HIV prevention research: The relay race continues

Researchers gathered at the International AIDS Conference focused on long-term efforts to control the spread of HIV

by Regina McEnery

If there is one thing researchers have come to realize in their search for a safe and effective vaccine, it’s that HIV doesn’t allow its victims—or science—much time to mount a successful defense.

Within six days after exposure to HIV—about the length of time the approximately 25,000 researchers, healthcare workers, and activists gathered at the XVII International AIDS Conference in Mexico City from August 3-8—the virus overcomes the body’s initial defenses and infects enough target cells to cause a ramping up of viremia that essentially turns HIV into the biological equivalent of a runaway train. This early, yet crucial, chapter in the life cycle of the virus was referenced in a number of key talks at the sprawling conference, reminding attendees at the biennial event why vaccines and other biomedical methods of preventing HIV represent enormous and thus far unmet challenges for scientists.

“We refer to [those six days] as a window of vulnerability,” said Anthony Fauci, director of the National Institute of Allergy and Infectious Diseases (NIAID). “But [those days] can also become a window of opportunity,” he added. “Our success or failure with vaccines, as well as with our ability to ultimately control [and] perhaps even cure HIV, will rest in that very short timeframe.”

Fauci’s remarks opened a session on new directions in AIDS research that could have served well as the back-up theme for the conference’s main mantra of “Universal Action Now.” Following the recent setbacks in the development of microbicides and vaccines, the field of HIV prevention was at the forefront of many discussions. The failure of Merck’s cell-mediated adenovirus serotype 5 (Ad5) vector-based vaccine candidate (MRKAd5) to show any efficacy in a large Phase IIb test-of-concept trial has steered researchers back to basic research, and the conference unexpectedly became a forum to showcase these shifting priorities.

The Mexico City conference, the first to be held in Latin America, squeezed in presentations...
on a disparate list of topics ranging from the study of better animal models for HIV pathogenesis, to the status of multiple approaches to HIV prevention, including vaccines, microbicides, oral pre-exposure prophylaxis (PrEP), and implementing safe male circumcision programs.

**A shifting pipeline**

The most vibrant example of shifting priorities in the AIDS vaccine field occurred last month when Fauci decided not to move forward with a Phase IIb test-of-concept trial known as PAVE 100A of a prime-boost vaccine regimen developed by researchers at the Vaccine Research Center, part of NIAID (see _PAVEing the way to a smaller trial_, page 1). Although Fauci is considering a smaller trial in place of PAVE 100A, the pipeline of vaccine candidates could still shrink in the coming months.

In its biennial 2008 AIDS Vaccine Blueprint, IAVI recommended that less promising vaccine candidates get weeded from the product development pipeline and that the freed-up resources be funneled instead to basic discovery research (see _Vaccine Briefs_, page 15).

A number of sessions at the conference also focused on ways to attract a new generation of researchers into AIDS vaccine research, an issue that has become in vogue ever since NIAID held a summit on HIV vaccine research and development earlier this year (see _Balancing AIDS Vaccine Research_, _IAVI Report_, March-April 2008). “Everywhere you go it is the same faces, all of us in our mid-50s,” said Mauro Schechter, chief of AIDS research at the Universidad de Federal do Rio de Janeiro in Brazil. “Where is the next generation? We are not giving the right message if we do not tell all the researchers that this is a relay race,” he said.

Though major strides have been made in the past decade in both the development of new antiretrovirals (ARVs) and in providing ARV treatment to more people living with HIV/AIDS, countries have been less successful in controlling the spread of new infections, particularly in high-risk populations. The US Centers for Disease Control and Prevention (CDC) released updated HIV incidence estimates at the conference showing that the annual number of new infections has been more than 16,000 higher in the US than the estimated 40,000 infections that had been continuously reported since the mid-1990s (see _A Static Epidemic_, _IAVI Report_, May-June, 2008).

The most recent estimates from the Joint United Nations Programme on HIV/AIDS (UNAIDS), which were released just prior to the start of the conference, indicate that 33 million people are currently living with HIV/AIDS and that 2.7 million new HIV infections occurred globally last year. Though the rate of new HIV infections has fallen in some countries, including in some of the hardest-hit regions of sub-Saharan Africa, this has been offset by increases in new infections in other countries, according to the UNAIDS report.

Moreover, the cost of treatment has grown astronomically since the advent of highly active antiretroviral therapy (HAART). To meet the goal of universal access, UNAIDS estimates it will cost approximately US$54 billion each year to provide ARVs to those in need in low- and middle-income countries by 2015. In Brazil, for instance, the cost of providing ARVs in 2008 is estimated to be $525 million—double the costs in 2004, according to the UNAIDS report.

UNAIDS Director Peter Piot said there are also some worrisome trends in the modes of transmission, creating even greater challenges for government-funded prevention programs. In Thailand, for instance, the highest rate of new infections is now among married women, said Piot. He also cited concern about increasing rates of HIV among injection-drug users (IDUs) and men who have sex with men (MSM) in some African countries.

“We have absolutely no choice but to continue to develop the science required for an HIV vaccine no matter how long it takes,” said Myron Cohen, associate director of the University of North Carolina’s Center for AIDS Research during his plenary talk on preventing the sexual transmission of HIV.

**Back to basics?**

Lack of efficacy prompted Merck to halt immunizations in the STEP trial 11 months ago. Unfortunately, the disappointing news didn’t stop there. Subsequent observations by researchers involved in the study suggested that the modified cold virus used to ferry the HIV antigens may have actually increased the risk of HIV acquisition in certain sub-groups of trial volunteers—mainly uncircumcised MSM who had pre-existing immunity to the Ad5 vector. While the results of the STEP trial may have slowed interest in the development of cell-mediated vaccines, clinical investigators attending the conference say there is still much to be learned from the trial volunteers. Susan Buchbinder, of the San Francisco Department of Public Health and a principal investigator in the STEP trial, said researchers are still awaiting data on human leukocyte antigen (HLA)-typing and herpes simplex-2 (HSV-2) status from trial volunteers who subsequently became HIV infected. They are also collecting behavioral data that could elucidate any possible sexual networks among uncircumcised men at certain trial sites, which are associated with an increased risk of HIV infection. Buchbinder noted that the retention rate in the STEP study, even after unblinding the volunteers, is still about 95%. “We explained to the study volunteers that this is a pivotal trial and that we need their continued participation and our retention rates have been very, very high,” said Buchbinder, adding that this was “a testament to the incredible dedication of our study volunteers.”

In a separate session focused solely on AIDS vaccine research, Buchbinder went into greater detail about the potential connection between susceptibility to HIV infection and circumcision status in MSM. Research suggests that the foreskin harbors an abundance of Langerhans cells, which can aid HIV transmission, but the protective effects of circumcision have only been established in hetero-
sexual men. In the STEP trial, researchers observed a high number of HIV infections among uncircumcised MSM who reported having unprotected, insertive anal intercourse. Buchbinder said one hypothesis is that HIV may have targeted activated Langerhans cells in these uncircumcised MSM, making the “less risky practice” of insertive anal sex much riskier.

**Clues from nonhuman primates**

A number of other talks at the conference focused on developing better animal models for studying HIV infection and searching for clues about virus control from different species of nonhuman primates.

Although nonhuman primates are the best animal model available, they are still an imperfect approximate for studying HIV infection in humans. This has led researchers to explore other options for preclinical evaluation of AIDS vaccine candidates, including mice that are genetically altered to express human immune cells, so-called humanized mice. Victor García-Martínez, a virologist at the University of Texas Southwestern Medical Center, presented on a humanized mouse model that he has been using to study the transmission and pathogenesis of HIV.

García-Martínez said the humanized mice developed human T cells at a furious pace after being injected with the bacterial toxin that causes toxic shock syndrome or Toxic 1—one measure of proof that their immune systems were reacting in a similar fashion to a human’s. They also measured the amount of time it took the mice to produce cytokines and found that it correlated with the time it takes to induce human inflammatory responses. As a final test of concept, they challenged the mice with Epstein-Barr virus and found that the responses closely resembled those seen in humans. García-Martínez said his laboratory is now using the humanized mice to study HIV pathogenesis in the gut, a central battleground for virus replication and CD4⁺ T-cell depletion early on in the course of HIV infection in humans.

While the humanized mouse model is being further developed, primates are still the best model available for studying HIV infection. Guido Silvestri, director of the clinical laboratory at the University of Pennsylvania, whose focus is the pathogenesis of HIV in different species of nonhuman primates, presented on the role the immune system plays in aggravating progression of HIV infection in humans.

In experiments with sooty mangabeys, natural hosts of SIV that can be infected with the virus without any deleterious consequences, Silvestri’s laboratory has observed an apparent lack of immune activation during infection, despite levels of virus replication comparable to those in other nonhuman primate species that do develop the monkey equivalent of AIDS. He compared sooty mangabeys to long-term nonprogressors—a rare subset of humans who are infected with HIV but do not progress to AIDS in the typical time frame, even without the aid of antiretroviral therapy. The difference between the two, said Silvestri, is that viral replication continued unabated in the primates, while it remains suppressed in long-term nonprogressors.

Silvestri believes his observations in sooty mangabeys have implications for vaccine development. “When it comes to vaccines, the main problem is that every time we try to mount an immune response against the virus, we are also creating new targets for the virus itself,” he said.

**A pill to prevent HIV?**

With no AIDS vaccine looming on the horizon, there is increasing attention being placed on the growing array of clinical trials evaluating oral PrEP—the use of ARVs to prevent HIV infection. Nowhere was this more evident than at the conference, where the status of PrEP trials and future concerns about its effectiveness and implementation were discussed at a broad range of sessions and received considerable media coverage.

An industry liaison forum sponsored by the International AIDS Society, which hosted the conference, included a discussion of PrEP, which was also the topic of a report released at the conference by the AIDS Vaccine Advocacy Coalition (AVAC).

There is no evidence yet from trials evaluating whether daily administration of the ARV tenofovir, or a combination pill of two ARVs known as truvada, will be effective at preventing HIV transmission, but the research and advocacy communities are gearing up for the results. Seven trials are currently underway or in the planning stages. The furthest along of these is a US trial funded by the CDC that is testing oral administration of tenofovir in 400 HIV-uninfected MSM. Results are expected next year on this study, according to Timothy Mastro, senior director of research at Family Health International, a sexual and reproductive health organization that is also funding a PrEP trial that will begin enrolling volunteers in Africa this year.

Mastro, who gave an overview talk on PrEP, said the primary purpose of the current batch of studies is to determine whether the ARV-based intervention prevents HIV infection and whether it is safe. Only then will researchers tackle some of the thornier issues involved with this prevention strategy. “Then we will evaluate risk behaviors, adherence, alteration of disease progression, and whether or not [HIV] resistance develops in those that become infected [during the] trial,” he said.

Four other trials are now enrolling volunteers, including a study of 2,400 IDUs in Thailand, a study of 1,200 heterosexual men and women from Botswana, and a study of 3,000 MSM in Brazil.
Ecuador, Peru, and the US, with additional sites to be added later. Another trial involving 4,200 women in southern Africa will evaluate a topical application of a gel-based microbicide containing tenofovir to determine its ability to block HIV infection.

In total, the seven PrEP studies will involve close to 18,000 volunteers and that number is likely to get even higher because study investigators in at least two of the trials have decided to expand enrollment in order to strengthen the statistical power of their trials. Mastro said the decision was made to expand enrollment in trials in both Thailand and Botswana after investigators observed a lower HIV incidence rate than what had been previously estimated in the study populations.

Mastro said studies have shown that oral PrEP can prevent transmission of SIV in repeat challenge studies in rhesus macaques, which is why advocates have high hopes for its efficacy in humans. If effective, though, there will be many obstacles to successful implementation of PrEP programs. “We may have an answer in 2-3 years and we have to make sure we are ready for the data,” said Mitchell Warren, executive director of AVAC.

An underutilized strategy

While the HIV prevention field awaits answers on PrEP, questions are mounting about why the implementation of male circumcision programs is lagging. Three years ago, researchers halted two large randomized trials after data showed that male circumcision reduced HIV transmission by as much as 65% in heterosexual men. Despite the plethora of favorable data, researchers and AIDS advocates at the conference reported that the intervention is underutilized, particularly in hard-hit regions in sub-Saharan Africa where heterosexual sex is the primary mode of HIV transmission.

New data was also released at the conference showing that adult male circumcision was not associated with increased promiscuity. Robert Bailey, an epidemiologist at the University of Illinois, reported that male circumcision did not appear to increase HIV risk behavior in a randomized control trial of 1,319 men in Kenya (PLoS ONE, 3(6):e2443, 2008).

Bailey, who has been studying circumcision for more than a decade, also presented data based on surveys of men in a Kenyan cohort suggesting that circumcision actually increases penile sensitivity and results in an enhanced ease of reaching orgasm among newly circumcised men as compared to men in an uncircumcised control group. He also observed a significant reduction in sexual risk behavior among both circumcised and uncircumcised men 6-12 months after enrollment in the circumcision study.

Population Services International (PSI), a nonprofit organization focused on alleviating major health problems in the developing world, is pushing for a scale-up of male circumcision in Africa, where efforts to provide the procedure to men have faced a number of cultural, religious, and even political barriers, despite solid evidence for its protective effects against HIV infection. And there is now some evidence that the tide may be turning.

Shortly after the close of the Mexico City Conference, President Yoweri Museveni of Uganda, who previously had opposed mass circumcision because he thought it would encourage the spread of HIV/AIDS, announced plans to circumcise more than 3,000 local youths between the ages of 12 and 18, according to Reuters news service.

Dvora Joseph, acting director of PSI, said there is still much stigma surrounding circumcision in some countries, while in others there are long lines of men waiting for the procedure due to shortages of trained healthcare workers. Warren acknowledged that circumcision is not a magic bullet, but in the absence of a vaccine, he said interventions like circumcision have become an important component in reducing the spread of HIV. PSI said widespread circumcision in sub-Saharan Africa—particularly in southern Africa, where the current circumcision rates are low—could prevent an estimated two million new HIV infections over the next 10 years and save as many as four million lives over the next 20.

Next-generation microbicides

The development of topical microbicides that women can apply before intercourse to prevent HIV transmission was a hot topic at the 2006 AIDS conference in Toronto, particularly after Bill and Melinda Gates specifically called for increased research efforts to develop microbicides and other new HIV prevention tools. But the announcement earlier this year that the microbicide gel Carraguard had no effect on HIV infection rates in women enrolled in a Phase III clinical trial made it just the latest in a string of candidates that have failed to provide protection against HIV (see Vaccine Briefs, LAVI Report, March-April, 2008).

Zeda Rosenberg, chief executive officer of the International Partnership for Microbicides (IPM), spoke at a number of sessions about the development of a new cadre of microbicides, which are comprised of existing antiretroviral drugs and therefore have a more precise mechanism of action against the virus. These ARV-based microbicides include gel formulations of tenofovir; dapivirine, a non-nucleoside reverse transcriptase inhibitor; and maraviroc, a CCR5 inhibitor.

The first results from efficacy trials of these second-generation candidates are not expected until 2010, when a Phase IIb test-of-concept trial testing a tenofovir gel will be completed in South Africa. However, a study released at the conference is cause for optimism. In a late-breaker abstract by CDC researcher Walid Heneine, he showed that a microbicide candidate consisting of truvada provided almost complete protection against repeated vaginal SHIV challenge in rhesus macaques. This supports other encouraging animal data on the topical use of ARVs in preventing HIV transmission.
Prevention (CDC). However, it was one of several trials delayed when Merck’s candidate vaccine, known as MRKAd5, failed to show any efficacy in the STEP trial.

Further fallout from the Merck trial sent the PAVE protocol team back to the drawing board. Based on analyses of the STEP trial, which indicated that male volunteers who received the vaccine candidate had a higher risk of acquiring HIV if they were uncircumcised and had pre-existing immunity to the Ad5 vector, two smaller PAVE trials were proposed. PAVE 100A was to include only 2,400 circumcised men who have sex with men in the US without pre-existing Ad5 immunity. The second part of the trial, called PAVE 100B was to be conducted in Africa. This arm of the trial was later deferred.

On May 30, AIDS vaccine experts met in Bethesda, Maryland, as part of the AIDS Vaccine Research Subcommittee (AVRS) to discuss whether to proceed with PAVE 100A, the US arm of the trial. At the conclusion of that meeting, a clear majority of the AVRS members recommended that NIAID should conduct the PAVE 100A trial. NIAID director Anthony Fauci recalls that only a few were not in favor of moving ahead with PAVE 100A.

But the final decision was his and in July Fauci announced that he had decided not to proceed with either part of the PAVE 100 trial. “A and B are out,” says Fauci. The main reason for his decision was the size of the proposed trial. Fauci concluded that an even smaller study than the 2,400-person trial would be sufficient to primarily study an effect on viral load, which is what most researchers consider to be the best hope for this regimen. “There are very few people who believe that a T-cell vaccine of this sort is going to actually block acquisition of [HIV] infection,” Fauci says. The original plan for PAVE 100A would also have studied in detail the immune responses to the vaccine. But Fauci argues that immune correlates should be studied later, only after the regimen’s ability to lower viral load is established. “I say do a trial that’s big enough to give you the answer if it works,” he says. “If it does work then you have the option of going back and amplifying the study. Why invest in a trial that’s so large that you’ll be able to [study] immunological correlates right away whether [the vaccine candidate] works or not?”

Even though Fauci’s final decision seemed to go against the recommendation of the AVRS, he says there was a “lack of firmness” in the opinions of some committee members. Some who voted yes publicly, later contacted him and said they were not sure. Fauci also took into consideration those who had a vested interest in the trial. Fauci says, after “taking all of this into consideration, there was still a majority that voted yes, but the margin was closer than the count at the meeting on May 30.”

Fauci also consulted another advisory committee, the Strategic Working Group (SWG) of NIAID’s Division of AIDS (DAIDS). “The vote of the strategic working group was unanimously not to go ahead with the trial,” says Fauci, adding that this group consists of “outside, unbiased investigators who have no stake in any of the [AIDS vaccine] clinical trials.”

**Defining a smaller trial**

Fauci has asked Scott Hammer, chair of the PAVE 100 protocol team and a professor of medicine at Columbia University, and Larry Corey, the principal investigator of the HVTN, to come up with an alternate trial design that focuses on whether the VRC vaccine candidates could lower viral load in vaccinated individuals who subsequently become HIV infected. The question now is how small of a trial will provide that answer. Fauci says the new trial would likely be “significantly” smaller than the PAVE 100A design, perhaps involving only 1,200 to 1,400 volunteers. “This would fall under the category of a STOC trial,” Fauci says, referring to a screening-test-of-concept trial. This design allows investigators to conduct smaller, less expensive trials than Phase Ib test-of-concept studies, and still collect information about a candidate’s ability to reduce viral load in infected vaccinees (AIDS 21, 2259, 2007).

STOC trials would only require about 1,000 study volunteers, depending on the HIV incidence in the trial cohort, and would provide answers about which candidates are worth pursuing in larger trials.

At the May 30 AVRS meeting, John Moore, a professor of microbiology and immunology at Weill Cornell Medical College, suggested doing a STOC trial, saying that he didn’t believe the VRC vaccine could be expected to have an effect on HIV acquisition, and therefore the initial trial didn’t have to be large enough to show that. Fauci agrees. “Moore actually recommended what we ultimately decided to do,” he says.

IAVI president and CEO Seth Berkley said he welcomed the fact that NIAID is considering a smaller trial. “These kinds of smaller
studies, such as the screening-test-of-concept trial proposed by IAVI, should become the norm, to test for a sign of promise before proceeding to large efficacy trials,” Berkley says.

But Eric Hunter, chair of the AVRS who expressed support for conducting the PAVE 100A trial at the May 30 meeting, says while a smaller trial is cheaper, it may delay the overall process. “There is a trade-off,” Hunter says. “Certainly such a [small] trial would be much more cost effective in the short term, but [it] has the downside that if it is successful then you have to go back and restart the whole thing again to get an explanation as to why it might have been successful.”

In a statement responding to NIAID’s announcement, the AIDS Vaccine Advocacy Coalition (AVAC) called for the Institute to “act swiftly to clarify the path ahead.” Fauci says he expects a recommendation from Hammer and Corey on a smaller trial design within weeks. “We are in the process of developing a protocol that we feel will respond to the challenges outlined by Dr. Fauci,” says Corey.

So far the response to his decision has been “overwhelmingly positive,” Fauci says, adding that when “you get 100 emails and 99 of them say you have made the right decision, that’s what I would consider [positive],” Stanley Plotkin, an advisor to Sanofi Pasteur and AVRS member, agrees with Fauci’s decision to consider a smaller trial. “I think that the decision is a reasonable compromise,” says Plotkin, who recommended conducting the PAVE 100A trial at the May 30 meeting. “I would say that the PAVE 100 trial was not cancelled, but rather scaled down to determine if the DNA/Adeno approach can do what it is supposed to do, reduce viral load after infection.”

Indeed Fauci does still think it is worth testing the VRC vaccine candidates. “I didn’t completely scrap it because I felt that [it was] different enough from the STEP product that it was interesting to see if it worked," he says.

**Sorting out the differences**

The VRC regimen is somewhat similar to MRKAd5 in that both use Ad5 to introduce HIV genes into the vaccinees. But while the STEP regimen involves three vaccinations with the same Ad5 candidate, the VRC prime-boost vaccine regimen involves three vaccinations with DNA encoding clade B Gag, Pol, Nef, and Env from HIV clades A, B, and C, followed by a vaccination with the Ad5 vector encoding clade B Gag and Pol, and Env from clades A, B and C. MRKAd5 did not contain HIV env. “I felt that the idea of a DNA containing an [HIV] envelope [gene] was an interesting enough concept,” Fauci says.

At the AVRS meeting, Julie McElrath, director of laboratories at the HVTN, presented a preliminary comparison of immune responses in humans collected from the STEP trial to data from a Phase II trial (HVTN 204) of the VRC vaccine regimen. This data showed some differences. The VRC regimen showed stronger CD4+ T-cell responses, but CD8+ T-cell responses and the breadth of responses—the number of HIV epitopes recognized—were similar for MRKAd5 and the VRC’s DNA/Ad5. However, the predominant responses were to different HIV proteins for the two regimens—DNA/Ad5 recipients responded predominantly to Env and Gag epitopes, whereas MRKAd5 recipients predominantly responded to Pol epitopes.

Hunter says antibody or CTL responses directed toward HIV Env epitopes might play a role in reducing viral load. “There is some preclinical data that would suggest addition of env might facilitate control of viral load even in the absence of a neutralizing antibody response,” he says.
Individual armor against HIV

Individuals who have an apparent resistance to HIV infection may hold important clues for AIDS vaccine research, but solving the mystery of this resistance is extremely complex

by Regina McEnery

For legions of scientists trying to unravel the mysteries of HIV and figure out ways to protect against infection, the notion that certain people possess a natural resistance to the virus represents both an intriguing hypothesis and a conundrum.

Two decades of research offer ample evidence that the phenomenon exists. More than 30 different high-risk cohorts have revealed individuals known generally as exposed seronegatives (ESNs), who have evaded infection despite known, sometimes repeated, exposure to HIV. Research involving these individuals has resulted in publication of more than 100 papers, according to Blake Ball, an immunogeneticist at the University of Manitoba involved with one of the longest-running and best-characterized of the cohorts, a group of commercial sex workers (CSWs) from Nairobi, Kenya. Ball works with one of the pioneers of ESN research, Francis Plummer, who began noticing early on in the epidemic that some women inexplicably resisted HIV infection despite participating in commercial sex work (Lancet 348, 1347, 1996). Moreover, HIV incidence among these women actually decreased with an increasing duration of potential exposure to the virus—each year these women participated in sex work, their risk of acquiring HIV decreased.

Other cohorts of ESNs have been identified as well, including men who have sex with men (MSM), the uninfected partners of serodiscordant couples, hemophiliacs who received HIV-contaminated blood products in the early 1980s before blood screening was implemented, and injection drug users. Still, after many years of research within this rare segment of high-risk populations, it is remarkably unclear how these individuals continue to dodge HIV. Is it luck or nature? Good genes that provide grade-A immunity? Most scientists ruled out mere coincidence after Plummer’s landmark study in 1996. At the conclusion of this study the researchers suggested that this highly-exposed subset of HIV-uninfected CSWs in Kenya may possess immunological traits absent in most of the population, which allowed them to fend off HIV or clear it before the virus could establish an infection. The chase has been on ever since to discover what those traits are and why only certain individuals have them.

But identifying and characterizing the possible immunological mechanisms of protection in these individuals has become an exhaustive enterprise that has yielded conflicting, inconclusive, and sometimes controversial results. Some researchers remain deeply skeptical that ESNs mount unique immune responses to HIV and instead favor the theory that these individuals possess some other factor that confers protection. Even those who remain committed to studying ESNs are unsure whether any valuable immunological clues will be unearthed that could contribute to the development of a preventive AIDS vaccine. Still Barbara Shacklett, a microbiologist at the University of California in Davis, thinks it’s important to continue searching for answers. “My view is that there are such individuals who are highly exposed and remain uninfected by our classical definition.”

What’s driving ESNs?

During the roughly two decades since epidemiologists began identifying and tracking ESNs, studies have looked extensively at both innate and adaptive immune responses against HIV in these individuals. These studies have uncovered numerous factors that could play a role in their apparent HIV resistance.

The innate factors have ranged from mutations on the CCR5 coreceptor that prevent HIV from entering cells; upregulation of chemokine production, including overexpression of RANTES, MIP-1α, and MIP-1β; polymorphisms in human leukocyte antigen (HLA) haplotypes that interfere with efficiency of antigen presentation and can figure in either increased susceptibility or resistance to infectious diseases; and autoimmune responses to CD4 or CCR5, which induce antibodies to these coreceptors that neutralize their function and therefore obstruct HIV’s ability to infect cells.

Studies have also looked at adaptive immune responses such as HIV-specific helper T cells, cytotoxic T lymphocytes (CTLs), and humoral immune responses, including mucosally available antibodies (AIDS Rev. 5, 87, 2003). Researchers have also analyzed the infecting virus in both hemophiliac and serodiscordant couple cohorts to determine if any viral mutations may be responsible for impeding transmission. The general belief is that the rare individuals who can demonstrate long-term resistance to HIV must draw on multiple mechanisms—both innate and adaptive immune responses.

“Protection against HIV transmission is probably going to be multifactorial,” says Wim Jennes, a microbiologist at the Institute
of Tropical Medicine in Belgium, who has also been studying ESNs in Africa for nearly a decade. “There probably will be several different factors playing a role. Some of them may have a minor impact or occur in a small number of people, while others occur in a higher percentage. That’s why it is important to continue these studies.”

But so far no evidence exists to suggest that any one of the known factors, or any combination of them, is actually responsible for the ability of some individuals to resist persistent HIV infection. Many of the associations between immune responses and HIV resistance that have been observed in ESNs have eventually been refuted. Among researchers who study these individuals there is still not even a widely accepted definition for ESNs or a standardized way of quantifying exposure in most high-risk groups. Further complicating matters is the fact that several of these reputed protective factors are also observed in HIV-infected individuals. However it is unclear if they are present at the time of HIV infection or only subsequently.

Identifying the factors that may play a protective role in ESNs is complex and the interpretation of studies involving these individuals has been hampered by several limitations. Previous studies have been criticized for the limited size of the study population, for omitting appropriate control groups, or for being unblinded. Other times investigators conducting the studies lacked the technological tools to detect low-level immune responses. As a result many of the studies involving ESNs have not been reproducible and the findings have therefore not been validated.

Researchers at the US Centers for Disease Control and Prevention conducted a meta-analysis in 2003 of all published articles relating to the study of highly-exposed persistently seronegatives (HEPS)—individuals repeatedly exposed to HIV who have undetectable levels of HIV antibodies by standard enzyme immunoassays—and found that some of the most compelling evidence points to an association between CTL activity and resistance to HIV infection in cohorts of CSWs and serodiscordant couples. There is also strong evidence for an association between chemokine receptor mutations, including the CCR5Δ32 mutation, and HIV resistance. This well-documented and studied mutation prevents the expression of CCR5, HIV’s preferred coreceptor, on the surface of cells and therefore can block the virus from infecting cells unless it mutates to gain entry via the CXCR4 coreceptor. But studies have shown that only a small proportion of individuals in the CSW or serodiscordant couple cohorts in Africa and Asia actually have the CCR5Δ32 mutation, suggesting this is not the only answer. Other receptor mutations have also been studied with regard to the HEPS phenotype, but according to the CDC’s analysis, none of them reached the level of being “strongly associated” with resistance to HIV infection. The CDC’s review also found that HIV-specific CD8+ T-cell responses against the Nef, Gag, Pol, and Env proteins of HIV have been detected in a proportion of ESNs from different cohorts, yet absolutely no evidence points to any of these responses as the explanation for an ESN’s apparent resistance to HIV.

**A lucky few**

With only between one in 700 and one in 1,000 estimated sexual acts between discordant couples resulting in HIV infection, it’s difficult to determine precisely what is special about high-risk individuals who remain HIV uninfected.

Shacklett abandoned her research of ESNs mostly for logistical reasons, after relocating from the Aaron Diamond AIDS Research Center in New York City, but she also admits getting cold feet over whether the ESN research would lead to anything conclusive. Shacklett says the HIV transmission study she was working on found what appeared to be CTL responses in some of the 16 HIV-uninfected women who were characterized as ESNs. “But I did feel it was kind of a stretch,” she says, reflecting on the preliminary findings. “The magnitude and breadth of responses was not that compelling so it was hard to know if they were truly protective.”

Within the spectrum of ESNs, Shacklett says there doesn’t appear to be one overwhelming mechanism that protects individuals. Take for example the association between the presence of the mucosal antibody immunoglobulin A (IgA) and resistance to HIV. Separate studies, using different methodologies, have shown that HIV-specific IgA may be present in the genital tracts of ESNs (AIDS 13, 23, 1999; J Immunol. 165, 5170, 2000).

Recently researchers conducted the first prospective, controlled study to look for IgA with direct HIV-neutralization capacity in a Kenyan cohort of CSWs and correlate its presence or absence with subsequent HIV acquisition (AIDS 22, 727, 2008). All immunologic assays in this trial were performed on blinded samples, which the study’s authors argue adds robustness to their data that has been absent from other studies characterizing the role of IgA in ESNs. This study showed that HIV-neutralizing activity in IgA from the genital tract secretions, as well as HIV-specific cellular immune responses in the blood, were significantly associated with HIV protection, says Rupert Kaul, University of Toronto’s research chair in HIV, who was a coauthor of the study.

But this association is not likely to end the debate over what protects ESNs from HIV infection. “I don’t think there is a good strong theory,” says Larry Corey, principal investigator of the HIV Vaccine Trials Network. “Different people have promulgated different ideas and there is no consensus that any are correct,” he adds.
This has been an ongoing controversy in the field for some time. “There is a very active debate over the issues,” Shacklett says. “I remember heated talks at Keystone meetings, almost arguments, over the validity and reproducibility of findings from one cohort to another.” In different cohorts the amount of possible HIV exposure or the route of transmission is variable, and Shacklett says this has made it hard to replicate findings or draw conclusions. Studies of discordant couples allow scientists to sequence the virus the HIV-uninfected partner is exposed to, which helps with the interpretation of HIV-specific T-cell or antibody responses. The HLA types of both partners are also known. But the level of HIV exposure among CSWs in high HIV-prevalence countries is usually greater because they have more sexual partners and use condoms less. This means that the HIV-resistant phenotype found in CSWs is more trusted than it is in HIV-serodiscordant couples and therefore more likely to have resulted from immunological factors within the exposed seronegative individual.

Collaborative study of ESNs

“I do think more collaboration is needed in the field and a consensus definition, as well as a consensus core set of assays, to define ESNs,” says Corey. “That would be helpful.” To better understand this phenomenon, some say an approach similar to the HIV Elite Controller study—a collaborative effort to study a subset of long-term nonprogressors who are HIV infected but are able to control viral replication at undetectable levels—is necessary. The Elite Controller study, led by Bruce Walker, director of the Partners AIDS Research Center at Massachusetts General Hospital, wants to recruit a cohort of 1,000 elite controllers to enable whole genome association studies in these individuals.

“The challenge with exposed seronegatives is defining the phenotype,” says Walker. “Different people have defined exposed seronegatives in different ways and there are fewer quantifiable parameters that are available to define the group than with elite controllers.” In Walker’s opinion, the best group of ESNs to study would be hemophiliacs because only in that case is there clear documentation of exposure to HIV. “That is the purest phenotype,” he says (see Turning to Hemophiliacs for Answers, below).

There are now a few collaborations—some in full swing and others in early stages—that are trying to bring much needed clarity to the study of ESNs by conducting case-control studies in larger, more well-defined cohorts, applying more sensitive methods of laboratory evaluation, and developing a clearer definition of what qualifies an individual as an ESN.

At the same time, the role of innate immunity in HIV infection is now a growing field of study that could ultimately redefine the current understanding of how the immune system operates with regard to HIV. Researchers are now focusing more on the role of innate immune responses in acute HIV infection, with considerable attention being paid to natural killer (NK) cells and their receptors that suggest a prominent role in the progression and

Turning to hemophiliacs for answers

While researchers have primarily concentrated on commercial sex workers and discordant couples in their research of exposed seronegatives, hemophiliacs may provide the most interesting clues about what may be responsible for resistance to HIV.

An alarming number of hemophiliacs were exposed to HIV during the early 1980s after receiving transfusions of HIV-contaminated blood plasma to control their bleeding disorder. Though the number of HIV-exposed hemophiliacs is small compared to other risk groups, their level of exposure was highest because HIV transmits most efficiently when injected directly into the bloodstream.

More than half of the 20,000 Americans with hemophilia were infected with HIV between 1979 and 1985, when an HIV test was finally developed and blood banks began safeguarding their supplies through routine screening of all blood. One of the most famous was US teenager Ryan White, who was diagnosed in 1984 with HIV and by his death in 1991 had already become an international symbol in the fight against AIDS.

But despite receiving regular injections of HIV-tainted blood products, not all of the hemophiliacs who were accidentally exposed to HIV became infected. This small number of individuals, classified as highly-exposed seronegatives, is now the focus of a research study being conducted by the Center for HIV/AIDS Vaccine Immunology (CHAVI), to identify any key genetic determinants of their apparent resistance to HIV.

Researchers are in the process of assembling a cohort of roughly 800 HIV-exposed, yet uninfected, hemophiliacs from the US, UK, Canada, Spain, and Germany who received Factor VIII concentrates between 1979 and 1984. The Factor VIII concentrates were derived from large pools of blood plasma collected from donors, some of whom were infected with HIV.

Managing to evade HIV infection is even more remarkable given the incredibly high risk of infection associated with this route of transmission. Jacques Fellay, a research associate at the IGSP Center for Population Genomics & Pharmacogenetics at Duke University, who is working on the CHAVI study, says a single transfusion or infusion of a blood product containing HIV carries an estimated risk of infection of more than 95%, compared to only a 1-3% estimated risk of infection from exposure through an HIV-contaminated needle.

Researchers affiliated with this CHAVI study will conduct whole-genome analyses of exposed, uninfected hemophiliacs and will compare them to a control group of approximately 1,000 HIV-infected individuals, not hemophiliacs in particular, who were recruited for other CHAVI studies. David Goldstein, a Duke University immunologist heading up the CHAVI study, says the genotyping for this study will employ technology that offers an unprecedented level of genetic information—around 1 million single nucleotide polymorphisms—to locate variants of higher frequency associated with resistance to HIV in the uninfected hemophiliacs.

Andrew McMichael of Oxford University, and principal investigator on this CHAVI study, thinks the genetic research on HIV-uninfected hemophiliacs holds the most promise for solving the ESN puzzle. Goldstein hopes this work will translate into better interventions for preventing HIV transmission, as well as better therapies. “The more we understand how people naturally vary in resistance to HIV, the more information we have to try and develop therapies,” says Goldstein. —RM

RM
perhaps inhibition of HIV. “There is increasing activity in this area in the HIV field, fueled by exciting genetic findings and discoveries about NK cells in other systems,” says Andrew McMichael, an Oxford University immunologist and investigator with the Center for HIV/AIDS Vaccine Immunology (CHAVI).

CHAVI launched a study last year to address many of the conflicting or inconclusive data surrounding ESNs. The project is using more sensitive detection assays to try and nail down whether ESNs from serodiscordant-couple cohorts in Uganda and the UK harbor any detectable levels of HIV. This prospective study will enroll up to 702 participants, making it one of the largest cohorts of ESNs ever evaluated. Identifying extremely low levels of HIV by ultra-sensitive assays might suggest that there is some immune response, either innate or adaptive, against HIV that is capable of preventing the virus from establishing a productive infection.

If traces of HIV are discovered, the CHAVI investigators will then sequence the viruses found in the individuals who were exposed to HIV but remain uninfected, by the traditional definition, and compare it to the virus in the infected partner. Analyses will hone in on the cellular immune responses in the ESNs in particular. Additionally, researchers will conduct genetic analyses of the cohort to see which individuals may have polymorphisms known to be associated with HIV resistance.

If the CHAVI study ultimately finds little difference between unexposed uninfected individuals and highly-exposed uninfected individuals, there would be a weaker argument for the role of HIV-specific immune responses in protecting these individuals from infection. But McMichael is cautiously optimistic. He thinks results from the CHAVI study will help quell some of the skepticism about ESNs. “We are finding some positive responses,” he says. “There may be something there, but the study is still blinded.”

Meanwhile other research groups continue the hunt for different factors that may be responsible for conferring protection against HIV. Ball is exploring the role of interferon regulatory factor 1 (IRF-1), a gene that belongs to a cluster of immunoregulatory genes thought to increase susceptibility to HIV by stimulating HIV transcription. However a recent study of 687 CSWs from the Kenyan cohort that Plummer’s laboratory has been tracking since the mid-1980s located a number of polymorphisms in IRF-1. It was the first report suggesting a viral transcriptional regulator, which is necessary for viral replication, might also contribute to HIV resistance (AIDS 21, 1091, 2007).

Everyone has slight variations of IRF-1 that may or may not impact the triggering of cellular immune responses, but with ESNs, the variations appear to be more complicated. Ball says the initial hypothesis was that ESNs would have higher concentrations of IRF-1 and therefore higher levels of cellular immune responses, but instead they found lower levels of IRF-1. Somehow, Ball says, even with reduced IRF-1 levels these ESN women still develop cellular immune responses against HIV.

“IRF-1 seems to be one of these multifunctional proteins, almost a double-edged sword,” says Ball. “In one case, it is important in triggering cellular immune responses. Secondly it is exploited by HIV, like many other host factors, to help it replicate.” Ball’s lab is now trying to define the precise mechanism of IRF-1 in ESN women.

Meanwhile Jennes and colleagues conducted an extensive genetic analysis of a small cohort of CSWs from Cote d’Ivoire and found unusual interactions between killer Ig-like receptors (KIR) and human leukocyte antigen (HLA) molecules in exposed seronegative CSWs. The group focused on this piece of the genetic puzzle because KIR and HLA genes function as reins on the functions of NK cells. The findings suggest that KIR/HLA interactions, which previous studies associate with slow disease progression, may also influence viral transmission (J. Immunol. 177, 6588, 2006).

In the study, the women were compared to HIV-infected CSWs and HIV-uninfected female blood donors from the same west African cohort. The analysis revealed that CSWs in the ESN group more often lacked the HLA ligand genes for their inhibitory KIR genes, compared to the HIV-infected CSWs. Also, ESNs more frequently possessed a B KIR haplotype, which contains a high number of activating KIR genes, than HIV-infected CSWs.

Jennes suggests that when HLA molecules are subtracted from the immunological equation, a chain reaction occurs that ultimately frees the NK cells to eliminate HIV-infected cells before a systemic infection is established. But scientists have been unable to determine the roles activating and inhibitory NK cells play against HIV, and efforts to replicate results observed in the cohort from Cote d’Ivoire have been limited by sample size and even political tensions. Researchers left the site in Cote d’Ivoire in 2004 because of ongoing civil unrest and now Jennes’ group is tracking an ESN cohort of discordant couples in Senegal.

Jennes says if researchers can better identify the mechanisms that drive NK cells, it might be possible to manipulate them to block the KIR/HLA interaction. “It is important to continue with these studies,” he adds. “Knowing that HIV protection is probably multifactorial, different ESN classes may reveal different protective factors,” says Jennes. “Every new finding may provide a new mechanism.”
We really ought to try heroic measures because we have nothing to lose,” says David Baltimore, a professor at the California Institute of Technology (Caltech), referring to AIDS vaccine research. Baltimore, who was a co-recipient of the Nobel Prize in 1975 for his work in the discovery of reverse transcriptase, is pushing the envelope in HIV research by pursuing gene therapy in an effort he refers to as “engineering immunity” against the virus.

So far researchers have had little success in inducing protective immunity against HIV. Early approaches to induce neutralizing antibody responses against the virus were unsuccessful, causing many researchers to shift strategies and focus instead on candidates that induced primarily cellular immune responses against HIV. But after the failure of Merck’s T-cell based vaccine MRKAd5 last year—regarded as one of the most promising cellular immunity vaccine candidates—this approach was also called into question. “I suspected that T cells were not going to be the whole answer,” says Baltimore. “I didn’t think they would turn out as badly as they did,” he adds, referring to the failure of MRKAd5. “Antibody approaches weren’t going anywhere [either], so it looked to me like we were in a position where it was possible we were going to end up with no AIDS vaccine,” Baltimore explains.

While many researchers are now focusing on innovative ways to develop improved AIDS vaccine candidates that can induce both neutralizing antibodies and cellular immune responses against HIV, Baltimore and others, including Philip Johnson, chief scientific officer at the Children’s Hospital of Philadelphia, are trying a novel approach. They are developing ways to introduce genes encoding antibodies into people that are capable of neutralizing a variety of HIV isolates in vitro. “The more standard measures [to develop an AIDS vaccine] were getting a lot of attention from a lot of people,” Baltimore says. “If there was a way to make them work they were going to get them to work, so they didn’t need me for that.”

Both Baltimore and Johnson are using viral vectors to introduce antibody genes, so far with varying degrees of success. Baltimore is leading a gene therapy project that uses an HIV-derived lentivirus as a vector to carry antibody genes into bone marrow-derived hematopoietic stem cells (HSCs). These, he hopes, will then become antibody-expressing B lymphocytes. Meanwhile, Johnson is working on a gene transfer approach, using an adeno-associated virus (AAV) as a vector to carry antibody genes. Johnson is collaborating with Ron Desrosiers, a professor at Harvard Medical School, who is using a monkey herpes virus vector to introduce antibody genes into cells.

So far Baltimore and his collaborators have been able to successfully incorporate genes into HSCs of mice, and are developing ways to evaluate the expression of antibody genes in human B cells after introducing them into HSCs. Meanwhile Johnson has shown expression of antibody genes in muscle cells of nonhuman primates that offer some protection against simian immunodeficiency virus (SIV).

Identifying antibodies

Although researchers are actively searching for broadly neutralizing antibodies against HIV, only about five have been identified so far from HIV-infected people. There is some disagreement among researchers about how many different isolates an antibody has to neutralize to earn the classification of being broadly neutralizing. “It depends where you draw the line,” says Dennis Burton, professor of immunology and molecular biology at the Scripps Research Institute. Even though the handful of already identified antibodies has been well studied, it’s still unknown how to induce them through vaccination. “Nobody knows how to design an immunogen that you can inject into people to give rise to these responses,” Baltimore says.

But there is evidence that if you could induce them in humans, they might do the trick. In passive immunization experiments, administration of the already identified broadly neutralizing antibodies has been shown to protect humanized mice—mice engineered to have human immune cells—as well as rhesus macaques from challenge with HIV or a hybrid simian/human immunodeficiency virus (SHIV), respectively (Nat. Med. 3, 1389, 1997; J. Virol. 73, 4009, 1999; Nat. Med. 6, 207, 2000; J. Virol. 75, 8340, 2001; Nat. Med. 9, 343, 2003).

Generally, relatively high serum antibody concentrations are required to provide complete protection in monkeys, according to Burton. In one study, intravenous transfer of 25 mg/kg body weight of the human broadly neutralizing monoclonal antibody b12 six hours prior to challenge protected four out of four monkeys from vaginal SHIV challenge, but a smaller dose of five mg/kg body weight only provided two out of four monkeys (J. Virol. 75, 8340, 2001). Similar challenge studies are obviously impossible in humans, but infusing antibodies into people already infected with HIV has been shown to delay rebound of viral load after interruption of antiretroviral (ARV) therapy (Nat. Med. 11, 615, 2005; J. Virol. 81, 11016, 2007). However, to ensure a constant supply of antibodies in humans, they might do the trick. In passive immunization experiments, administration of the already identified broadly neutralizing antibodies has been shown to protect humanized mice—mice engineered to have human immune cells—as well as rhesus macaques from challenge with HIV or a hybrid simian/human immunodeficiency virus (SHIV), respectively (Nat. Med. 3, 1389, 1997; J. Virol. 73, 4009, 1999; Nat. Med. 6, 207, 2000; J. Virol. 75, 8340, 2001; Nat. Med. 9, 343, 2003).

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That's why researchers, including Baltimore and Johnson, are developing ways to produce a continuous supply of these antibodies by introducing the genes that encode them into human cells. At the same time, researchers are also trying to engineer improved antibodies or antibody-like molecules that will perhaps be even better than the naturally occurring ones at inhibiting HIV from infecting its target cells.

Success in mice

A few years ago, Baltimore showed in experiments with mice that it is possible to use a retrovirus to deliver T-cell receptor genes specific for certain kinds of tumors into HSCs isolated from their bone marrow. When the HSCs were transferred back into the bone marrow of mice, they developed into T cells that expressed the tumor specific T-cell receptor (Proc. Natl. Acad. Sci. 102, 4518, 2005). "I said well, if you can do it with T-cell receptors, you should be able to do it with antibody genes," says Baltimore.

He is now testing this approach with an HIV-derived lentivirus to carry antibody genes into CD34+ HSCs. He hopes these cells will multiply and develop into antibody producing B cells, which could then provide a lifelong supply of antibodies. Ultimately, Baltimore's goal is to utilize this gene therapy approach as a strategy to prevent HIV infection, but for now he is focusing on testing this approach in HIV-infected people because he says it is easier to conduct gene therapy trials when it is a therapeutic approach.

Still, even as an experimental therapy, this is an elaborate strategy. For example, Baltimore's approach would require a bone marrow transplant—researchers would remove bone marrow, insert the antibody genes into HSCs isolated from the marrow ex vivo, and then transfer them back into the volunteer. Each procedure could cost as much as a couple of thousand dollars for a single volunteer and could be especially difficult to do in developing countries, says Pin Wang, an assistant professor at the University of Southern California, who is collaborating with Baltimore's group.

Wang is currently trying to develop a way to introduce the antibody genes with a simple injection, rather than a transplant. To accomplish this he has engineered an HIV-derived lentivirus vector that he hopes can target CD34+ HSC target cells following injection directly into the bone marrow. The vector carries two proteins on its surface, an antibody that recognizes the CD34+ receptor on the HSCs, and a fusion protein that enables it to fuse with the HSCs so it can introduce its genetic payload. This approach would lower the cost substantially to about US$100 per procedure, Wang estimates.

To provide proof of concept Wang is conducting experiments using injections of the engineered lentivirus vector to try to introduce a luciferase gene into the bone marrow of humanized mice that have CD34+ HSC target cells following injection directly into the bone marrow. The vector carries two proteins on its surface, an antibody that recognizes the CD34+ receptor on the HSCs, and a fusion protein that enables it to fuse with the HSCs so it can introduce its genetic payload. This approach would lower the cost substantially to about US$100 per procedure, Wang estimates.

For more information, see Figure 1. Testing for gene expression in humanized mice. Human CD34+ hematopoietic stem cells (HSCs) can be seen expressing a luciferase gene in the bone marrow and spleen of a humanized mouse. The luciferase gene was introduced by injecting an HIV-derived vector into the bone marrow of the mouse, providing proof of concept that this simplified approach can work. The vector has proteins on its surface that enable it to target CD34+ HSCs and therefore a bone marrow transplant is not required to introduce modified HSCs. The mice are generated by injecting human cord blood CD34+ HSCs. After 60 days they are injected with the vector and then two weeks later, Luciferase expression can be seen in the bone marrow and the spleen (shown at day 14). Red areas show the highest level of Luciferase expression and blue shows the lowest level. Image provided by Pin Wang, University of Southern California.

It’s impractical to inject someone for the rest of their lives with an antibody

Pamela Bjorkman

That's why researchers, including Baltimore and Johnson, are developing ways to produce a continuous supply of these antibodies by introducing the genes that encode them into human cells. At the same time, researchers are also trying to engineer improved antibodies or antibody-like molecules that will perhaps be even better than the naturally occurring ones at inhibiting HIV from infecting its target cells.
into the genome of the HSCs, it’s still unclear if the B cells derived from these human HSCs can express the antibody proteins the same way they do naturally. Baltimore says experiments are underway to test this in vitro, as well as in humanized mice. To test it in vitro, Baltimore matures human HSCs into B cells by treating them with a certain combination of growth factors. In humanized mice, researchers can infect human CD34+ cells with the lentivirus carrying antibody genes in vitro, and then inject the HSCs into the mice, where they mature into human B and T cells, Baltimore says. If the antibody proteins are expressed by the human B cells, then researchers can test if they provide protection by infecting the human T cells in these mice with HIV. “We are in the process of doing that,” Baltimore says. “If we can make that work then the next step might be monkeys or maybe even humans depending on how it looks.”

But even if successful in nonhuman primate studies, gene therapy has a checkered past and conducting this type of study in humans might raise safety concerns. Several years ago, a gene therapy trial to treat children with x-linked severe combined immunodeficiency (X-SCID) using a retroviral vector caused leukemia because the vector integrated into the region of an oncogene. Baltimore says there is less of a concern of cancer with using an HIV-derived lentivirus as a vector because HIV integration happens millions of times every day in HIV-infected people without causing cancer. “That is one reason I consider our method safer than the trials for X-SCID,” he says.

**Better than nature**

Baltimore’s group is currently working with the broadly neutralizing monoclonal antibody b12, among others, and if his gene therapy approach proves successful, eventually researchers could also utilize this method to deliver modified antibodies that are even better than the naturally occurring ones that have already been characterized. “If you could deliver antibody genes to HSCs and have them function in B cells,” Baltimore says, “then you would liberate the whole scientific community to use their design methods to make better antibodies or antibody-like proteins.” He is currently collaborating with Bjorkman’s group, which is trying to engineer improved antibodies that Baltimore’s lab will then test in animal models. “We can introduce whatever we want into our synthetic gene,” Bjorkman says. She expresses her engineered proteins in cultured cells and then, in a neutralization assay, infects cultured cells with HIV to test if HIV can still infect these cells in the presence of the engineered proteins. “We dream up strange things that we then test for neutralization,” Bjorkman says.
One effort to improve upon nature involves combining structures from several naturally occurring antibodies into one protein (see Figure 2). Developing a combination protein from several antibodies might help prevent HIV escape mutants, Bjorkman says, likening it to the way ARV therapy is most effective with a combination of three or more drugs. In addition, a combination antibody protein will bind to HIV proteins like gp120 more tightly than just one antibody, because it would be more difficult to dissociate all of them. “If you link five things together, it’s almost impossible to remove it,” Bjorkman says. “So it’s kind of glued there.”

Bjorkman is now trying to combine the genes for binding sites from several of the known broadly neutralizing antibodies to see if they are better at blocking HIV from infecting its target cells or are effective on a wider range of strains. She is also trying to engineer smaller versions of the known antibodies because she says they are often too big to fit in the narrow space between the target cell and HIV. “We can link [several of] them together in a smaller format,” Bjorkman says.

Some antibodies will only bind to gp120 that is bound to the CD4 receptor, so another option is to link the antibody proteins to a CD4 receptor, which would bind to the HIV Env protein and induce a conformational change. Additionally, her lab screens for mutants in antibody genes that allow them to bind more tightly to gp120, and uses 3D protein imaging to identify structures that would fit better. This might result in engineered antibodies that would be effective at lower concentrations, Bjorkman adds. “We are still far from a complete understanding,” she cautions. “Using 3D protein imaging to enable a rational design of new architectures is still quite challenging.”

So far, improving on nature has not been so easy. Some of the engineered proteins, for example, are impossible to express in cultured cells. In addition, Bjorkman says, “We keep managing to make things that decrease the ability to neutralize HIV [in vitro].” However, she says there are a few promising leads. “We have some multimeric versions of existing antibodies that work better [than the parental antibodies].”

Muscling their way in

Meanwhile Johnson is working on a different approach that involves using AAV to carry DNA encoding antibody into muscle tissue, to make the cells express the HIV antibodies. In contrast to Baltimore’s gene therapy approach, the DNA carried by AAV does not integrate into the genome of the muscle cells. Instead, it stays in the nucleus as a so-called episome, and the cell then expresses the antibody genes, Johnson says.

He says the primary goal of this work is to develop a way to prevent HIV infection. In some ways this may be easier than treating HIV-infected individuals, according to Johnson. Once HIV has mutated extensively it is more likely to develop escape mutations to the antibody. In contrast, he says, there are very few different viruses that are responsible for actually establishing an HIV infection in a single person. A recent analysis of env sequences in HIV-infected people shows that often a single virus is responsible for establishing infection (Proc. Natl. Acad. Sci. 105, 7552, 2008). “There are very few viruses that make it through the bottleneck,” Johnson says. “You have an Achilles’ heel for the virus if you have the antibodies there at the right time, and that is at the time of initial infection.”

Johnson’s approach has already been tested in mice and rhesus macaques with encouraging results. His group showed a few years ago that an injection of AAV carrying DNA encoding the broadly neutralizing monoclonal antibody b12 into mice leads to expression of antibodies in their blood (J. Virol. 76, 8769, 2002). And in preliminary, still unpublished experiments, his group injected AAV with DNA encoding three different antibodies that are all known to neutralize SIVmac316 in vitro, into rhesus macaques. One year after vaccination the nonhuman primates are still expressing high levels of antibody in their serum. “We are very encouraged that this will go on for a very long time,” Johnson says.

He vaccinated three groups, each of three monkeys, with slightly different antibody genes and one month later challenged them with intravenous injection of SIVmac316, a derivative of SIVmac239. All six control monkeys became infected, and four of them have since developed AIDS. But of the vaccinated monkeys, all three in one group were protected, two of three were protected in a second group, and one of three in the third group remained uninfected. The reason for the variation in the level of protection is still being investigated.

In his next experiments, Johnson wants to determine the dose of antibody that will be necessary to achieve protection. He also plans to challenge the vaccinated macaques either rectally or vaginally to more closely mimic the primary mode of HIV transmission. To be protected from such a mucosal challenge, the antibodies would have to be present in mucosal tissues. Johnson says a recently approved protein-based vaccine against human papilloma virus provides evidence that a vaccine injected intramuscularly can protect against sexually transmitted viruses, suggesting antibodies are available at the site of infection.

His current goal is to conduct a clinical trial to see if injecting humans with an AAV vector carrying genes for broadly neutralizing antibodies like b12, results in production of the antibody. The proof that this can work is already there from monkey experiments, he says. “We have clearly shown that we can do this in monkeys and that it’s effective,” Johnson says, adding that the challenge now is to show the approach is safe in humans. Due to issues surrounding regulatory approval, it will be at least three years until a clinical trial of this approach will be underway, says Johnson. “The challenge is how you answer the safety questions the [US Food and Drug Administration] and others are going to have.”
AIDS Vaccine Blueprint launched: A challenge to the field

The AIDS Vaccine Blueprint 2008, IAVI’s biennial report on the state of AIDS vaccine research and development and a roadmap for the field, was released at the XVII International AIDS Conference in Mexico City August 3-8. It issues several challenges to AIDS vaccine researchers, setting interim goals and key milestones by which the field can measure progress.

Among the key scientific challenges it highlights are determining the mechanisms responsible for control of HIV infection in elite controllers, individuals who naturally suppress HIV replication to undetectable levels without the help of antiretroviral therapy, and elucidating the mechanism by which live-attenuated simian immunodeficiency virus (SIV) protects nonhuman primates against viral challenge. The Blueprint also recommends shifting resources away from the development of the majority of AIDS vaccine candidates that are currently in the clinical pipeline, since they are unlikely to be effective.

The publication, which IAVI has been producing since 1998, strikes a different theme and tone than two years ago, when more than two dozen AIDS vaccine candidates were moving through the clinical pipeline, including Merck’s cellular immunity vaccine MRKAd5, which many researchers regarded as the most promising. This vaccine candidate was based on a genetically altered adenovirus serotype 5 (Ad5) vector that carried HIV gag, pol, and nef genes.

Merck stopped immunizations in the Phase Ib test-of-concept STEP trial last September after the vaccine candidate failed to prevent transmission of HIV or reduce the viral load in vaccine recipients that subsequently became HIV infected.

“Two years ago, we all thought we had a signal of hope from Merck,” said Seth Berkley, president and CEO of IAVI. “What has happened is we’ve learned a lot about the science.” Because most of the AIDS vaccine candidates in clinical development employ similar strategies to Merck’s, the IAVI Blueprint urges stakeholders to “review their portfolios and drop candidates considered to have a low probability of success.”

IAVI suggests that the resources being spent on the current pipeline of candidates should be reallocated to developing a more diverse clinical pipeline. “Science is not a straight line,” said Alan Bernstein, president of the Global HIV Vaccine Enterprise, commenting about the recent setbacks in the AIDS vaccine field. “Failure is part of the game. It’s clear after 25 years that we are on a long journey.” Bernstein added that any long journey requires a roadmap, and that the Blueprint fulfills that role.

To develop new and improved vaccine candidates, the Blueprint suggests greater focus be placed on research to identify the best HIV immunogens for inducing both cellular and antibody responses against the virus. “Until now, the field has focused more on how to deliver antigens and less on the antigens themselves,” the document’s authors write.

But the Blueprint also recommends the exploration of live replicating viral vector-based vaccine candidates. To determine which of these candidates to advance into clinical trials, IAVI suggests that they be evaluated in comparative studies in the nonhuman primate model and that a “useful initial yardstick” would be comparing them against the Ad5 vector used in Merck’s vaccine candidate.

Other recommendations set forth include enhancing discovery efforts to identify new broadly neutralizing antibodies and translating the information about the binding sites of these antibodies into the design of new HIV immunogens, conducting small efficacy trials on leading candidates to see if they achieve pre-determined criteria, establishing incentives to enhance innovation in AIDS vaccine discovery, and training the next generation of AIDS vaccine scientists.

Omu Anzala, an AIDS vaccine researcher at the University of Nairobi School of Medicine, said it would be a big mistake to abandon research efforts in developing countries. “We cannot afford not to be part of [vaccine discovery],” said Anzala.

Peter Piot, the executive director of the Joint United Nations Programme on HIV/AIDS (UNAIDS) said despite declines reported by his agency in the number of HIV infections in some of the hardest-hit countries, there are still 33 million people living with the virus and an estimated 7,500 new HIV infections reported every day. “We are making progress through antiretroviral therapy, but there is no doubt we need a vaccine,” said Piot. — Regina McEnery
Passage of PEPFAR

President Bush recently signed into law a revised version of the President’s Emergency Plan for AIDS Relief (PEPFAR) authorizing US$48 billion in funding over the next five years to expand existing HIV/AIDS prevention, treatment, and care efforts worldwide. The original five-year, $15 billion plan was due to expire in September. The revised version more than doubles the amount of funding for HIV/AIDS prevention, treatment, and care programs, and also authorizes $9 billion in funding for malaria and tuberculosis programs.

The reauthorization aims to prevent 12 million new HIV infections and provide antiretrovirals (ARVs) to at least three million HIV-infected people in need over the next five years. It also allows for spending on support services for 12 million people affected by HIV/AIDS, including five million orphans and vulnerable children, and for training 140,000 new healthcare workers.

The US Senate passed the legislation last month after lengthy discussions concerning appropriate funding levels for prevention, treatment, and care efforts. The new plan does not require that one-third of total funding be spent on abstinence programs, a controversial stipulation in the original legislation, and instead calls for the Global AIDS Coordinator to provide “balanced funding” for prevention programs. However, another controversial component remains in the new legislation—all recipients of PEPFAR funding must sign a pledge demonstrating their opposition to prostitution.

The reauthorization took an important step in the process of overturning a two-decade restriction that banned visitors or immigrants living with HIV/AIDS from entering the US by removing from the bill a provision of the Immigration and Nationality Act. This restriction was partly why the US has not hosted one of the large biannual International AIDS Conferences since 1990, when it was held in San Francisco. The US Department of Health and Human Services now must remove HIV from the list of “communicable diseases of public health significance” for the ban to be completely lifted.

A section of the new PEPFAR bill also contains provisions related specifically to facilitating the development of vaccines, including those against HIV/AIDS, tuberculosis, and malaria. The reauthorization allows the US to negotiate with organizations—including the World Bank and the GAVI Alliance—to pursue the use of advanced market commitments, a novel market incentive that is meant to stimulate vaccine research and development efforts for diseases that primarily affect developing country populations.

The PEPFAR legislation also requires the US President to report to Congress within one year on a strategy for accelerating the development of vaccines for HIV/AIDS, malaria, and tuberculosis, as well as other infectious diseases. This strategy would include details on creation of economic incentives for research, development, and manufacture of these vaccines, as well as the efforts taken by the US to support clinical trials of vaccines in developing countries and to prepare these countries for the introduction of new vaccines.

During the deliberation process, many individuals and organizations involved in HIV/AIDS prevention, treatment, and care praised President Bush and Congress for initially authorizing PEPFAR, as well as for the plan’s accomplishments to date. The original bill in 2003 was the largest single funding commitment by any government to combat a single disease. Over the past five years, PEPFAR has supported the provision of life-saving antiretroviral treatment for approximately 1.7 million HIV-infected people. —Jonathan Grund, contributing writer

Treating people to prevent the spread of HIV

Researchers from the British Columbia Centre for Excellence in HIV/AIDS presented a study at the XVII International AIDS Conference, held in Mexico City from August 3-8, that used mathematical modeling to determine that expanding access to life-saving HIV/AIDS treatment, and care efforts worldwide. The revised version more than doubles the amount of funding for HIV/AIDS prevention, treatment, and care programs, and also authorizes $9 billion in funding for malaria and tuberculosis programs.

The reauthorization aims to prevent 12 million new HIV infections and provide antiretrovirals (ARVs) to at least three million HIV-infected people in need over the next five years. It also allows for spending on support services for 12 million people affected by HIV/AIDS, including five million orphans and vulnerable children, and for training 140,000 new healthcare workers.

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Researchers from the British Columbia Centre for Excellence in HIV/AIDS presented a study at the XVII International AIDS Conference, held in Mexico City from August 3-8, that used mathematical modeling to determine that expanding access to life-saving highly-active antiretroviral therapy (HAART) could potentially reduce the number of new HIV infections by up to 60% over the next 25 years (J. Infect. Dis. 198, 550, 2008).

Researchers involved with the study say they included three major high-risk populations for HIV infection in the model—men who have sex with men (MSM), injection-drug users (IDUs), and MSM who were also IDUs. They also varied the model to account for different adherence rates and guidelines for initiating therapy, and still their mathematical model consistently predicted that providing ARVs to at least 75% of individuals worldwide who are clinically eligible for treatment would result in a substantial decrease in the number of new HIV infections. “Basically the more people you treat and the faster you engage people in treatment, the greater the impact you will have on the epidemic,” said Julio Montaner, president-elect of the International AIDS Society.

HIV-infected individuals on HAART who adhere to treatment often decrease their plasma HIV RNA levels to below the levels of detection by currently available assays. Initiation of HAART has also been associated with marked reductions in HIV RNA levels in genital secretions in men and women, suggesting they will be less likely to transmit HIV to others (AIDS 14, 415, 2000). More recently, HAART has been shown to be associated with a decrease in HIV transmission between serodiscordant heterosexual couples, despite repeat exposure (J. Acquir. Immune Defic. Syndro. 29, 275, 2002; J. Acquir. Immune Defic. Syndro. 40, 96, 2005).

Montaner and his colleagues on this study advocated strongly at the Mexico City conference for a major expansion of access to ARVs in developing countries as a way of reducing the number of new HIV infections. —Regina McEnery