published a Commentary addressing this issue; *Nat. Med.* **10**, 806, 2004).

McElrath highlighted some of the reasons why CD8⁺ T cells (cytotoxic T-lymphocytes or CTLs) typically fail to control HIV replication in infected individuals. First she discussed the impact of immune escape, wherein regions of HIV targeted by CTLs mutate to avoid recognition. McElrath's group studied the evolution of the HIV-specific CTL response in 21 seroconverters and (in collaboration with Jim Mullins) conducted full length sequencing of the HIV genome at multiple timepoints post-infection to look for evidence of CTL escape mutations. The first HIV proteins to be recognized by CTLs were Tat and Vpr, and epitopes within these proteins were also the first to show evidence of CTL escape mutations. McElrath pointed out that studies in macaques have suggested that the efficiency of CTL recognition of a particular epitope (the technical term is functional avidity) may be correlated with the incidence of escape mutations, but so far she has been unable to confirm these observations. In McElrath's study, escape occurred in epitopes within all viral proteins and appeared unrelated to the magnitude or functional avidity of the CTL response.

She went on to discuss the possible role of CTL dysfunction in HIV infection. Following up on published work by Mark Connors (*Nat. Immunol.* **3**, 1061, 2002), McElrath has begun to investigate whether the ability of CTLs to proliferate in response to HIV antigens is correlated with control of viremia. In the two seroconverters studied to date, HIV-specific CTLs appeared to maintain their proliferative capacity for the first few months post infection, but this functional property was lost over time. McElrath hypothesized that the apparent loss of CTL function may relate to the rapid diminution of HIV-specific CD4⁺ T-cell activity that typically occurs in acute HIV infection. Her preliminary finding was echoed in a late-breaker presentation by Marcus Altfeld from Bruce Walker's group in Boston; in a study of 18 acutely infected individuals, he also found that HIV-specific CTLs initially possessed the ability to proliferate but that the function was lost over time. Supporting McElrath's hypothesis,

Altfeld also reported that the addition of autologous CD4⁺ T cells isolated from an early timepoint in infection restored the proliferative capacity of the dysfunctional HIV-specific CTLs while, conversely, depletion of CD4⁺ T cells almost totally abrogated the proliferation. As McElrath explained at the close of her presentation, these data suggest that the functional properties of HIV-specific T cells may be an important correlate of the immune system's ability to control viral replication, but the critical question is whether the pre-exposure induction of T-cell responses by vaccination will allow seemingly important functions (such as proliferation and IL-2 production) to be better maintained if an individual becomes HIV-infected.

Conclusion

The next International AIDS Conference will take place in 2006 in Toronto (www.aids2006.org). While it's likely that the meeting will continue to have to juggle the many different topic areas relevant to the global AIDS pandemic, vaccine science and policy will undoubtedly remain on the agenda. The conference co-chairs are Helene Gayle, recently appointed president of the International AIDS Society, and former IAS president Mark Wainberg, both of whom have a strong interest in accelerating the progress of AIDS vaccine research. 

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

Topical CCR5 inhibitor prevents vaginal transmission of SHIV

HIV is primarily transmitted across mucosal barriers, so understanding the earliest events of infection and how the virus breaches these barriers are essential to microbicide development. HIV uses the cell surface chemokine receptor CCR5 as a coreceptor to enter target cells, and individuals who have a deletion in their CCR5 receptors rarely become infected with HIV. Furthermore, CCR5-tropic (R5) viruses predominate in the early stages of infection, so CCR5 would seem to be a promising target for a microbicide.

RANTES is a chemokine that prevents HIV infection *in vitro* by attaching to the CCR5 receptor on the surface of susceptible cells. Chemically-modified versions of RANTES have been designed to increase its ability to block HIV infection. In a recent study (*Science* **306**, 485, 2004), Michael M. Lederman from Case Western Reserve University and colleagues treated female macaques with a topical vaginal application of PSC-RANTES, one such analog that has *in vitro* inhibitory concentrations for some HIV isolates in the picomolar range.

Thirty animals were treated with different concentrations of PSC-RANTES or with saline and then challenged vaginally with a virulent form of the chimeric simian/human immunodeficiency virus (SHIV) SF162 that has an R5 HIV envelope. Animals were followed for up to 24 weeks for signs of infection. At the highest dose (1mM) of PSC-RANTES all five animals were protected from SHIV infection; 330 μ M protected 4 out of 5 macaques and 100 μ M protected 3 of 5 animals. There was a statistically significant dose-effect relationship when they analyzed the whole range of concentrations.

The results are encouraging but there are still some hurdles ahead. The 1 mM concentration is several orders of magnitude higher than the subnanomolar used to inhibit SHIV SF162 *in vitro*. The authors speculate that this dose disparity might be simply due to the high dose of SHIV used *in vivo* to ensure that all control animals became infected. Also, it is still not known if HIV crosses the mucosa as free virus or as cell-associated virus; if the latter, incoming virus may be protected from any CCR5 inhibitor. Lederman told the *San Francisco Chronicle* that PSC-RANTES could be tested in humans in about a year, to assess safety and ensure that it does not cause inflammation.

Is HIV a main player in the gut?

Experiments in monkeys reported several years ago (*Science* **280**, 427, 1998) showed that simian immunodeficiency virus (SIV) initially depletes CCR5⁺ CD4⁺ T cells in the intestine within days of infection, long before targeting the peripheral lymphoid tissue, thereby demonstrating that the gastrointestinal (GI) tract appears to be a major target for SIV. In part because these types of experiments in gut-associated lymphoid tissue (GALT) are invasive and difficult to perform in humans, CD4⁺ T cell levels are usually measured in human peripheral blood to assess disease progression. Peripheral blood CD4⁺ T cells are not significantly depleted until months or even years after infection.

Two papers published in September have now shown that depletion of CCR5⁺ CD4⁺ T cells occurs in the GALT of HIV-infected humans throughout infection. Martin Markowitz and colleagues from the Aaron Diamond AIDS Research Center studied 13 subjects identified at very early infection by taking endoscopic biopsies from the rectum, and found that significant mucosal CD4⁺ T cell depletion occurs in primary infection (*J. Exp. Med.* **200**, 761, 2004). The depletion was not as severe as that seen previously in macaques, probably because the rectum has immune inductive sites such as Peyer's patches and lymphoid follicles containing naïve, resting CD4⁺ and also CD8⁺ T cells. *In situ* hybridization studies localized HIV RNA in the inductive compartment of the mucosa and not in the effector lymphoid tissue (diffuse lamina propria). This was consistent with

the SIV experiments although here HIV RNA could be found very early (7-14 days) in the infection. The authors assumed that the target CD4⁺ T cells were already depleted in human effector tissue by the time biopsies were taken. The authors also found that although peripheral blood CD4⁺ T cell levels could be recovered with suppressive antiretroviral therapy, even after five years there was still a significant depletion of this cell population in the GI mucosa.

Daniel Douek and colleagues at the Vaccine Research Center performed similar studies in a group of 14 HIV-infected therapy-naïve individuals (as well as seven noninfected persons). They also found that the GI tract has the most substantial CD4⁺ T cell depletion at all stages of HIV disease and that the depletion occurs preferentially within the subset of cells also expressing CCR5 (*J. Exp. Med.* **200**, 749, 2004). These account for the majority of GI tract CD4⁺ T cells.

The implications of these findings are several. The confirmation in HIV-infected patients of acute depletion of activated CCR5⁺ CD4⁺ T cells in the gut mucosa as previously seen in monkeys strongly suggests that these are HIV-infected cells killed directly by the virus, and debunks once again the idea of early HIV disease being latent. The consequences of this early loss of mucosal immune cells are unclear since opportunistic infections do not usually appear until peripheral CD4⁺ T cells fall significantly. Since the mucosal CD4⁺ T cells seem to be perhaps the earliest targets of HIV, an AIDS vaccine that induces mucosal immunity might be more effective than one that elicits only relatively strong systemic immunity.

Cellular enzyme crucial to HIV replication discovered

A single HIV RNA transcript, whether spliced or unspliced, must make its way from the cell's nucleus to the cytoplasm to function as full length genomic RNA or to be translated into the various structural and accessory proteins. Fully spliced cellular RNA has no nuclear restriction, but unspliced or partially spliced eukaryotic RNAs are normally retained in the nucleus. HIV mRNAs containing functional introns and HIV genomic RNA must be exported to the cytoplasm for the expression of many viral proteins, and all lentiviruses encode the Rev protein that is essential for post-transcriptional transport of the unspliced and incompletely spliced viral mRNAs from nucleus to cytoplasm. Rev functions by binding to an RNA structural element known as the Rev responsive element (RRE). HIV also makes use of CRM1, a cellular nuclear export receptor protein that directly interacts with Rev, and uses a cellular nucleus-cytoplasm shuttle pathway to export HIV mRNA transcripts. This pathway is different from the pathway used by fully spliced HIV RNAs and cellular mRNAs: the inhibition of one does not affect the export of RNA by the other. Kuan-Teh

Jeang and colleagues now report (*Cell* 119, 381, 2004) that an additional cellular protein, DDX3, is required in the Rev/RRE export pathway.

The researchers were initially studying cellular factors induced by Tat, which may be involved in viral transcription or post-transcription steps, when they found that HIV Tat upregulated DDX3, an RNA-dependent helicase that unwinds RNA molecules.

Using several functional and mutagenesis assays they came to the conclusion that DDX3 is important and limiting for HIV Rev/RRE/CRM1 function. In parallel experiments they found that DDX3 had no effect on constitutive transporter element-dependent expression used by simpler retroviruses, such as the type-D Mason-Pfizer monkey virus (MPMV), to solve the nuclear export problem.

How exactly the DDX3 helicase works with Rev and CRM1 is not yet understood, although one possibility is the existence of a Rev/RNA/CRM1/DDX3 complex. The discovery of this new player in HIV transcription has opened a potential new target for small molecule inhibitors of RNA helicases. However, there is the possibility that complete inhibition of DDX3 could abrogate an essential cellular function. On the positive side, targeting a cellular protein would avoid the almost certain problem of resistance caused by rapid viral mutations.

Vaccine-induced CD4⁺ T cells restore HIV-specific CD8⁺ T cell proliferation

HIV-specific CD8⁺ T cell activity is a major component of the host immune response associated with the control of virus replication following primary, acute HIV-1 infection. There is a temporal association of the HIV-specific cellular immune response and initial control of viremia after primary infection. This is in contrast to the chronic phase of infection when strong polyclonal HIV-specific CD8⁺ T cell responses are broadly diversified, but there is no correlation to viral load and untreated patients are unable to contain HIV disease. Researchers have not been able to dissect the functional and phenotypic parameters of this failure using current antigen-specific interferon- γ T-cell assays.

In a new report by Lichterfeld and collaborators (*J. Exp. Med.* 200, 701, 2004), researchers have been able to study this defect in more detail and taken steps to rectify it. They first determined the fate of HIV-specific CD4⁺ and CD8⁺ T cell lymphoproliferative responses mounted during primary HIV infection, by following the evolution of these responses *ex vivo*. As found previously, these data indicate a parallel evolution of lymphoproliferative HIV-specific CD8⁺ and CD4⁺ T cell responses. *Ex vivo* proliferation of HIV-specific CD8⁺ cells critically depended on interleukin (IL)-2. Neutralization of IL-2 by IL-2-specific mAbs resulted in an almost complete abrogation of *ex vivo* proliferative activities of HIV-specific CD8⁺ T cells obtained from patients with acute infection.

Furthermore, HIV-specific CD8⁺ T cells in PBMC samples were stimulated with HIV-specific CD8⁺ T cell epitopic peptides in the presence or absence of a simultaneous stimulation of HIV-specific CD4⁺ T cells with peptides that, when used alone, elicited a

selective CD4⁺ T cell-mediated lymphoproliferative response. They found that the enhancement of the *ex vivo* proliferation of HIV-specific CD8⁺ T cells by synchronized stimulation of HIV-specific CD4⁺ T cells was almost entirely blocked by adding IL-2-neutralizing antibodies. Therefore, antigen-specific lymphoproliferative CD4⁺ T cell responses significantly enhance the *ex vivo* proliferative activity of HIV-specific CD8⁺ T cells in an IL-2-dependent fashion.

They also found that the CD4⁺ T cells harvested during acute infection can rescue the *ex vivo* proliferative capacity of autologous HIV-specific CD8⁺ T cells isolated in chronic infection. This was also an IL-2-dependent mechanism because the enhancement of HIV-specific CD8⁺ T-cell lymphoproliferative responses by those CD4⁺ T cells was almost entirely abrogated by IL-2-neutralizing antibodies.

Finally, they demonstrated *in vivo* restoration of the proliferative activity of HIV-specific CD8⁺ T cells by vaccine-induced, IL-2-secreting, HIV-specific CD4⁺ T cells. Patients had been immunized in a previous Phase II trial with Env-depleted inactivated HIV particles. After immunization, HIV-specific CD4⁺ T cells in vaccinees, but not in control individuals, developed strong proliferative capacities. Also, strong lymphoproliferative activities were observed in HIV-specific CD8⁺ T cells from vaccine recipients, but not in control individuals. These data indicated that the *in vivo* augmentation of virus-specific CD4⁺ T cell responses can lead to the reconstitution of HIV-specific CD8⁺ T cell lymphoproliferative immune responses *ex vivo*. Although these results confirm the *ex vivo* experiments outlined above, ultimately the success of a therapeutic vaccine such as this one will require evidence of attenuated HIV replication or disease progression.

Disappointing results from DNA/MVA AIDS vaccine candidates

Interim safety and immunogenicity data from clinical trials of two AIDS vaccine candidates was presented at the AIDS Vaccine 04 conference which took place 30 August to 1 September in Lausanne, Switzerland. The candidates, DNA.HIVA and MVA.HIVA, were developed as a prime-boost pair by a partnership led by the International AIDS Vaccine Initiative (IAVI). The partnership includes the University of Oxford/UK Medical Research Council, the University of Nairobi/Kenya AIDS Vaccine Initiative and the Uganda Virus Research Institute. DNA.HIVA is a bacterial plasmid containing the p17 and p24 regions of the HIV *gag* gene, plus 25 cytotoxic T lymphocyte epitopes from across the HIV genome. MVA.HIVA consists of a copy of the same HIV genetic material inserted into a modified vaccinia Ankara (MVA) virus vector. The HIV sequences were derived from an HIV subtype A, the subtype which accounts for most infections in East Africa.

The data presented at Lausanne involved 205

volunteers in small-scale Phase I clinical trials in Kenya, Uganda and the United Kingdom. Generally, the two vaccines were safe and well tolerated. However, only about a quarter of all volunteers showed HIV-specific cellular immune responses, and these were not long-lasting. Immune responses in volunteers were assessed by interferon (IFN)- γ ELISPOT assays conducted at the trial sites on fresh peripheral blood mononuclear cells.

Clinical trials of the same two vaccine candidates that have already started will be completed over the next few months. These trials, currently taking place in Kenya and the United Kingdom, are studying the highest dose of MVA.HIVA and the administration of a third dose of MVA.HIVA. The trials will also measure vaccine-induced immune responses using multiple laboratory tests in addition to IFN- γ ELISPOT assays. Emilio Emini, IAVI's Senior Vice President for vaccine development, has said that unless there are new data on immune responses that are dramatically different IAVI will not develop DNA.HIVA and MVA.HIVA further.

Seven European countries demand push on AIDS vaccine

In Paris last June, health ministers and representatives from seven European countries (United Kingdom, France, Germany, Italy, the Netherlands, Spain, and Sweden) plus representatives of the European Union's Commission and the Eurovac consortium issued a joint call for better coordination in the development of an AIDS vaccine. The meeting, hosted by French Health Minister Philippe Douste-Blazy, aimed to formulate a single European view before a Group of Eight (G8) meeting in the US that same week (see *Enterprise* article below).

The representatives fell short of committing more funding to the AIDS vaccine effort, but did call for

strengthening research collaboration among vaccine scientists and working together to attain a critical mass to push prototype vaccines through the lengthy, costly process of clinical trials. The statement described an AIDS vaccine as "an absolute necessity." Attendees formulated a European "scientific diary" under the authority of a scientific committee that Michel Kazatchkine, director of the French ANRS, outlined: to identify and prioritize the best candidate vaccines for testing in clinical trials; to establish a network of research laboratories and centers of clinical evaluation for Phase II trials in particular; to support collaboration with developing countries to take part in Phase III trials; to mobilize private sector vaccine research in Europe.

Crucell grants IAVI exclusive license to use AdVac technology for AIDS vaccine

Dutch biotechnology company Crucell NV has signed an agreement with IAVI whereby Crucell will develop AdVac vectors for use in IAVI's AIDS vaccine development program.

Earlier this year Crucell and IAVI entered into an exclusive license agreement to develop an AIDS vaccine based on Crucell's AdVac technology. The AdVac vectors are developed from adenovirus serotypes 11 and 35 and have shown promising results as vectors for AIDS vaccines in a series of

studies by Crucell in collaboration with Harvard Medical School. The technology is also being used by Crucell in the development of a malaria vaccine in collaboration with GlaxoSmithKline, Walter Reed Army Institute of Research and the US National Institute of Allergy and Infectious Diseases, and a tuberculosis vaccine in collaboration with the Aeras Global TB Vaccine Foundation.

With the AdVac technology agreement, Crucell adds to the AIDS vaccine candidate collaboration it already has with Merck & Co. "Our technologies are now supporting two of the programs best positioned to bring an AIDS vaccine to the market," Crucell's Chief Scientific Officer Jaap Goudsmit said in a statement.

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

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

IAVI is a global not-for-profit organization whose mission is to ensure the development of safe, effective, accessible, preventive HIV vaccines for use throughout the world. IAVI's financial and in-kind supporters include the Bill & Melinda Gates, Rockefeller, Alfred P. Sloan and Starr foundations; the governments of Canada, Denmark, Ireland, the Netherlands, Norway, Sweden, the United Kingdom and the United States; multilateral organizations including the European Union and the World Bank; corporations such as BD (Becton, Dickinson & Co.), Continental Airlines and DHL; leading AIDS charities such as Crusaid and the Until There's a Cure Foundation; and other private donors such as the Phoebe W. Haas Charitable Trust B.

ANRS launches new clinical trials of preventive AIDS vaccine candidates

The French National Agency for AIDS Research (ANRS) announced the launch of a new trial of a candidate preventive AIDS vaccine at the AIDS Vaccine 04 Conference in Lausanne, Switzerland. The Phase I trial ANRS VAC16 will compare the intramuscular and intradermal (ID) routes of injection of a vaccine preparation of lipopeptides to determine safety and immunogenicity. Enrollment for this trial started in July at six sites in France; the target is a total of 70 non-infected volunteers. This is the first time that the ID route is being assessed for this type of vaccine preparation. Encouraged by results obtained in macaques, the investigators hope that the ID route may enhance the production of HIV-specific cytotoxic T lymphocytes (CTLs).

The vaccine candidate, LIPO-4T (LPHIV-1), is a mixture of 4 lipopeptides containing CTL epitopes from HIV-1 subtype B proteins (from strains MN and LAI). The epitopes are from Gag, Pol-RT, Pol and Nef. Each epitope contains a CD4⁺ T cell epitope from tetanus toxin in colinear sequence, and a monopalmitoyl group at

the N-terminal position to form the lipopeptide. The vaccine is manufactured by Biovector SA.

A second trial involving a lipopeptide vaccine candidate started enrollment in September. The Phase II ANRS VAC18 trial will test the safety and compare the cellular immunogenicity of three doses of the LIPO-5 candidate versus placebo in 132 non-infected volunteers in 6 sites in France. LIPO-5, manufactured by Aventis Pasteur, is a mixture of 5 lipopeptides containing CTL epitopes from HIV-1 subtype B (Gag, Pol and Nef). Each peptide is modified in the C-terminal position by the addition of a lipid moiety to form the lipopeptides.

The LIPO-5 vaccine is currently being tested in a different trial in the United States. Earlier this year the US National Institute of Allergy and Infectious Diseases in collaboration with the ANRS began the Phase I/II trial HVTN 042/ANRS VAC19 to evaluate LIPO-5 combined with a canarypox vector (vCP1452). This trial is based on preliminary results obtained from the ANRS Phase I trial VAC10, which suggested that the combination of lipopeptides and canarypox vector could induce a significant cellular immune response. For more information on these and other trials, visit the IAVI database of AIDS vaccines in human trials (www.iavireport.org/trialsdb).

Global HIV Vaccine Enterprise Scientific Plan Meeting

The Coordinating Committee of the Global HIV Vaccine Enterprise met on 21 October in Washington, DC to finalize the scientific plan that will outline the priorities needed to achieve the goal of developing a safe and effective AIDS vaccine and to discuss its implementation. A total of 52 participants from 16 countries attended the meeting, including the Joint United Nations Programme on HIV/AIDS (UNAIDS), the World Health Organization (WHO), the European Commission, as well as other stakeholders. Also present were leaders of the six working groups that helped draft the scientific plan.

The Global HIV Vaccine Enterprise arose from a concept and broad proposal developed by Richard Klausner, from the Bill and Melinda Gates Foundation, and other scientists and advocates. The proposal was published in the journal *Science* in June 2003 (www.sciencemag.org/cgi/content/full/300/5628/2036) and was endorsed by the G8 nations during their meeting in Sea Island, Georgia last June.

In general terms, the Enterprise is an alliance of independent agencies and groups who are working to jointly develop the scientific plan, which will be published early next year. The idea is to identify and prioritize the most critical scientific problems and encourage donors to deploy resources to solve them. There will be common standards for assessing laboratory and clinical research results and an emphasis on the sharing of data, resources, and expertise.

Participants at the meeting discussed the best ways the Enterprise could help address critical areas needed for the development of a vaccine. The major components of the plan are fourfold. First, encouraging the development of AIDS vaccine development centers or AIDS vaccine consortia, analogous to the Vaccine Research Center at the US National Institutes of Health or IAVI's Neutralizing Antibody Consortium, to develop and test novel candidate vaccines that can elicit enhanced humoral and cellular immunity. Second, standardization of the laboratory and clinical assays and parameters used to evaluate preclinical and clinical immunogenicity of candidate vaccines, so that the collected laboratory and clinical data can be compared even if trials are conducted in different geographical locations and with different vaccine candidates; the goal is to develop a fully integrated clinical trials system. Third, to improve the utility of non-human primate models in fundamental research efforts to better define immunological parameters associated with protection. Fourth, ensuring that increased, dedicated vaccine manufacturing capacity is available for use once an effective AIDS vaccine has been licensed.

The Coordinating Committee will also develop plans for a support framework for the Enterprise. This will comprise four major elements: a permanent secretariat; expert working groups that will provide guidance to steer the scientific plan; a forum for engaged funders willing to devote additional resources to the scientific plan; and a broader stakeholders' forum to enable engagement of the wider community and provide a mechanism for feedback and dialogue. The Coordinating Committee will be meeting again in the first quarter of 2005 to discuss progress.