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RV144 ■ STEP ■ POL ■ CELLULAR MEDIATED IMMUNITY ■ ANTIBODIES ■
IMMUNE CORRELATES ■ ANTIBODY DEPENDENT CELLULAR MEDIATED
CYTOTOXICITY ■ T CELLS ■ ANTIBODY DEPENDENT CELLULAR VIRAL
INHIBITION ■ VECTORS ■ ANTIGENS ■ CANARYPOX ■ GP120 ■ GAG ■
PHAMBILI ■ ADENOVIRUS ■ ENV ■ CMV ■ MOSAIC ANTIGENS ■
NON-HUMAN PRIMATE MODELS ■ HOST GENETICS ■ AIDSVAX ■ EARLY
EVENTS ■ PRIME-BOOST ■ CLADES ■ MUCOSAL IMMUNITY ■ NATURAL
KILLER CELLS ■ NEF ■ PRE-EXISTING IMMUNITY ■ ALVAC ■ ELISPOT ■
BROADLY NEUTRALIZING ANTIBODY ■ BROADLY NEUTRALIZING ANTIBODY ■ CYTOKINES ■
REPLICATING VECTORS ■ CD4 T CELLS ■ BREAKTHROUGH
INFECTIONS ■ MRKAD5 ■ RECEPTORS ■ INNATE IMMUNITY

**The Mysteries
of Protection**

DETAILS OF THE THAI TRIAL ■ AIDS VACCINE 2009 UPDATE ■ REPORT FROM KEYSTONE MEETING ON TB AND HIV

EDITOR'S LETTER

ON HIS WALL AT THE University of Washington, Larry Corey, principal investigator of the HIV Vaccine Clinical Trials Network (HVTN), has a plaque that reads, “If at first you succeed, try hard not to look astonished.”

There weren't many researchers who met that challenge after hearing the initial results of RV144 on September 24. The canarypox vector-based ALVAC in combination with a genetically engineered version of gp120 known as AIDSVAX showed a modest 31.2% efficacy in preventing HIV acquisition—the first evidence of efficacy in any AIDS vaccine clinical trial. In the hours and days following the announcement, we interviewed scores of researchers and many of them shared the same sentiment: surprise. Even some of the study's investigators were caught off guard when they were whisked away to an army base in California to be briefed on the results during what they thought was a trip to attend the annual Interscience Conference on Antimicrobial Agents and Chemotherapy.

The results from RV144 were unexpected in part because of how these candidates had performed in previous trials. RV144 also got off to a rocky start. Just after its launch, the trial was publicly scrutinized by a cadre of leading AIDS vaccine researchers, who published a policy forum in *Science* magazine questioning the scientific rationale for the trial.

But now another sentiment seems to prevail among many AIDS vaccine researchers: optimism. This best describes the mood at the AIDS Vaccine 2009 conference, which took place from October 19-22 in Paris. At the conference, detailed analyses of the data from RV144 were shared and discussed, and the emphasis shifted from the controversies of the past to the way forward. This trial provides a clue, actually 8,000 of them, that may help solve the mysteries of immunological protection against HIV. This is the first chance researchers have had to try to dissect the correlates of protection in humans—a finding that would undoubtedly lead to the development of improved candidates and a renewed sense of hope that vaccine-induced protection against HIV is not an insurmountable task.

Additional research findings showcased in Paris provided more promising news. Significant advances with other vectors, newly discovered neutralizing antibodies against HIV, and lessons from trials of failed candidates were also shared and contributed to the overall sense of optimism. Although much work remains to be done, it may be that in Paris the AIDS vaccine field picked up a much needed dose of *joie de vivre*.



KRISTEN JILL KRESGE



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The International AIDS Vaccine Initiative (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. Founded in 1996 and operational in 24 countries, IAVI and its network of collaborators research and develop vaccine candidates. IAVI's financial and in-kind supporters include the Alfred P. Sloan Foundation, the Bill & Melinda Gates Foundation, the Foundation for the National Institutes of Health, The John D. Evans Foundation, The New York Community Trust, the James B. Pendleton Charitable Trust, The Rockefeller Foundation, The Starr Foundation, The William and Flora Hewlett Foundation; the Governments of Canada, Denmark, India, Ireland, The Netherlands, Norway, Spain, Sweden, the United Kingdom, and the United States, the Basque Autonomous Government, the European Union as well as The City of New York, Economic Development Corporation; multilateral organizations such as The World Bank; corporate donors including BD (Becton, Dickinson & Co.), Bristol-Myers Squibb, Continental Airlines, Google Inc., Henry Schein, Inc., Merck & Co., Inc., Pfizer Inc, and Thermo Fisher Scientific Inc.; leading AIDS charities such as Broadway Cares/Equity Fights AIDS and Until There's A Cure Foundation; other private donors such as The Haas Trusts; and many generous individuals from around the world. For more information, see www.iavi.org.

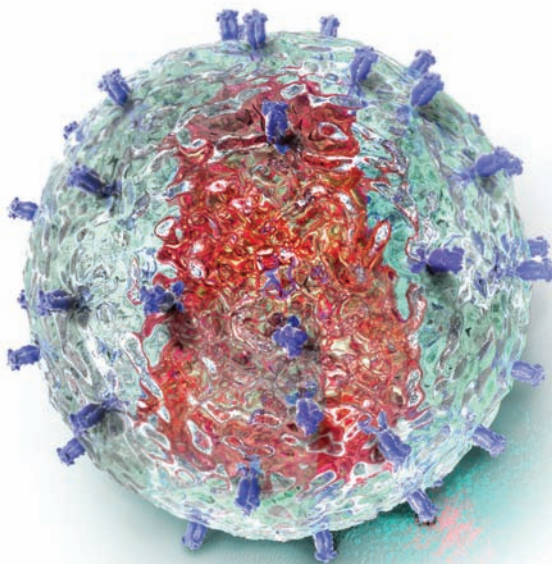
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Raft of Results Energizes **RESEARCHERS**

The AIDS Vaccine 2009 conference drew a record number of attendees to one of the most positive meetings in years

By Kristen Jill Kresge

DURING THE AIDS VACCINE 2009 conference, which was held from October 19-22 in Paris, there was a renewed sense of optimism among the nearly 1,000 researchers and policymakers in attendance, the largest crowd in the conference's nine-year history. "It's not time for being pessimistic. This should be a conference of hope," said Michel Sidibé, executive director of the Joint United Nations Programme on HIV/AIDS (UNAIDS), who spoke at the opening session.

This optimism was fueled in part by recent results from clinical trials. Less than a month earlier, the initial results of the RV144 trial in Thailand provided the first evidence of possible protection against HIV infection through vaccination (see *RV144 in Detail*, page 6). "We have the first signal, modest as it may be, of efficacy. Now that I see this very small signal, I believe an HIV vaccine is feasible," said Anthony Fauci, director of the US National Institute of Allergy and Infectious Diseases (NIAID).

Although RV144 grabbed the most headlines, several other advances in clinical and pre-clinical research were also showcased in Paris. Details about newly discovered antibodies against HIV and data from a replicating cytomegalovirus (CMV) vector-based vaccine in nonhuman primates (NHPs), among other findings, also served to energize researchers. Alan Bernstein, executive director of the Global HIV Vaccine Enterprise, which co-

hosted the conference, called the quest to develop an AIDS vaccine "a robust, active field of research that is moving ahead very rapidly."

This is quite a transformation from two years ago when the field was grappling with the sobering results of the STEP trial—a Phase IIb trial of an adenovirus serotype 5 (Ad5)-based vaccine candidate (MRKAd5) developed by Merck. The STEP trial showed not only that MRKAd5 did not reduce the risk of HIV infection or set point viral load in individuals who became infected despite vaccination, but that there was actually a trend toward an increased risk of HIV infection among certain sub-groups of vaccinated volunteers.

While researchers will now focus on trying to understand why the vaccine candidates tested in RV144 may have provided some protection against HIV infection, researchers affiliated with the STEP trial are still trying to unravel the reasons why MRKAd5 failed. This information, together with data from new vectors and antigens, will likely contribute to the design of improved HIV vaccine candidates in the future. "We are at the beginning of a new phase of HIV vaccine research," said Yves Levy, co-chair of AIDS Vaccine 2009.

Data from RV144 unveiled

RV144, a Phase IIb trial involving more than 16,000 Thai volunteers, tested Sanofi Pasteur's canarypox vector-based candidate ALVAC-HIV

(vCP1521) in a prime-boost combination with AIDSVAX B/E, a genetically engineered version of HIV’s gp120 surface protein. In September, researchers from the US Military HIV Research Program (MHRP) and the Ministry of Public Health in Thailand reported that at the conclusion of the six-year trial, this prime-boost regimen reduced the risk of HIV infection by about 31%, but had no effect on set point viral load in those who became HIV infected despite vaccination.

In Paris, additional data from RV144 was presented to a standing room only crowd in a special session that was added last minute to the meeting agenda. Supachai Rerks-Ngarm, the trial’s principal investigator, explained the statistical analyses of the trial results based on different populations of volunteers (see Table 1, this page), which were also published online in the *New England Journal of Medicine* at the conclusion of the session (*N. Engl. J. Med.* 2009; doi:10.1056/NEJMoa0908492).

Depending on the number of volunteers, the efficacy estimates for the different statistical analyses ranged from 26.2% to 31.2% (see Table 2, page 7). The highest efficacy was observed using the modified-intent-to-treat (mITT) population. This was also the only statistically significant result, with a two-tailed p-value of 0.04. Rerks-Ngarm called the mITT the “most preferred analysis.”

The mITT analysis excluded seven volunteers (five in the vaccine group and two in the placebo group) who were included in the intent-to-treat analysis (ITT) because after the six-month period in which injections were administered, investigators discovered that they were actually HIV infected at the start of the trial.

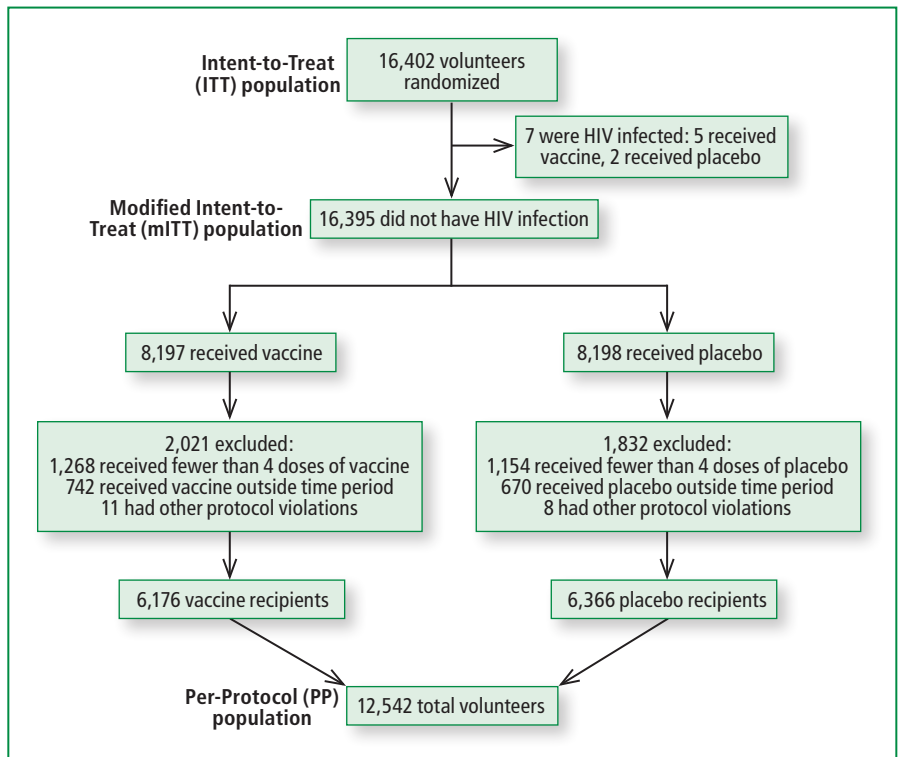
The lowest efficacy was seen with the per-protocol (PP) analysis, although Rerks-Ngarm said the PP and mITT results were “qualitatively consistent.” The PP analysis excluded 3,853 volunteers who did not receive all of the injections on schedule, as well as those who became HIV infected during the six-month period in which injections were administered. The fact that the ITT analysis provided a better result was puzzling to some researchers. However, the PP analysis for this trial excluded 39 HIV-infected volunteers. “Roughly half of the infection endpoints occurred in the first six months and therefore weren’t counted [in the PP analysis],” said Nelson Michael, director of MHRP, who also presented on the findings. Additionally, volunteers who missed a study visit, even by a single day, were also excluded.

Leading up to the AIDS vaccine conference, there was some controversy surrounding the decision made by trial investigators to release only the most favorable mITT analysis when they first announced the results in September. But according to Nelson, the mITT was always the priority. “The trial was powered based on the mITT. This was always the core analysis of the study. We were going to use it regardless of what the outcome was,” said Michael. “The bottom line is all three analyses showed the same trend and one [the mITT], which included the most data and the least bias, was statistically significant.” Fauci agreed. He said that regardless of the analysis, the findings from RV144 appear to be biologically significant and warrant further study.

Intriguing observations

At the conference, Michael pointed out two intriguing questions that have already emerged from the RV144 data. One is whether the modest protective effect of the vaccine candidates was limited to individuals who were at the lowest risk of HIV infection. In the trial, the efficacy of the prime-boost regimen seemed to be higher among individuals who reported being at low risk of HIV infection, as

TABLE 1
RV144: Populations for the Three Statistical Analysis Plans



RV144 in Detail



THE TRIAL

A Phase IIb test-of-concept trial, based on the expected number of HIV infection endpoints, conducted by Thailand Ministry of Public Health

Co-primary endpoints: Prevention of HIV infection and ability to reduce viral load

Duration: Six years

- September 2003—screening starts
- October 2003—first vaccination
- July 2009—data analysis begins

Sponsor: US Army, Surgeon General

Trial Cost/Funders: US\$105 million; US National Institute of Allergy and Infectious Diseases (NIAID) (75%), US Army (25%)



THE VOLUNTEERS

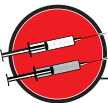
16,402 Thai citizens (60% male, 40% female) enrolled, 16,395 received at least one dose of vaccine or placebo

Inclusion criteria:

- Male or female Thai citizen, 18-30 years of age
- Available for participation for 3.5 years
- Can understand study and give written informed consent
- Completed enrollment in screening protocol

Exclusion criteria:

- HIV infected, active tuberculosis, or chronic use of immune-modifying therapy
- History of anaphylaxis or other serious adverse reactions to vaccines



THE VACCINE CANDIDATES

Prime

ALVAC-HIV (vCP1521)

A live, recombinant, non-replicating canarypox viral vector vaccine encoding clade B *gag/pro* and clade E *env*
(Vaccine Developer: Sanofi Pasteur)

Boost

AIDSVAX gp120 B/E

A genetically engineered version of HIV gp120 (*env*) from clade B and E (Vaccine Developer: Genentech; its spin-off, VaxGen, tested AIDSVAX previously; intellectual property rights now owned by Global Solutions for Infectious Diseases)

Dosing schedule



THE TEAM

Key collaborators:

- NIAID
- Sanofi Pasteur
- Global Solutions for Infectious Diseases
- US Military HIV Research Program, a branch of Walter Reed Army Institute of Research

Other collaborators:

- Mahidol University in Thailand
- Armed Forces Research Institute of Medical Science—US and Thai components

Principal investigator: Supachai Rerks-Ngarm, Thailand Ministry of Public Health



compared to those who said they were at high risk or who had engaged in what is considered a high-risk activity (sharing a needle, having sex with an HIV-infected partner, working as a commercial sex worker, or having multiple sex partners, among others) in the previous six months (see Table 3, page 8). However, this observed difference in vaccine efficacy among different risk groups was not statistically significant. “What we have is preliminary and hypothesis generating,” said Jerome Kim, deputy director of science at MHRP. Only 2.2% of participants in both the vaccine and placebo groups reported having a same-sex partner and only 0.8% of volunteers in both groups reported sharing needles. The majority of volunteers were heterosexual men and women recruited from the general population, which according to Fauci allowed investigators to study a low-dose mucosal exposure to HIV.

This has generated hypotheses about whether the route and level of HIV exposure may have been instrumental to the protection seen in this trial. In NHP studies, Genoveffa Franchini, chief of the animal models and retroviral vaccines section at the National Cancer Institute, has observed that protection against acquisition of simian immunodeficiency virus (SIV) with a prime-boost regimen similar to what was tested in RV144 is only achieved at a lower challenge dose (see *Evidence for Protection*, page 8).

The mode of exposure may also be important, as evidenced by studies of early HIV infection. George Shaw, a professor in the department of medicine at the University of Alabama at Birmingham, and Eric Hunter, a professor at Emory University, have both shown that in the vast majority of cases of heterosexual transmission, a single founder virus is responsible for establishing an infection (see *HIV Transmission: The Genetic Bottleneck*, IAVI Report, Nov.-Dec. 2008). Only 20% of the HIV transmissions in heterosexual couples that have been studied so far are linked to more than one founder virus; whereas 40% of infections in men who have sex with men (MSM) and 60% in injection drug users (IDUs) are linked to more than one founder virus (see *Deadly Synergy*, page 14). This seems to support the hypothesis that different routes of exposure may be fundamentally more difficult to protect against, as Raphael Dolin, the Maxwell Finland Professor of Medicine at Beth Israel Deaconess Medical Center, suggested in an editorial published in the *New England Journal of Medicine* in response to RV144. “Perhaps the requirements for protection against transmission in low-risk, heterosexual persons are considerably different or less stringent than

those in high-risk subjects,” he wrote. “This observation may be important in the design of future clinical trials.”

The two previous Phase III trials of AIDSVAX alone, which showed no protection against HIV infection, were conducted in MSM and IDUs, and the STEP trial primarily involved MSM. RV144 was the first AIDS vaccine efficacy trial that recruited volunteers from the general population, and this was because investigators were interested in community-level sexual risk. “I think it is really important to understand where the US Department of Defense comes from and why they are working on vaccines. They are working on vaccines because of the sexual risk to US soldiers,” said Debbie Birx, former director of MHRP who now heads up the US Centers for Disease Control and Prevention’s Global AIDS Program.

Another provocative question raised by the RV144 data is whether the protective effect of the vaccine candidates waned over time. Data presented by Michael suggests that the efficacy of the vaccine candidates may have decreased after the first year following vaccination (see Table 4, page 9). “There was a 50% to 60% efficacy in the first year,” said Donald Francis, executive director of Global Solutions for Infectious Diseases, who was involved in the two earlier Phase III trials of AIDSVAX while at VaxGen and whose organization now holds the intellectual property rights to the product.

However, because the trial was not designed to look at whether a certain number of injections were effective or if the protective responses waned with time, investigators cannot draw any conclusions about this observation. “These hypotheses merit further investigation and we are assembling experts to interpret the results and to maximize the knowledge gained through this study,” said Michael.

The search is on

The primary task now for researchers will be trying to identify possible immune correlates of protection associated with the results of RV144. Dolin called the establishment of immune correlates “the central question in HIV vaccine development.”

Everyone agrees that identifying immunological correlates would propel HIV vaccine research and lead to the development of improved candidates. “If those insights emerge, this will be a tremendous advance,” said Mark Feinberg, vice president of policy, public health, and medical affairs at Merck.

Four scientific working groups have already been set up to prioritize data analysis for RV144

TABLE 2

The different results for the three statistical analyses of RV144 after 3.5 years of follow up.

Intent-to-Treat Analysis	
Cumulative # of Infections / Total # of Volunteers	
Placebo	76 / 7,325
Vaccine	56 / 7,347
Vaccine Efficacy: 26.4%	
95% Confidence Interval: -4.0 to 47.9	
p-value: 0.08	
Modified Intent-to-Treat Analysis	
Cumulative # of Infections / Total # of Volunteers	
Placebo	74 / 7,325
Vaccine	51 / 7,347
Vaccine Efficacy: 31.2%	
95% Confidence Interval: 1.1 to 52.1	
p-value: 0.04	
Per-Protocol Analysis	
Cumulative # of Infections / Total # of Volunteers	
Placebo	50 / 6,002
Vaccine	36 / 5,874
Vaccine Efficacy: 26.2%	
95% Confidence Interval: -13.3 to 51.9	
p-value: 0.16	

[EVIDENCE FOR PROTECTION]

The first data that hinted that ALVAC encoding Env could protect against simian immunodeficiency virus (SIV) challenge was published in 2005. In this study, a version of ALVAC expressing Gag, Pol, and Env from simian immunodeficiency virus (SIV) demonstrated protection against a low-dose oral inoculation of SIVmac251 in 10 out of 16 neonate rhesus macaques (*J. Acquir. Immune Defic. Syndr.* 38, 124, 2005). This study was designed to mimic HIV transmission to infants during breast feeding. A similar result was observed in neonate macaques with modified vaccinia Ankara, another poxvirus vector.

Genevieve Franchini, chief of the animal models and retroviral vaccines section at the National Cancer Institute, has been involved in several studies of ALVAC in non-human primates (NHPs). She says that the partial protection seen in neonate macaques was encouraging, although there are obvious differences between the infant and adult models. In older macaques, her group first observed some protection against non-pathogenic virus (HIV-2) infection with ALVAC, but against a more pathogenic challenge virus (SIVmac251), there was only a transient reduction in viral load.

However, now Franchini says that based on recently collected and still unpublished data from her lab, there is some evidence that protection against infection with a prime-boost regimen of ALVAC and gp120, similar to that tested in RV144, can also be demonstrated by altering the SIVmac251 challenge dose in NHPs. "If we decrease the dose of virus challenge, we start to see protection from acquisition and from [high] virus load," she says, adding that this is only true in the vaccinated animals because all control animals get infected. —KJK

TABLE 3

HIV Incidence and Vaccine Efficacy by Risk Group in the Modified Intent-to-Treat Population

Risk Group	Vaccine (N=8,197)			Placebo (N=8,198)			Vaccine Efficacy
	# Evaluated	# Infected	HIV Incidence	# Evaluated	# Infected	HIV Incidence	% (95% CI)
Low	3,767	17	0.135	3,837	29	0.227	40.4 (-8.5 to 67.2)
Medium	2,297	12	0.157	2,222	22	0.299	47.6 (-6.0 to 74.0)
High	1,896	22	0.349	1,929	23	0.364	3.7 (-72.7 to 46.3)

(see Figure 1, page 10). These groups report into a scientific steering committee, which is led by Barton Haynes, director of the Center for HIV/AIDS Vaccine Immunology (CHAVI). Given that there are limited samples available from RV144, these working groups will be carefully selecting which assays should be performed as well as the companion studies that can be conducted in NHPs to try to identify the immune correlates. The RV144 investigators are also accepting ideas or suggestions from the public, which can be submitted at www.hivresearch.org.

Some early immunological data from RV144 was presented in Paris by Mark de Souza, laboratory director at the Armed Forces Research Institute of Medical Sciences. So far, researchers have analyzed a subset of samples from RV144 for cellular immune responses and the presence of binding antibody. De Souza reported that 17% (26 out of 152) of cell samples analyzed had a positive interferon (IFN)- γ ELISPOT assay response, defined as greater than 55 spot-forming cells per million peripheral blood mononuclear cells (PBMCs), when exposed to either Env or Gag antigen. De Souza said these data are identical to what was observed in an earlier Phase I/II trial with this prime-boost regimen. But when compared to the ELISPOT assay results observed in the STEP trial (see page 9), de Souza said the data here are "actually quite shameful."

Response rates for CD4⁺ Env-specific intracellular cytokine staining showed that 33% of samples analyzed from vaccinees responded to either Env or Gag antigen by secretion of IFN- γ and/or interleukin-2, compared to only 2% of placebo recipients. Responses in vaccinees were predominantly directed toward Env.

The lymphoproliferation assay, which measures the ability of cells to proliferate in the presence of antigen, showed that 90% (61 of 68) of samples from vaccinees collected two weeks after the final immunization responded to clade E gp120, compared to 17% of samples from pla-

cebo recipients. And 89% (51 of 57) of samples from vaccinees responded to clade B gp120, compared to 19% of placebo recipients.

Nearly all (99%) of the 142 individuals analyzed had detectable binding antibody to clade B or E gp120, but only 52% of these individuals had detectable binding antibody to clade B p24. De Souza said that overall the cellular and humoral responses were comparable to those seen in earlier trials.

Data still emerging from STEP

If the STEP trial is any indication, it may take some time before researchers are able to fully decipher the results of RV144. Investigators working on the STEP trial are still collecting data from volunteers and generating hypotheses about the effects of MRKAd5 two years after immunizations were stopped early because the vaccine was found to be ineffective.

Initially, investigators observed that a subgroup of vaccine recipients who were uncircumcised and who had pre-existing antibody immunity to the Ad5 vector used in MRKAd5, were at a higher risk of HIV infection compared to placebo recipients with these same characteristics. When the data was analyzed a year ago, vaccinees who had only one risk factor—were either uncircumcised or Ad5 seropositive—had an intermediate level of risk. "That held across multiple multivariate models," said Susan Buchbinder, principal investigator of the STEP trial.

However, after 15 months of additional follow up, the picture has changed. From October 2007 to January of this year, 48 additional HIV infections have occurred among male STEP trial volunteers—26 among vaccinees and 22 among placebo recipients. Additionally, 12 new HIV infections have occurred among female volunteers, split evenly between vaccine and placebo groups. In Paris, Buchbinder reported that there is still an increased risk of HIV infection among uncircum-

TABLE 4

Cumulative Vaccine Efficacy Over Time in RV144

Month	Modified Intent-to-Treat Analysis		Per-Protocol Analysis	
	HIV Infections	Vaccine Efficacy	HIV Infections	Vaccine Efficacy
6	16	54%	n/a	n/a
12	42	60%	21	68%
18	67	44%	41	41%
24	82	36%	53	27%
30	95	36%	62	31%

cised men who received the vaccine as compared to those who received placebo through January of this year, but the trend toward more infections among those with pre-existing Ad5 immunity is no longer evident. “Whatever effect we were seeing [with Ad5] appears to have gone away,” she said.

It is still unclear whether this indicates that the increased risk of HIV infection associated with Ad5 immunity occurred early and then waned over time or that Ad5 immunity actually did not have any effect on risk of HIV infection, added Buchbinder. She cautioned that all of this data must be interpreted carefully because it was collected after the trial was unblinded, which could affect risk behaviors taken by the volunteers. Investigators initially observed a decline in self-reported risk behavior among vaccinees with Ad5 immunity following unblinding, but the level of risk-behaviors has increased again with time, according to Buchbinder. “We’re not able to more thoroughly reduce risk in these study participants even when we tell them that there is a potential increased risk of [HIV] acquisition,” she said.

One possible explanation for the correlation between Ad5 seropositivity and increased risk of HIV acquisition is that the Ad5 vector activates T cells, which then migrate to the mucosa, thereby increasing the number of cells susceptible to HIV at the portal of entry. This hypothesis was recently investigated by Adel Benlahrech, a research associate at Imperial College London. In Paris he presented results from a study of 20 healthy volunteers, 60% of whom were Ad5 seropositive and 5% who were Ad11 seropositive, a lower prevalence serotype. Dendritic cells, generated from monocytes that were derived from these individuals, were pulsed *ex vivo* with replication-defective Ad5 or Ad11 and then co-cultured with autologous lymphocytes. Both Ad5 and Ad11 induced the proliferation of CD4⁺ T cells. The expansion of CD4⁺ T cells was greatest in Ad5 seropositive indi-

viduals and these cells expressed high levels of alpha 4 beta 7 and CCR9, which indicates potential homing to the mucosal tissues. Benlahrech also reported that the expanded Ad5- and Ad11-specific T cells showed increased CCR5 expression and higher susceptibility to infection by CCR5-tropic

HIV. He concluded that adenoviral-based vaccines in individuals with pre-existing immunity may result in preferential expansion of CD4⁺ T cells that home to mucosal tissues, which could lead to an increased susceptibility to HIV infection.

Nicole Frahm, associate laboratory director at the HIV Vaccine Trials Network, presented on the growing body of immunological data that is being collected from the STEP trial. Frahm reported that although 90% of 551 male vaccinees in the STEP trial showed an HIV-specific T-cell response as measured by IFN- γ ELISPOT assay, the median number of epitopes these cells targeted in Gag, Nef, and Pol (the antigens included in MRKAd5) was two. She contrasted this to results from studies in rhesus macaques that have shown protection against heterologous SIV challenge following vaccination with Ad5-based vaccines. In these studies, animals that

Non-neutralizing Antibody Function

For now, it’s anybody’s guess what led to the modest level of protection seen in RV144. But most researchers are betting on antibodies. “Most people are pinning this efficacy to antibodies,” says Nelson Michael, director of the US Military HIV Research Program.

Neither ALVAC nor AIDSVAX has previously induced broadly neutralizing antibodies against HIV. This has raised questions about the possible role of binding, non-neutralizing antibodies as well as other non-binding antibody functions, including antibody-dependent cellular cytotoxicity (ADCC) in the protection observed in RV144. Through ADCC, antibodies can link HIV-infected cells with effector cells, including natural killer cells, which can then kill the HIV-infected cells and help stop the spread of the virus.

“If it’s true that there was transient sterilizing immunity then that puts a finger on antibodies in a way which we have never imagined before,” says David Baltimore, a professor at the California Institute of Technology. “It could be a kind of ADCC effect or some other secondary effect rather than direct neutralization.”

There is some evidence from a Phase I/II trial in Thailand that suggests the ALVAC/AIDSVAX prime-boost regimen can induce ADCC, while ALVAC alone does not (*Vaccine* 23, 2522, 2005). In this study, there was a significant difference between the magnitude of ADCC responses between vaccinees who received the prime-boost regimen and placebo recipients. —KJK

FIGURE 1

In the Hunt for Correlates, Making the Most of a Precious Lot

Four scientific working groups have been charged with a tall order—how best to utilize the limited number of samples from RV144 to try to pinpoint potential correlates of immunity. The samples are so few and so important that scientists frequently describe them as precious, and choosing the best assays will not be an easy task. These four committees all report into a scientific steering committee, led by Barton Haynes, director of the Duke Human Vaccine Institute and head of the Center for HIV/AIDS Vaccine Immunology (CHAVI), comprised of the heads of each working group and additional members yet to be named. There is also a product development group—led by Stanley Plotkin, executive advisor to the CEO of Sanofi-Pasteur, and Phil Gomez, director of PRTM Management Consultants—that will consider future clinical trials of ALVAC and AIDSVAX. Listed below are the scientific working groups, their leaders, and the main issues they will likely focus on in the months ahead. *Source: Barton Haynes*



Humoral and Innate Immunity

Barton Haynes, director of the Duke Human Vaccine Institute and head of the Center for HIV/AIDS Vaccine Immunology (CHAVI)

This group will focus on both traditional neutralizing antibodies as well as those that require an Fc receptor to induce activation of natural killer cells to offer cell-mediated immunity, such as antibody-dependent cellular cytotoxicity. Specific human monoclonal antibodies of interest will be made and tested in nonhuman primates against a mucosal simian immunodeficiency virus (SIV)/HIV hybrid, or SHIV.

The humoral/innate and T-cell immunity teams will also be conducting pilot studies on samples from 50-100 volunteers who received either vaccine or placebo to establish relevant reagents, determine assay variability and reliability, and evaluate the immune responses. Scientific committees will then select the most relevant assays for a case-controlled study of 51 HIV-infected volunteers who received the vaccine and a larger control group of uninfected vaccinated individuals to measure the quantity and breadth of immune responses. Controls may also include participants who received the placebo.



Animal Models

Genoveffa Franchini, chief of the animal models and retroviral vaccines section at the US National Cancer Institute at the US National Institutes of Health

This group will look at how best to model the results of RV144 in nonhuman primates (NHPs) to obtain a model that can best determine the correlates of protection. Monoclonal antibodies from the humoral/innate team will be tested for their ability to modify infection in NHPs challenged mucosally with SHIV.



T-cell Immunity

Julie McElrath, director of the Fred Hutchinson Cancer Research Center/University of Washington HIV-1 Vaccine Evaluation Unit

This group will explore the phenotype and function of CD4⁺ and CD8⁺ T-cell responses in pre- and post-vaccination samples. Additional case-control studies will depend upon initial findings. The humoral/innate and T-cell teams will coordinate immune cell phenotypic studies.



Host Genetics

David Goldstein, Director of the IGSP Center for Human Genome Variation at Duke University

This group will decide whether a sufficiently powered analysis of the role of genetics in responses obtained in RV144 can be studied. Specific analyses under consideration include Fc receptor allotype analysis, killer immunoglobulin-like receptor typing, and human leukocyte antigen typing.

were protected targeted on average 12.5 epitopes in Gag, Nef, and Pol (*J. Virol.* 2009; doi:10.1128/JVI.01441-09). Frahm concluded that the breadth of the responses induced by MRKAd5, as well as the specific epitopes they targeted, which were in less conserved regions, may have been suboptimal, and this might explain why the vaccine candidate failed. “We don’t know at this point whether it’s just better to have more [epitopes targeted], or if it’s more important to make responses to the right epitopes,” she said.

Several ongoing studies are also shedding light on the breakthrough virus infections that are occurring among STEP trial volunteers. James Mullins, a professor in the school of medicine at the University of Washington, presented results from an analysis of whole genomes derived from 64 individuals (39 vaccinees and 25 placebo recipients) in the STEP trial during acute infection.

Mullins reported that in 75% a single founder virus was detected, with no observable difference between vaccine and placebo recipients. The remaining 25% were infected with two, and in one case four, viral variants. When these viruses were characterized, Mullins found that the viruses infecting vaccinees were more likely to differ from the epitopes included in MRKAd5 than those infecting placebo recipients. Based on this observation, Mullins offered two possible conclusions: either MRKAd5 blocked the outgrowth of specific HIV variants that were similar to the vaccine, or vaccine-elicited cytotoxic T lymphocytes may have driven specific mutations among viruses in vaccine recipients following infection. When Mullins and colleagues drilled down further and looked at each amino acid, they identified 10 significant amino acid differences between virus sequences in the vaccine and placebo recipients. Buchbinder reported that there was a

trend toward a lower acute viral load in vaccinees whose founder virus sequence matched the vaccine at any of these 10 amino acid sites in Gag, Nef, and Pol, as compared to placebo, but she said this analysis was still in the exploratory phase.

Interim analysis of Phambili

Immunizations in the Phambili trial, a Phase IIb companion study to the STEP trial conducted in South Africa, were also halted in September 2007. At that time, only 801 volunteers were enrolled of a planned 3,000. About two-thirds of the volunteers had received at least one injection when unblinding occurred, but fewer than 10% of volunteers had received all three injections. Despite unblinding, retention rates of volunteers in Phambili remain high at 92%. Glenda Gray, principal investigator of the Phambili trial, reported some interim analyses from these participants.

To date, 60 participants in the Phambili trial have become HIV infected—33 in the vaccine group and 27 in the placebo group. The HIV incidence among vaccinees is 4.7%. The majority of HIV infections (50/60) have occurred in individuals with pre-existing Ad5 antibody immunity (29 among vaccinees and 21 among placebo recipients). More infections have also occurred among women (40/60, with 21 in the vaccine group and 19 in the placebo group).

However, Gray reported that neither the baseline Ad5 antibody titer nor the number of doses of vaccine had any impact on HIV infection risk. Although the vaccine candidate was immunogenic with 89.2% and 77.4% of individuals mounting HIV-specific T-cell responses to clade B and clade C HIV respectively, as measured by IFN- γ ELISPOT, there was overall no difference between viral load set point in HIV-infected volunteers in the vaccine and placebo groups. There was, however, a 0.57 log reduction in set point viral load in female vaccinees as compared to placebo recipients, but this difference was not statistically significant. Still, Gray said, this observation warrants further study.

New vectors

In addition to canarypox and Ad5, several other viral vectors are also in various stages of clinical and preclinical development. Dan Barouch, a professor of medicine at Beth Israel Deaconess Medical Center and Harvard University, presented immunogenicity data from a Phase I trial of an Ad26 vector involving 36 Ad26 seronegative volunteers. Barouch said the Ad26 vector was developed to avoid potential prob-

lems with pre-existing immunity to the more prevalent Ad5 serotype. Three doses of the Ad26 vector encoding HIV clade A Env were administered over 24 weeks. After eight weeks, the mean IFN- γ ELISPOT response was 381 spot-forming cells per million PBMCs in the low-dose group (10^9 viral particles) compared to 365 in the high-dose group (10^{11} viral particles). Barouch said he is also exploring several additional functional methods to characterize the immune responses induced by the Ad26 vector, including its ability to inhibit HIV replication in a viral inhibition assay.

Several other serotypes of adenovirus are also being explored as potential vectors by researchers at the Vaccine Research Center (VRC) at NIAID. In addition to the DNA/Ad5 prime-boost regimen that is now being tested in a Phase II trial known as HVTN 505, scientists at the VRC are also considering Ad28, Ad35, and Ad41 vectors that could be tested in heterologous adenovirus prime-boost regimens. However, Gary Nabel, director of the VRC, acknowledged that the T-cell responses to Ad vectors complicate the immune response to HIV. “As much as possible we’d like to be working with non-human adenoviruses in the next few years,” he said.

To that end, researchers at the VRC are also developing simian and chimp Ad vectors, as well as other non-Ad vectors, including integrase-deficient lentiviral vectors. Nabel briefly discussed the development of a replication-defective recombinant lymphocytic choriomeningitis virus (LCMV) vector. Researchers at the VRC have been able to remove the glycoprotein gene of LCMV and replace it with a vaccine antigen. According to Nabel, this LCMV vector is immunogenic and potent—1,000-fold fewer virus particles are required than the standard dose of 10^8 viral particles of Ad5. This LCMV vector is now being advanced into NHP studies. “On the T-cell side, I think we can look forward to a lot of improvements,” said Nabel.

Some of the candidates that are generating interest are replicating viral vectors. Several effective vaccines have been based on live-attenuated viruses, but since this approach is not feasible for HIV, researchers are focusing instead on replication-competent viral vectors (see *Go Forth and Multiply*, IAVI Report, May-June 2008). At a satellite symposium organized by IAVI, researchers gathered to discuss the progress and prospects related to the development of replicating viral vectors. Although these vectors may stimulate more robust and durable immune responses, they may not be as safe as replication-

We are at the beginning of a new phase of HIV vaccine research.

– Yves Levy

On the T-cell side,
I think we can
look forward
to a lot of
improvements.

– Gary Nabel

deficient viral vectors. Therefore, one of the main questions regarding the development of replicating vectors is what type of safety studies will be required by regulatory authorities, such as the US Food and Drug Administration or the European Medicines Agency, before clinical trials of these candidates can move forward.

One replicating viral vector has already been tested in clinical trials. At the satellite symposium, Yiming Shao, chief expert of the National Center for AIDS/STD Control and Prevention in China, presented on the replicating vaccinia virus (based on the Tiantan strain) that was previously used to vaccinate against smallpox and is now being explored as an HIV vaccine vector. A Phase I trial of this replicating vector was recently conducted in China. The Phase Ia trial, which involved 12 volunteers, was used to evaluate a low and high dose of the vector (either 20,000 or 40,000 particle-forming units). The Phase Ib segment of the trial, which involved 36 volunteers, was designed to evaluate the safety and immunogenicity of a DNA prime, followed by a replicating vaccinia vector-based candidate. Shao said that alone, the replicating vaccinia vector did not induce a T-cell response, but that in combination with a DNA prime there was an HIV-specific T-cell and antibody response. A second generation of this vector is now being developed for a Phase II trial in China that is expected to begin next year.

Other replicating viral vector-based HIV vaccine candidates are also scheduled to enter clinical trials soon. A replicating but attenuated vesicular stomatitis virus (VSV) vector-based candidate, which was originally developed by Wyeth and is now licensed to Profectus Biosciences, is scheduled to be tested alone in a Phase I trial starting next year. David Clarke, director of vaccine vectors at Profectus, said there is also interest in exploring a heterologous DNA/VSV prime-boost regimen in the future.

A replication-competent Ad4 vector developed by Marjorie Robert-Guroff, chief of the immune biology of retroviral infection section at the vaccine branch of the National Cancer Institute, is also being considered for a Phase I clinical trial. Replicating Ad4 has already been administered to approximately 10 million military recruits to prevent acute respiratory disease, according to Robert-Guroff, and in pre-clinical studies, she said both intranasal and oral administration of the Ad4 vector encoding HIV immunogens induced strong effector and central memory T-cell responses.

Effector memory T-cell responses are the key to the impressive control of viral replication afforded by the replicating CMV vector, according to Louis Picker, associate director of the Vaccine & Gene Therapy Institute at Oregon Health & Science University. In Paris, Picker presented data that expanded on an earlier study, which provided the first evidence that a replicating rhesus CMV (RhCMV) vector could effectively control viral replication in four out of 12 vaccinated monkeys (*Nat. Med.* 15, 293, 2009).

This larger study compared three vaccine regimens in rhesus macaques: a RhCMV/RhCMV prime-boost regimen (12 macaques), a RhCMV/Ad5 prime-boost regimen (12 macaques), and a DNA/Ad5 prime-boost regimen (10 macaques). These animals were compared to 27 unvaccinated controls. All animals were CMV infected at the start of the trial and were vaccinated with RhCMV vectors including SIV *gag*, *rev/nef/tat*, *env*, and *pol*. All animals were challenged intra-rectally on a weekly basis with a low dose of SIVmac239. At the time of the conference, all but three animals, two controls and one animal in the DNA/Ad5 group, were SIV-infected.

Picker said that the DNA/Ad5 regimen delayed acquisition of infection in this model—animals in the DNA/Ad5 vaccine group developed a viral load greater than 30 copies of SIV/ml plasma after a median of 9.5 virus challenges compared to a median of five in the other groups, a statistically significant difference. All of the DNA/Ad5 vaccinated animals had binding antibody responses, according to Picker, which he said “may have accounted for their delay of acquisition.” The DNA/Ad5 regimen also lowered viral load set point, but eventually all of the animals that were infected developed progressive infection.

While the RhCMV vector vaccines had no effect on acquisition of infection, “what was remarkable was what happened once they got infected,” said Picker. Over half (54%) of the animals in the RhCMV vaccine groups developed undetectable plasma viral loads, compared to 26 of 27 control animals who developed progressive infection. Picker called this an “unprecedented control of infection.” One animal had a peak viral load of 40 million copies of SIV/ml of plasma and occasional lower-level viral blips followed by periods of complete control of viral replication.

The protection afforded by the replication-competent RhCMV vector also seems to be stable—12 of 24 animals have sustained “high-level

elite control” through a median of 147 days of follow-up, with “viral blips decreasing in frequency,” Picker said. One macaque that originally exhibited control of viral replication developed progressive infection after 11 weeks. Picker postulates that this control is mediated by effector memory T cells, which he referred to as “trained combat troops” that wane in the absence of persistent antigen.

The virologic control in this experiment correlates most closely with peak SIV-specific CD8⁺ T cells in blood. This finding indicates to Picker that the CMV vector has the capacity to act earlier than prime-boost approaches and therefore “extends the T cell and HIV vaccine paradigm.”

New broadly neutralizing antibodies

Scientists were also encouraged by good news related to HIV antibodies. Only four antibodies that are widely considered to be broadly neutralizing had been identified previously (b12, 2G12, 2F5, and 4E10), and efforts to induce these antibodies through vaccination have been unsuccessful so far. Now, for the first time in a decade, researchers have discovered five new broadly neutralizing antibodies against HIV that may lead to the design of new AIDS vaccine candidates.

Two of these antibodies, known as PG9 and PG16, were identified by IAVI scientists in collaboration with researchers from The Scripps Research Institute in La Jolla, California (see Figure 2, this page). These findings were published in *Science* magazine in September and presented at the conference by Sanjay Phogat, a principal scientist at IAVI’s AIDS Vaccine Design and Development Laboratory (*Science* 326, 285, 2009).

The PG9 and PG16 antibodies are the first to be isolated from an African donor and were identified through an effort known as Protocol G that involved collecting blood samples from 1,800 HIV-infected individuals at clinical research centers in Thailand, Australia, the US, the UK, and several sub-Saharan African countries. Using a standard neutralization assay that could detect the ability of sera to directly block HIV infection, researchers at the biotechnology company Monogram Biosciences and IAVI were able to identify the top 10% of samples with broad neutralization capabilities. Then, a high-throughput B-cell activation strategy developed by Theraclone Sciences, a biotechnology company in Seattle, was used to generate immunoglobulin (Ig) G containing supernatants from

approximately 30,000 activated B cells from serum from a single HIV clade A infected donor. B-cell supernatants were then screened using a high-throughput micro-neutralization assay, developed by scientists at IAVI and Monogram. This led to the discovery of five antibodies of interest, two of which, PG9 and PG16, exhibited broad and potent neutralization activity. PG9 and PG16, both of which failed to bind gp120 or gp41, neutralized 127 and 119 of 162 viruses respectively. The potent neutralization capabilities of PG9 and PG16 frequently exceed those of the four previously identified broadly neutralizing antibodies.

Researchers at IAVI and Scripps then determined that PG9 and PG16 target epitopes primarily located in regions of the V2 and V3 loops of the HIV Env trimer. These epitopes differ by only a single amino acid substitution from those in HIV clade B consensus sequences, suggesting these epitopes are part of a relatively conserved structure within these variable loops. These epitopes may also be more readily accessible to antibodies and therefore are a promising target for vaccine researchers. “It’s fair to say that it [this epitope] is a new vaccine target,” said Phogat. The focus now is on immunogen design. “The aim is to design vaccine candidates that prompt the immune system to produce similar neutralizing antibodies,” said Dennis Burton, a professor of immunology at Scripps and scientific director of the IAVI Neutralizing Antibody Center.

Now that this method for isolating antibodies has been identified, scientists predict that it may lead to the discovery of other new antibodies and additional epitope targets for scientists to exploit. “We expect to identify additional antibodies and novel targets on HIV in the near future,” said Burton.

Nabel presented on three additional broadly neutralizing antibodies, one of which is a variant of another, which were also recently discovered by researchers at the VRC. The strategy used to isolate these antibodies involved designing resurfaced stabilized cores by introducing specific mutations into both the inner and outer domains and the bridging sheet of HIV Env and then knocking out the CD4 binding site. B cells isolated from HIV-infected individuals whose serum had broadly neutralizing activity were then incubated with the resurfaced cores, and CD4 binding site specific B cells were selected by flow cytometry. Nabel presented in detail on one of the three new monoclonal antibodies, known as VRC01, which was isolated in this manner. VRC01 binds to wild-type gp120 and

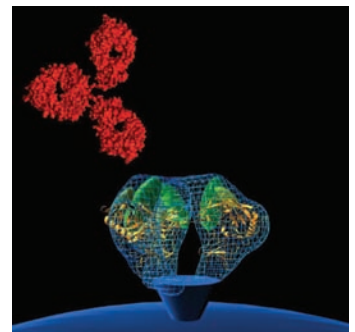
Continued on page 17

FIGURE 2

Modeling the PG9 and PG16 Epitopes onto the HIV-1 Trimer

The model below is adapted from a recent cryo-electron tomographic structure of the HIV-1 trimer. The V1/V2 and V3 loops, which are not resolved in the crystal structure, are represented in green and yellow.

The approximate locations of gp41 and the viral membrane (not resolved in the structure) are shown in blue. The red structure located above the trimer is a human immunoglobulin (Ig)G molecule representative of PG9 and PG16.



Courtesy of The Scripps Research Institute

Deadly SYNERGY

A Keystone symposium on TB and HIV emphasized the need to tackle the diseases in tandem

By Andreas von Bubnoff

ALTHOUGH TUBERCULOSIS (TB) is thought to be the leading cause of death among people with HIV/AIDS, scientific conferences often focus on these two deadly diseases separately. But this changed recently, when over 300 scientists and clinicians from all over the world gathered in Arusha, Tanzania, from October 20-25 at a Keystone Symposium on “Overcoming the Crisis of TB and AIDS.”

TB is responsible for between one-third to a half of AIDS deaths, and at least a quarter of the approximately two million people who died of TB last year were coinfecting with HIV, conference co-organizer Anne Goldfeld of Harvard Medical School said in the opening session. “Each infection and its solution cannot be separated from each other,” she added. “By bringing together scientists and clinicians who work at the cutting edge of each disease, it’s the aim of this conference to serve as a catalyst to generate new ideas and to identify new ways of solving our global humanitarian disaster.”

The presentations at the Keystone symposium spanned many topics including the natural history of the two diseases, the mechanism of their synergy, and their treatment and prevention. The meeting’s location in Africa was also important to help build partnerships, said co-organizer of the conference Stefan Kaufmann of the Max Planck Institute for infection biology.

A lethal dance

Given that the meeting convened both TB and HIV experts, one central topic was how the two infections synergize in individuals that are coinfecting. In such patients, each infection enhances the other infection’s pathogenicity: HIV by compromising the immune system, and TB by driving HIV replication.

Goldfeld showed the results of *in vitro* studies that address how TB infection increases viral load of HIV by inducing virus replication. Her data suggest that TB infection in monocyte-derived macrophages directly induces a novel transcription factor that binds to the long terminal repeat enhancer element of HIV, thereby activating HIV replication. This is just one of several transcription factors induced by TB infection that activates HIV replication.

Goldfeld is also involved in the CAMELIA clinical trial in Cambodia that is studying the timing of HIV therapy in coinfecting individuals who are already on TB therapy. Starting coinfecting people on highly active antiretroviral therapy (HAART) too late results in high mortality because they become too immunosuppressed, but there have also been concerns that starting HAART early might result in adverse drug reactions and interactions. “[There] was a tremendous bias that people couldn’t take seven different types of medicines at once,” Goldfeld said. “So the recommendation was to wait until after the intensive

phase of TB therapy was finished to initiate HAART.”

Another concern is the worsening of TB symptoms in people who start HAART during TB treatment as a result of immune reconstitution inflammatory syndrome (IRIS), an inflammatory disease thought to be associated with the increase in the number of CD4⁺ T cells that occurs once people start HAART. It typically occurs in 7-34% of coinfecting people according to the published literature, six to eight weeks after initiation of HAART, Goldfeld said.

The aim of the CAMELIA trial, which just finished enrolling 661 coinfecting volunteers, is to see if early HAART initiation will increase survival in coinfecting, immunosuppressed individuals despite the perhaps more complex initial clinical management that might involve dealing with IRIS, Goldfeld said. In the trial, some coinfecting volunteers are started on HAART two weeks after starting TB therapy, while others delay initiation of HAART until two months after starting TB therapy. Results of the trial, which evaluates survival one year after initiation of TB therapy, are expected in mid-2010.

Some volunteers enrolled in CAMELIA are also enrolled in another trial called CAPRI-T, which is designed to evaluate whether distinct characteristics of CD4⁺ T cells are involved in causing IRIS. Investigators are prospectively analyzing blood samples from participants at several time points after they start TB therapy and at the time IRIS occurs, if this condition develops. Results of this trial are also expected in 2010.

Alan Sher, chief of the laboratory of parasitic diseases at the National Institute of Allergy and Infectious Diseases (NIAID), presented data from a study of IRIS in mice. He said that recent studies suggest that IRIS might be caused by an enhanced expansion of TB-specific effector memory CD4⁺ T cells, although longitudinal studies have called this association into question.

But to his surprise, Sher did not observe expansion of T cells in a mouse model for IRIS. He infected T-cell deficient mice with *Mycobacterium avium*, which causes a TB-like infection in these mice. When given CD4⁺ T cells from a normal mouse, the mice indeed got a rapid IRIS-like wasting disease and died. But the injected T cells did not expand more in infected mice compared to uninfected mice. Instead, they became more activated. This may induce chemokine production that leads to recruitment of myeloid cells, such as macrophages, from blood into tissues including the lung, where they produce the

inflammatory cytokine TNF- α and cause damage.

“[The myeloid] cells are what causes disease,” Sher concluded. “T cells themselves don’t cause disease.” This suggests that the expansion and tissue recruitment of myeloid cells by these activated T cells might also be a potential target for the prevention of IRIS, Sher said, adding that the current treatment with steroids is not ideal.

Insights on HIV transmission

The meeting also featured many presentations that dealt with HIV and TB separately. George Shaw, a professor of medicine and microbiology at the University of Alabama, presented an update on his analyses of tracing clinically productive HIV infections to the transmitted founder viruses that caused them (see *HIV Transmission: The Genetic Bottleneck*, IAVI Report, Nov.-Dec. 2008). The additional data confirmed previous results that showed that the majority of heterosexual infections can be traced back to a single transmitted founder virus, while about 20% involve more than one transmitted founder virus. By contrast, more than one transmitted founder virus is evident in about 40% of men who have sex with men (MSM) and in about 60% of injection drug users (IDUs; see *Capsules from Keystone*, IAVI Report, March-April 2009).

Shaw reported that the highest number of transmitted founder viruses observed in IDUs is now at least 17. “There [are] so many we can’t count [precisely],” he said. He pointed out that in light of the observation that heterosexual infections result in a lower number of transmitted founder viruses than MSM or IDU infections, it was interesting that in the recently published results of the RV144 HIV vaccine trial, the vaccine may have been more effective in subjects at lower risk of HIV infection (*N. Engl. J. Med.* 2009; doi:10.1056/NEJMoa0908492). “It will probably be interesting to [quantify] the numbers of transmitted viruses [in the vaccinees] and their neutralization susceptibility [and] correlate that with the [risk behavior],” Shaw said. “Something might come out.”

Since the majority of HIV/AIDS infections are the result of sexual transmission, researchers are also focusing on the role of semen in HIV transmission. Frank Kirchhoff, a professor of virology at the University of Ulm, gave an update on his studies of the enhancing effect of semen on HIV transmission. Previously, he had found that the semen peptide prostatic acidic phosphatase forms amyloid fibrils called semen-derived enhancer of virus infection (SEVI) that can capture HIV virions and promote

**Each infection
and its solution
cannot be
separated from
each other.**

– Anne Goldfeld

[SICK CHIMPS]

Tanzania's national parks were the safari destination for many attendees of the Keystone symposium on "Overcoming the crisis of TB and AIDS" in Arusha. One, Gombe National Park, is also home to the wild-living chimpanzees that Beatrice Hahn, a professor of medicine and microbiology at the University of Alabama, and her colleagues have been studying for more than nine years. At the conference, Hahn presented the results of her studies with chimpanzees infected with simian immunodeficiency virus (SIV)cpz, which is thought to have given rise to HIV-1 group M in humans, which accounts for the majority of HIV-1 infections. She found that wild-living SIVcpz-infected chimpanzees are 10-16 times more likely to die than uninfected chimpanzees (*Nature* 460, 515, 2009). "[There] is no doubt that the infected group died faster," Hahn said. This challenges the prevailing view that all natural SIV infections are non pathogenic (see *SIV May Be Much Younger Than Previously Thought, Research Briefs, IAVI Report*, May-June 2009).

The study also found that SIVcpz-infected females are less likely to give birth and had a higher infant mortality rate than uninfected females. In addition, the spleens and lymph nodes of three infected animals showed CD4⁺ T-cell depletion, and one female had histopathological signs of end-stage AIDS. The same analysis in sooty mangabeys, which are thought to not get sick from SIVsmm infection, confirmed that indeed, being infected does not affect the lifespan of sooty mangabeys. However, Hahn said there may be other nonhuman primate species that get sick from SIV infection. "I think that gorillas are probably also negatively impacted," she said. "I have no proof [but] if I had to bet I would say yes." —AvB

their attachment to target cells. This can enhance the infection rate of HIV in limiting dilution assays by up to 100,000-fold (see *Clues from CROI, IAVI Report*, Jan.-Feb. 2008).

Kirchhoff found that semen itself also increases the infectiousness of HIV by about two- to 50-fold, a variability that was first observed when Kirchhoff compared semen samples from different members of a local soccer team. This variable enhancement correlated with the amount of SEVI. But in contrast to SEVI fibrils, the effect of semen cannot be tested under ideal conditions because it contains cytotoxic compounds, Kirchhoff said. To protect the target cells from the cytotoxic effect, he removes the virus and semen from the target cells after two hours. As a result, however, much of the virus is also removed, which is part of the reason why the enhancing effect of semen is smaller than the effect of isolated SEVI fibrils.

The cytotoxic effect of semen could also explain why a recent study found that sperm appears to inhibit, and not enhance, HIV infection (*FASEB J.* 23, 3609, 2009). Kirchhoff said that the researchers involved in that study incubated target cells with seminal plasma for one day, long enough for the cells to be harmed. "Some people push the system so that they work at the threshold of cytotoxicity," he said, adding that many common semen treatments such as heating or preincubation of semen with target cells also reduce the ability of semen to enhance HIV infection and do not reflect the *in vivo* situation.

Kirchhoff also presented data suggesting that the presence of semen counteracts the inhibition of HIV transmission by microbicides, potentially eliminating their protective effect. He found that semen generally reduces the efficiency of microbicides and antiretroviral agents. In some cases this means that they are inactive at concentrations applied *in vivo*. But in the case of microbicides containing the CCR5 inhibitor Maraviroc, Kirchhoff said it should still be possible to achieve the effective dose that inhibits HIV even in the presence of semen. "I think Maraviroc is still very promising because it's relatively efficient even in the presence of sperm," he said.

That's good news for Ronald Veazey of Tulane University, who provided an overview of microbicide research. He and his collaborators are currently evaluating a microbicide containing Maraviroc in rhesus macaque experiments. Veazey said that unpublished data suggest that the microbicide shows "remarkable efficacy" in preventing transmission from vaginal challenge

with an R5-tropic SIV/HIV hybrid (SHIV) challenge virus in rhesus macaques.

When asked if the effects of sperm should be measured in nonhuman primate experiments testing microbicides, Veazey said it would be difficult to get semen from macaques, and using human semen in macaque experiments would be like "dealing with apples and oranges." Kirchhoff disagreed. "The studies in the monkey models are a model for HIV transmission in humans," he said, "so I think it would actually be good to use human semen because we want to know how human semen affects virus transmission."

Targeting with aptamer-siRNAs

One potential strategy to prevent or treat HIV infection in the future is the use of siRNAs, small RNA molecules that can be designed to inhibit HIV replication by silencing certain genes in HIV or in host cells infected with HIV (see *Interfering with HIV, IAVI Report*, Sept.-Oct. 2006). But introducing siRNAs into HIV target cells is difficult, said Judy Lieberman, a professor of pediatrics at Harvard Medical School, who is developing vaginal microbicides that contain siRNAs to prevent HIV infection. However, she reported that using aptamers could overcome that obstacle. Aptamers are RNA molecules that fold in such a way that they bind to specific target proteins. Lieberman uses aptamers that bind to the CD4 receptor and has fused siRNAs that silence HIV or host cell genes to these aptamers. She said that the resulting aptamer-siRNAs enter HIV target cells such as macrophages and CD4⁺ T cells, and can inhibit HIV replication in human cervicovaginal tissue isolated from hysterectomy patients. Initial experiments in humanized mice look promising, she added. "At least in a few mice it looks like we are getting gene silencing *in vivo* in the genital tract," she said. "We are very excited about this new approach to microbicides."

Ramesh Akkina, a professor of microbiology, immunology, and pathology at Colorado State University, is also using aptamer-siRNA molecules, but in this case to treat HIV infection. Akkina, along with John Rossi of the Beckman Research Institute of City of Hope, and colleagues are using a gp120 binding aptamer that allows entry into HIV-infected cells. They fused the aptamers to siRNA, which represses HIV replication by silencing the HIV *tat* and *rev* genes (*Nucleic Acids Res.* 37, 3094, 2009). Akkina said that intravenous injection of these aptamer-siRNAs lowers viral load in HIV-infected humanized Rag-hu mice from 100,000 viral parti-

cles/ml to less than 50 within one week. It also reversed CD4⁺ T-cell loss. Akkina compared the approach to a guided missile. “The missile head is the aptamer, the real bomb is the [siRNA tail].”

According to Akkina, such targeting of specific cells makes it possible to use 100 times lower siRNA doses than previous approaches using untargeted siRNAs. Akkina said there are plans to do Phase I clinical trials with aptamer-siRNAs as a potential treatment.

Antibody PrEP

A much discussed strategy to prevent HIV transmission is the administration of antiretrovirals to uninfected individuals, an approach known as pre-exposure prophylaxis (PrEP). David Ho, a professor at Rockefeller University and the director of the Aaron Diamond AIDS Research Center in New York City, is developing a novel PrEP strategy which involves infusing people with a monoclonal antibody called Ibalizumab. The antibody binds the CD4 receptor—the primary receptor used by HIV—thereby preventing HIV from infecting CD4⁺ T cells. This strategy, he said, requires much less frequent dosing than with ARVs and is also typically associated with fewer adverse events. So far, Ibalizumab has been extensively tested in HIV-infected people as a therapeutic agent, Ho said. Trials involving more than 200 HIV-infected people who were largely on salvage therapy regimens have shown that Ibalizumab given intravenously is safe, can decrease viral

load by about 1 log, and can increase CD4⁺ T-cell counts by about 50 cells/ml. “The effect is quite reasonable since it is virtually monotherapy in advanced-stage patients,” Ho said. Currently, Ibalizumab treatment is being tested in Phase IIb clinical trials.

But Ho said the antibody should also be tested in healthy, uninfected people to see if it has any utility in HIV prevention. One concern with antibodies that bind to the CD4 receptor is that they could inhibit normal immune function, for which the CD4 receptor is important. But Ibalizumab doesn’t seem to interfere with normal immune function, Ho said, probably because it binds to a face of domain 2 of CD4, which is opposite the side of domain 1 where the immune function is carried out. In addition, it is an immunoglobulin (Ig) G4 antibody, which means that it almost has no Fc receptor binding ability and can therefore not recruit immune cells that are active in antibody-dependent cellular cytotoxicity. Because gp120 binds to domain 1, Ibalizumab doesn’t directly interfere with gp120 binding, so just how it blocks HIV infection is still unknown.

Ho is now testing the ability of Ibalizumab to prevent infection in monkey experiments and is planning to launch a Phase I study in healthy volunteers. He also plans to further improve the potency and the pharmacokinetic profile of the antibody so that it would only need to be administered every few months, and wants to deliver the DNA encoding the Ibalizumab antibody by using adeno-associated virus as a vector. ■

Continued from page 13

neutralizes clade A, B, and C viruses at very low concentrations. When tested against a panel of 89 tier-2 viruses, VRC01 neutralized over 90%.

Binding of VRC01 is competed by CD4 binding-site antibodies like the previously identified antibody b12, but unlike b12, VRC01 actually stimulates the binding activity of CD4-induced antibodies.

Nabel also reported results that indicate that it is possible to induce CD4 binding-site antibodies in animal models. “We’ve now been able to make progress in eliciting such antibodies through immunization,” said Nabel. Scientists at the VRC administered a first-generation trimeric immunogen to rabbits and were able to elicit antibodies that bind CD4 and will neutralize viruses that are considered easier to neutralize. “These are not broadly neutralizing antibodies but really this is the first time in animals that we’ve had the ability to immunize and elicit antibodies that will neutralize through this

site,” added Nabel. He said these advances “serve as guides for vaccine development.”

Fauci agreed. In his concluding remarks he highlighted the discovery of new antibodies by IAVI and the VRC as one of the key findings of the year. He also noted other promising advances, including research from Burton’s group that shows that high levels of neutralizing antibodies may not be required to block HIV infection and the observations that HIV that establishes an infection may be easier to combat because it is much less glycosylated than virus that has been replicating for a long time. Together, these findings go a long way toward inspiring optimism. “As most of us have been discussing over the past few days, we are at the threshold of where we want to be,” Fauci said. ■

Additional reporting by Andreas von Bubnoff and Regina McEnery.

Vaccine BRIEFS

Economy Threatens World Progress on Immunization

THE THIRD EDITION OF THE State of the World's Vaccines and Immunization brought some good news about efforts toward immunizing children against vaccine-preventable diseases and the development of new vaccines, but also some dire warnings about how the global economic downturn might impede progress in immunization programs.

The report, issued in October by the World Bank, the World Health Organization (WHO), and the United Nations Children's Fund (UNICEF), noted that there are now 106 million children receiving the required three doses of DPT (diphtheria-pertussis-tetanus) vaccine before their first birthday—a 74% increase in coverage since 2000. Despite this progress, 24 million children a year still fail to receive even a single dose of the DPT vaccine—a gap global health authorities fear will only widen if donor countries fail to sustain investments in immunization programs, particularly in developing countries. The global economic downturn is causing concern that the United Nations' Millennium Development Goal to reduce deaths among children under age five by 66% between 1990 and 2015 will not be met if countries are forced to curtail their immunization campaigns.

"This report is really a call to action aimed at everyone," said Graeme Wheeler, managing director of operations at the World Bank, at an October 21 report launch in Washington, D.C. Wheeler said an estimated US\$1 billion is needed annually to ensure that new and existing vaccines will be delivered to all children in 72 of the world's poorest countries, and that a failure to continue to support campaigns will cause many diseases to come roaring back. "We need to stick with it," he said.

Polio illustrates the power of global immunization campaigns particularly well. The disease, which caused widespread panic in industrialized countries during the 1940s and 1950s and until recently was endemic in many developing countries, has ebbed, largely due to immunization campaigns. There were just 1,247 new polio cases worldwide this year, according to the WHO, and there are now only four countries where polio is still endemic: Nigeria, Pakistan, India, and Afghanistan.

Along with immunizing children against DPT, MMR (measles, mumps, and rubella), and polio, there is also a growing stable of new vaccines that protect against rotavi-

rus, meningitis, the highly pathogenic H5N1 avian influenza virus, pneumococcal disease, and certain strains of the human papillomavirus that can lead to the development of cervical cancer. The WHO estimates that pneumococcal disease and rotavirus infection together account for 1.3 million deaths in children annually—mostly in developing countries.

The report noted that continued investments will also be needed to accelerate the development of vaccines against tuberculosis, AIDS, and malaria, which are responsible for more than four million deaths a year, mainly in developing countries. The report estimates the cost of developing a new vaccine to be \$500 million. There are currently about 80 vaccines in the late stages of clinical testing—40 of them are aimed at diseases for which a vaccine does not yet exist. Of those, the malaria vaccine candidate RTS,S/AS01 being developed by GlaxoSmithKline Biologicals, which is being tested in a recently launched Phase III trial in Africa, was cited as a high-impact vaccine that was the furthest along in clinical testing. —Regina McEnery

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WHO Meeting to Evaluate Test-and-Treat Strategy

NEARLY A YEAR AFTER a quintet of researchers from the World Health Organization (WHO) published an article in *The Lancet* describing the results of a mathematical model that predicted that a combination of annual HIV testing and immediate antiretroviral (ARV) treatment could potentially end the AIDS epidemic in 50 years, scientists, public health officials, and community activists gathered from November 2-4 to talk exclusively about the strategy dubbed test and treat (*Lancet* 373, 48, 2009). The widely publicized *Lancet* study used South Africa to model a generalized HIV epidemic (see *Test and Treat on Trial*, *IAVI Report*, July-Aug. 2009).

The WHO convened the meeting in Geneva, Switzerland, to stimulate discussion about the ethical implications, acceptability, and feasibility of implementing the test and treat approach in various populations. Researchers considered some of the ways to study a strategy that looks promising based on mathematical models, but which has not yet been subjected to the rigors of a randomized, controlled clinical trial.

The experts who gathered in Geneva included Julio Montaner, president of the International AIDS Society, who is a vocal advocate of early initiation of ARV treatment and has been studying the impact that expansion of ARVs has had on lowering community viral load and HIV incidence in Vancouver, British Columbia. Community viral load reflects the mean viral load of a group of HIV-infected individuals. Montaner said a study that looked at the effect of expanding ARV treatment from 3,500 HIV-infected individuals to 5,000 in a community in Vancouver appears to have had an impact on transmission. "All I am prepared to say right now is that new HIV infection rates are going down," said Montaner. There are about 13,000 HIV-infected individuals in British Columbia who are eligible for ARVs.

"There are huge opportunities to address some very important questions related to the effectiveness of ARVs, both in terms of morbidity and mortality outcomes as well as HIV transmission," said Montaner. "Those opportunities will become even greater with the anticipated release of new WHO guidelines," he added. According to Montaner, the WHO is expected to increase its benchmark for initiating ARV therapy from 200 CD4⁺ T cells to 350 CD4⁺ T cells later this year.

US researchers are hoping to launch a pilot study next spring to evaluate the feasibility of implementing test and treat in Washington, D.C., which has the highest prevalence of HIV in the country, and the Bronx in New York City, which has the highest AIDS death rate of the city's five boroughs due to the fact that so many HIV-infected individuals are diagnosed late. The three-year study will occur in high-risk communities where poverty, racial discrimination, AIDS stigma, distrust of doctors, and other factors can be barriers to accessing medical care. Wafaa El-Sadr, the director of the Center for Infectious Disease Epidemiologic Research at Columbia University's Mailman School of Public Health, will be heading up

the pilot study, which is being funded by the US National Institute of Allergy and Infectious Diseases (NIAID) and reflects a collaborative effort between NIAID, the US Centers for Disease Control and Prevention, and local health departments in the two cities.

El-Sadr said the goals of the study are to determine the best way to link HIV testing and treatment programs, to retain HIV-infected individuals in treatment programs, and to ensure individuals adhere to their daily ARV regimens.

"What I got from the [WHO] meeting was a collective commitment of the importance of continuing to expand access to treatment," said El-Sadr. "Only about 40% of people who need treatment today can obtain it. We have a long way to go."

Mark Harrington, an activist who heads the Treatment Action Group in New York City, said he left the WHO meeting more optimistic about the approach. At the very least, Harrington said, test and treat may provide better linkage between prevention and treatment. "Care and treatment and prevention need to be done altogether."—*Regina McEnergy*

Call for novel HIV-1 immunogens

In an effort to speed up the screening process of novel immunogens against HIV-1, the INTERNATIONAL AIDS VACCINE INITIATIVE is placing an open call for a collaborative screening and characterization process of any potential HIV-1 immunogen to all researchers engaged in HIV vaccine research.

If you have an HIV-1 immunogen that has produced promising results in small-animal or non-human primate studies, we would like the opportunity to independently reproduce the data and advance the candidate.

For more information regarding the IAVI novel immunogen screening program, please e-mail:

novelimmunogens@iavi.org



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