HIV prevalence estimates: Fact or fiction?

Better data have led to a drop in the estimated global HIV prevalence in some countries, but the epidemic still continues to outpace international responses

by Kristen Jill Kresge

Science and politics often clash—consider embryonic stem cell research or the even more quotidian debate over global warming and its consequences. These and many other issues are hotly contested in both political and scientific circles.

Politics has always been at the forefront of the HIV/AIDS pandemic. Even before it had a name, HIV was a political issue. In the days when it first started spreading in the US, rapidly killing those who became infected, the people who would soon be branded AIDS activists implored the US government to openly discuss and actively confront this new disease. As a result there is more legislation singly devoted to HIV/AIDS than any other disease.

Now some are suggesting that science and politics may be colliding again—this time in the fundamental way the scope of the global HIV/AIDS epidemic is measured. Some epidemiologists have called into question the accuracy of global HIV prevalence estimates, which represent the total number of people who are thought to be infected with the virus in a region or country at a specific point in time. Prevalence figures are used by governments, public-health agencies, and donor organizations to gauge the severity of the pandemic and this, in turn, drives decisions about how and where money is spent on both HIV prevention and treatment. These estimates are regularly updated by the The Joint United Nations Programme on HIV/AIDS (UNAIDS), in partnership with the World Health Organization (WHO), but the responsibility of collecting the data falls to the individual countries.

In recent years many of the HIV prevalence estimates have been revised based on improved data and in almost all cases the new estimates are lower than previously thought, sometimes dramatically so. As a result the total number of people in the world thought to be infected with HIV keeps going down. A few years ago UNAIDS estimated that 42 mil-

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lion people were HIV infected. Now the number stands just below 40 million, according to the 2006 Report on the Global AIDS Epidemic. The question about the accuracy of the estimates was pushed to the forefront recently when India cut its HIV prevalence numbers by half. UNAIDS had previously estimated that India had over five million HIV-infected individuals, meaning it had more people living with HIV than any other nation.

This has led some to accuse UNAIDS of crying wolf about the severity of the global pandemic—some of the harshest critics even suggest UNAIDS is purposely exaggerating prevalence figures to sustain political momentum and funding for HIV prevention and treatment programs. But even if a more accurate picture of the global HIV/AIDS pandemic shows that prevalence is lower than originally thought, everyone agrees it still warrants urgent attention. “Even if you cut the [HIV prevalence] numbers in sub-Saharan Africa in half, it’s still a huge problem,” says James Chin, a retired epidemiologist and faculty member at the University of California, Berkeley who is one of the most outspoken critics.

Getting better data

HIV prevalence estimates are generated by epidemiologists using HIV infection data from small subsets of the population that can be extrapolated using mathematical models. These models combine national population estimates and epidemiological data collected in a country and then churn out estimates of national HIV prevalence, based on a series of assumptions. In South Africa, where there is the largest number of HIV-infected individuals, the national HIV prevalence among adults between the ages of 15 and 49 is estimated by UNAIDS to be nearly 19%. The number of HIV infections is not evenly distributed within the population—many countries have epidemics that are still mainly contained within certain regions or in groups that are at especially high risk, such as injection-drug users (IDUs) or commercial-sex workers (CSWs). In some regions of South Africa or in high-risk populations, the prevalence estimates can be twice as high as the national estimate.

Since its inception in 1995, UNAIDS, along with WHO, has released annual estimates of regional HIV prevalence and bimannual estimates of national HIV prevalence that serve as the standard measure of the extent of the pandemic. These numbers are one of the primary drivers behind decisions about funding for AIDS-related prevention and treatment programs worldwide and therefore receive a great deal of international attention.

Over recent years and months, many of the UNAIDS estimates have been revised. Most of these revisions reflect substantial downgrades in the numbers, indicating that there are fewer HIV-infected individuals than previous estimates suggested (see Figure 1). Several factors contribute to this revision of HIV prevalence, including the increased or improved surveillance of HIV infection in many countries, better population estimates, and more accurate models for estimating prevalence on a population basis. The positive influence of HIV prevention campaigns also plays a role, though it is often difficult to directly pinpoint. In Uganda, many epidemiologists have suggested that the dramatic drop in HIV prevalence in the mid-1990s was at least partly due to the high death toll of individuals who were infected early on in the epidemic.

But in most cases the revisions to the UNAIDS estimates are based on the collection of better data that more accurately represents the burden of HIV infection in individual countries. In its 2006 update on the pandemic, UNAIDS said that “new systems, including greatly improved surveillance, tell us with increasing accuracy where and how the epidemic is moving.” Many countries are conducting more rigorous surveillance of their epidemics, both in the general population and in high-risk groups, by either increasing access to voluntary counseling and testing services or conducting household surveys that are part of the broader demographic and health surveys (DHS). These population-based surveys allow researchers to track the spread of several diseases in developing countries and monitor trends in overall health. In DHS surveys, researchers randomly visit households in a community and collect medical information from the available family members. Recently this survey was altered to include collection of a saliva sample that could later be used to conduct an HIV test.

Previous prevalence estimates have been based primarily on sentinel surveillance data collected from pregnant women who visited antenatal clinics, one of the few settings where there is mandatory HIV testing. The original method of projecting prevalence
based on data from antenatal clinics was established in the 1980s by Chin when he was working at the Global Program on AIDS at WHO, years before the job of tracking the pandemic came under the purview of UNAIDS. He thought HIV prevalence data collected from sexually-active women would be a good surrogate for national prevalence.

But in most cases this data was not representative of HIV infection for the entire population. Most antenatal clinics are located in urban areas, where HIV prevalence is generally much higher, and the pregnant women who would take advantage of healthcare generally have a higher income, which introduced another bias. When Zambia conducted the country’s first population-based health study it found that estimates for HIV prevalence based on antenatal clinic data correlated very closely in urban areas, but that neglecting rural populations led to a gross overestimation of national HIV prevalence.

“Data from antenatal clinics help monitor trends over time,” says Karen Stanecki, a senior advisor at UNAIDS in Switzerland. “The intent [with data from pregnant women] is to monitor changes, not to predict the actual number of people who are infected,” says Prabhat Jha, professor of epidemiology at the Center for Global Health Research at the University of Toronto.

Watch out for falling estimates

Following pressure from donor organizations to come up with more accurate prevalence estimates, more countries began conducting population-based surveys, often leading to a drop, sometimes precipitous. Kenya reduced its estimated HIV prevalence in 2003 after conducting a population-based survey, from 2.3 million HIV-infected individuals to 1.2 million. “That was a huge reduction,” Chin says.

Following that, more than a dozen other countries conducted population-based surveys that led to revisions in the UNAIDS prevalence estimates. In Ethiopia the number of HIV-infected individuals was cut by half to one million. Cambodia also lowered its national prevalence estimate, from 1.8% of the population to less than 1%. India is one of the latest countries to release new figures indicating that the estimated national HIV prevalence is only half that previously projected by UNAIDS.

Chin suspects that prevalence figures might also have been overestimated in the US. In 1986 it was estimated that there were between 1 and 1.5 million people infected with HIV nationally, and the fact that the numbers have not changed since then suggests to Chin that the original figures were an overestimate.

Now 30 countries have conducted population-based surveys to help better gauge the extent of their HIV/AIDS epidemics. In Benin, Mali, and Niger the results from these surveys were very similar to the figures estimated using sentinel surveillance data from antenatal clinics, but in the majority of cases the new figures were lower. “There are still plenty of countries that haven’t done these types of

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Figure 1. Adjusted HIV prevalence estimates. The first column shows the HIV prevalence estimates for 2003 that were reported by UNAIDS in 2004. The middle column shows the adjusted estimates based on data from household surveys in these countries. The latest prevalence figures are shown in the third column. Data provided by UNAIDS based on the 2004 and 2006 Report on the Global AIDS Epidemic. For more information, go to http://www.unaids.org/en/HIV_data/2006GlobalReport/default.asp.
The number of adults and children estimated to be living with HIV/AIDS in 2006, according to the 2006 UNAIDS Report on the Global AIDS Epidemic.

The onus of collecting better data falls on the individual countries that have to finance and conduct population-based surveys. “We [UNAIDS] don’t do any surveys,” says Stanecki. “Surveillance is done by the countries themselves.” UNAIDS and WHO just work with countries, holding regional training workshops on the modeling tools, and assist with calculating national HIV prevalence estimates.

Politics at play

There are obvious political reasons both for and against individual nations collecting better data on the scope of the HIV/AIDS epidemic. Some countries are motivated to conduct household surveys to show that the epidemics are not as bad as estimates suggest and to prove to the international community that the government is handling the epidemic. Other countries may be leery of showing that there is less of an HIV/AIDS problem because it could result in funding cuts for the country’s AIDS-related programs. This controversy was reignited when India’s National AIDS Control Organization (NACO) released new prevalence estimates in July, in cooperation with UNAIDS and WHO.

NACO reported that the new estimates were the result of a considerable increase in the number of HIV testing sites in both rural and urban areas and in low-prevalence Indian states, as well as the conduct of comprehensive household surveys. Most agree that these new estimates are more accurate than before, Jha refers to the previous prevalence estimates in India as “guessimates” and says that the “sources for the new data are better, but still not perfect.” He also points to two supporting pieces of evidence that
corroborate that the Indian epidemic is not as extensive as originally thought. A study published in 2006 showed that HIV prevalence in Southern Indian states, where two-thirds of the HIV-infections are located, were declining and other surveys indicated that AIDS mortality rates were less than original UNAIDS/WHO estimates (*Lancet* 367, 1164, 2006). But there is still a risk that basing the new prevalence estimates on household surveys, which limit access to high-risk individuals, may underestimate the scope of the problem.

Staneczki defends the new estimates for India. “The estimates that were done in the past were based on limited data and we now have better information,” she says. “We recognize uncertainty in the estimates with ranges and [for India] it’s now a much smaller range.” But Jha says it is still a pretty wide confidence interval for the Indian prevalence estimates.

**Strange bedfellows**

As HIV prevalence estimates continue to be downgraded, some epidemiologists are questioning whether politics might be interfering with the science of tracking the pandemic. “Each year we get numbers from UNAIDS, but we don’t have easy access to the supporting analyses and calculations,” says David Ho, director of the Aaron Diamond AIDS Research Center in New York City. “Those [analyses] should be put out there for the entire scientific community to comment, along with the conclusions and projections.”

Staneczki says this process is already in place. UNAIDS appoints a reference group, including outside scientists and experts, to review the models and publishes all of the findings from this group, she says. But the exact methodology that was used to establish the new prevalence figures for India has not yet been released publicly. Jha says that if anything the Indian experience should argue for making the prevalence numbers “completely transparent in the future.”

Chin argues that UNAIDS is reticent to lower the estimates even further because it will only make it more difficult to sustain political momentum and funding for HIV prevention and treatment programs. This suggestion is controversial. “We’ve been continuously lowering our numbers over the past years and we do this in collaboration with countries,” says Staneczki. “We don’t have any agenda.” Chin isn’t convinced. “Regardless of who they’ve been working with, they are making gross overestimates,” he says. “They’re an advocacy organization but they shouldn’t ignore the science.”

Whether or not the numbers are too high, funding and expanding HIV prevention and treatment programs remains critical—only a minority of HIV-infected individuals in developing countries currently receives life-saving antiretrovirals (ARVs) and last year alone four million people were newly infected with the virus. In a recently published book, Chin provocatively accuses UNAIDS of misapplying mathematical models to produce exaggerated estimates and then giving credit to the agency’s prevention programs for the declining prevalence. Jha emphasizes the fact that the new lower prevalence estimates in India are not a direct result of HIV prevention programs. “It’s not due to a control program, but to a computer program,” he says.

Stephen Lewis, the former UN Special Envoy for AIDS in Africa, and Paula Donovan, who was Lewis’s senior advisor at the UN, wrote in a review of Chin’s book that it poses an “open challenge to the UN’s role in the most eviscerating plague in human history,” (*Nature* 447, 531, 2007).

Others also view some of the dire predictions and projections previously issued by UNAIDS on the potential expansion of the HIV/AIDS epidemic in Asia as exaggerated. In a 2002 publication called “HIV/AIDS China’s Titanic Peril,” UNAIDS projected that there would be 10 to 20 million HIV infections in the country by the end of this decade. “Although we still have two to three years to go, I do not think China will come close to that figure,” says Ho. China already lowered its prevalence to 650,000 from around a million and, according to Chin, it could be cut in half again as more data is collected.

Many researchers and epidemiologists now agree that the Asian HIV epidemic is unlikely to bring about the devastation that was initially predicted. Yet the 2006 UNAIDS update report contained another dire warning about the spread of HIV in Asia, referring to “rapidly growing epidemics in regions such as Eastern Europe and South-East Asia that may come to rival that of sub-Saharan Africa in scope.” Staneczki says that UNAIDS doesn’t like issuing projections. “We don’t really project to say where the epidemic is going,” she says.

**Mind the gap**

While better surveillance has allowed epidemiologists to collect data that most agree are more representative of the scope of the epidemic, there is still an enormous gap between what is needed to control and eventually end the HIV/AIDS pandemic and what is currently being done. “The numbers are lower, but there’s still the possibility of explosive growth,” says Jha. There is an overwhelming need for improving the availability of ARVs to HIV-infected individuals in developing countries and new prevention methods, including AIDS vaccines, to help prevent the millions of new HIV infections that still occur each year.

“It’s great to get better estimates, but from an epidemiological point of view it isn’t over,” says Berkley, who points out that even in countries with a stable epidemic like the US, there are still a substantial number of new HIV infections each year. “What India, and the rest of the world, should do is focus on prevention, especially for high-risk populations, and continue accelerating vaccine research,” says Jha.
Cahn then turned to the political controversy sparked by Australian Prime Minister John Howard’s statement in April that his country should refuse entry to migrants or refugees who are HIV infected. After saying that “good research drives good policy, and we have never been more in need of good policy,” Cahn said that “epidemics are not stopped by immigration officers. We are confronting HIV, not people living with HIV.” He also touched on the need for greater emphasis on prevention saying that “the epidemiology does not lie; we are falling behind in preventing HIV.”

Conference co-chair David Cooper of the University of New South Wales, Sydney, welcomed attendees to the first major international HIV/AIDS conference in Australia and called for an open dialogue and focus on research. He also talked about the recent politics and said that “pointing fingers of blame will do nothing to curtail infection, and threatening to demonize people living with HIV will not help, making recent immigrants from developing countries the alleged culprits will not help,” calling for a return to proven prevention and treatment measures underpinned by “political leadership that builds on success rather than undermining it.” He also urged the Australian government to recognize that “HIV knows no borders.”

Tony Abbott, Federal Minister for Health and Ageing, countered that no conference delegates had been tested to enter the country, and said that this is the way it should remain. But he also noted that permanent residents are required to undergo HIV testing and they will now “have to give enforceable undertakings about treatment,” saying that this is to identify and help infected people, not to judge and quarantine them.

**Keynote**

The first keynote speaker, Michel Kazatchkine of the Global Fund to Fight AIDS, Tuberculosis, and Malaria, talked about how prevention and treatment mutually reinforce each other and are now seen as parts of an integrated approach to the HIV/AIDS response, and said that “health is no longer seen as a happy by-product of development” but as “a necessary investment for development.” Similarly, he also stated that the non-profit sector is now seen as a necessary investment for development, and he hoped that, in the near future, access to health would be seen as a human right.

Tony Fauci of the US National Institutes of Health (NIH) talked about pathogenesis as the bedrock of information from which all advances in treatment, prevention, and, ultimately, vaccine development springs. He made the point that “for every person put on treatment, six more are infected,” indicating that treatment is unsustainable on a global scale. With regard to prevention he said there was “an awful lot to do,” with less than 10% of individuals at risk of HIV infection currently able to access prevention programs, and said that “half of the 60 million HIV infections that we project will occur by 2015 could be averted with a comprehensive scale up of proven prevention strategies.” Fauci then talked about the evolving concept of an AIDS vaccine, and said there was some optimism that a “less than perfect vaccine” can be developed that will not prevent infection but instead lower viral set-point, prevent disease progression, and lower the risk of transmission to others.

**Vaccine research**

Alas, despite the warm words about prevention by most of the speakers at the opening session, Fauci was the only one to mention vaccines, and this was perhaps the high point of recognition for AIDS vaccines at the conference. Outside of the satellite symposium on replicating viral vectors (see Reproducing protection, page 8) there was only one 90-minute session devoted to oral presentations on AIDS vaccine research and, other than that, not a lot of emphasis was placed on fundamental immunological research.

During the vaccine session, Dennis Burton of The Scripps Research Institute gave a broad summary of HIV-specific neutralizing antibodies (NAs). He began by saying there were two related questions dominating the field: what specificities and levels of antibody would provide benefit on HIV exposure, and how to design immunogens to elicit and sustain these levels of antibody? Burton said that “we have a good handle” on the specificities of the broadly-neutralizing antibodies (BNAs) required but that the levels that will be needed were yet to be determined. He does not share the recent enthusiasm for non-neutralizing antibodies, saying they might provide some benefit but in the absence of NAs he doesn’t think this will be substantial. Burton concentrated on the immunogen design question and outlined three approaches. The first is trimeric Env mimics,
but the major stumbling block is that the Env trimer is very unstable. Burton said that “everyone is waiting for the crystal structure of the trimer” to engineer more stable forms and so enable better design of mimics; that structure should be available in two to three years. The second approach is entry intermediates that essentially attempt to target the co-receptor binding site. This is difficult because this step is immediately prior to membrane fusion and so the site may not be spatially accessible to antibody. This approach is currently not being pursued aggressively by many research groups. The third approach is epitope mimics that look to use the molecular information from structural studies of BNAbs in complex with Env to design immunogens, and this is where a lot of the current effort is focused.

Burton then ran through the broadly neutralizing epitopes and their potential to serve as immunogens. The CD4-binding site is “the natural target” for vaccine development since it is highly conserved and accessible, at least to CD4. However, NAAb b12 has been the only evidence that this site is immunogenic. The crystal structure of b12 in complex with gp120 core was published recently by Peter Kwong’s lab at the Vaccine Research Center at the NIH (Nature 445, 732, 2007), which Burton called “a great advance” that has spurred the design of mimics. Further NAAb directed against the CD4-binding have recently been isolated (Nature Medicine 13, 1032, 2007) that should further accelerate progress with this target.

HIV’s glycan shield is also a target, and NAAb 2G12 is the prototype that targets this carbohydrate epitope comprised of mannos residues. Researchers are looking to reproduce the array of manno residues and use that to elicit similar antibodies. Raymond Dwek’s group at University of Oxford has found a naturally-occurring homolog with a similar structure in Candida albicans that they are investigating, and Burton’s own work in collaboration with others is focusing on trying to synthesize mimics in multivalent presentations, including on phage Qβ; both approaches have generated mimics that do bind 2G12.

The other broadly neutralizing epitope of interest is the membrane proximal external region (MPER) which is targeted by a number of NAAb, including 2F5 and 4E10. There are ongoing attempts to reproduce these peptide epitopes, including some in Burton’s lab where they’ve been able to construct peptides that bind well to 4E10 but do not elicit 4E10-like antibodies when inoculated in to mice or rabbits. Burton said that there are a number of possible reasons for this, one of those being the controversial hypothesis advanced by Bart Haynes and colleagues that 2F5 and 4E10 are polyspecific autoantibodies that bind to cardiolipin and so may be susceptible to tolerance (Science 308, 1906, 2005). Burton said he didn’t agree with that interpretation, and his group has data that suggests cardiolipin autoactivity does not explain the difficulties in eliciting MPER-directed antibodies.

Erin Scherer of University of Oxford presented that data in another session. She measured the lipid reactivity of 2F5 and 4E10 in a number of assays, including one developed for the diagnosis of antiphospholipid syndrome—a human autoimmune disease that can include antibodies against cardiolipin—and another to measure general lipid affinity using liposomes of different composition. She found that 2F5 did not bind cardiolipin and that 4E10 had a generalized affinity for lipids, concluding that neither antibody would be a target for tolerance and therefore this mechanism was unlikely to explain the difficulty in eliciting NAAb against HIV.

Burton’s presentation was followed by Bruce Walker of Harvard Medical School, who began by warning that he was going to be “a little bit provocative” about the direction in which cytototoxic T lymphocyte (CTL; or CD8+ T cell) research should go. Emphasizing that the first generation AIDS vaccines will control HIV infection rather than completely prevent it, he asked a question he thought that many people were now asking: whether we were “essentially measuring the equivalent of binding non-neutralizing antibodies” with current CTL assays. He questioned the physiologic relevance of ELISPOT assays since they do not involve infectious virus, antigen processing and presentation, or MHC Class I presentation, nor do they measure cytotoxic activity or inhibition of virus production. Rather, they simply reflect engagement of a peptide with its receptor and subsequent interferon (IFN)-γ expression.

Walker thinks that measuring the ability to inhibit virus replication and inducing these responses with a vaccine will be vital to successful vaccine strategies. He then described an in vitro virus neutralization assay his group is developing that uses CD4+ T cells exogenously infected with HIV as a substrate to
measure the relative ability of CTLs to inhibit virus replication. They have seen between 1000- and 10,000-fold reduction in virus replication over a 7 to 10 day assay period, and Walker emphasized again that this is measuring something that is very likely functionally relevant.

To ask questions more relevant to vaccine development, his group has also enriched Gag- or Env-specific CTL responses from peripheral blood mononuclear cell (PBMC) samples and compared their relative virus neutralization capacities. A previous publication from Walker and collaborators suggests that Gag-specific CTL responses are associated with lowered viremia in HIV-infected individuals, whereas CTL responses against other proteins, including Env, are associated with higher viremia (Nature Medicine 13, 46, 2007). Similarly, they now see that Gag-specific CTL responses are better at inhibiting virus replication than the Env-specific responses from the same individuals; for every individual tested, “when we enriched for Env-specific responses we got less virus neutralization, when we enriched for Gag we got more virus neutralization,” said Walker. This was not due to virus mutants arising within the assay.

Walker concluded by asking if these data were “completely irrelevant because it’s done in vitro, or is this something that we really need to think about in terms of where we’re heading with vaccines,” and what might be the implications for vaccine testing? The virus neutralization assay is laborious but his group is working with IAVI to develop a high-throughput assay for testing samples from vaccine trials. He doesn’t envisage that it will ever fully replace simpler assays like the ELISPOT, but it will be very useful to assay a subset of samples from a clinical trial to directly measure virus inhibition by CD8+ T cells.

Reproducing protection

Satellite symposium at the Sydney conference gathers researchers and regulators to discuss the benefits and risks of replicating viral vectors for AIDS vaccines

By Simon Noble

Fingers are crossed that the most promising of the vaccine candidates currently in clinical testing will show some benefit to vaccinated volunteers who later become HIV infected. However, since virtually all of those candidates focus on eliciting cell-mediated immunity, the smart money seems to be on the outcome that, at best, they will have low-to-moderate efficacy, leading to a lower viral set-point that ameliorates disease progression and potentially lowers the likelihood of transmission. If that’s likely the best that can be expected from the leading candidates, that means there’s still a need for better vectors that induce more robust immune responses. Since most of the obvious options for replication-deficient viral vectors are under active development, some researchers are turning to a more classical approach with a novel twist—replicating viral vectors.

But replicating viral vectors bring with them a new set of risks associated with their ability to propagate. So the regulatory authorities and the general public will have to be convinced that the risk inherent in such a vaccine approach is outweighed by the benefit. These risk-benefit analyses will depend greatly on local conditions; an acceptable risk in a very high incidence environment like Lesotho might well be different from the risk acceptable in Australia, for instance.

To get the conversation started, IAVI held a satellite symposium at the Sydney Conference entitled ‘Accelerating the Development of Replicating Viral Vectors for AIDS Vaccines.’ The intention was to bring together scientists and regulators to discuss and better understand the issues around the development and evaluation of replicating vectors, and to identify risks that should be addressed during development.

Bang for your buck

Ian Gust of the University of Melbourne introduced the satellite in his role as chair, pointing out that vaccine development is now “much more complex than in the days of Jenner and Pasteur, when you could revolutionize medicine with a vaccine tested in a single study with an n=1.” Since many of the licensed vaccines today are intended
Many are beginning to accept that we need to think about novel vectors with a bit more ‘oomph’

Wayne Koff

Chris Parks of IAVI gave an overview of the current pipeline of replicating viral vectors. Listing the potential vaccine strategies in order of increasing immunogenicity/efficacy—nucleic acids, protein/peptide subunits, virus-like particles, inactivated virus, nonpropagating viral vectors, replicating viral vectors, ‘Jennerian’ vaccines (i.e. related nonpathogenic animal viruses), live attenuated virus—Parks pointed out that, contrarily, this corresponds with a decrease in safety.

But historically the most successful vaccines have come from either live-attenuated

Wayne Koff
Pharmaceutical companies now must demonstrate that a vaccine is nearly ‘risk-free’ and this is extremely complex, lengthy, and costly

vaccines or Jennerian vaccines. Since live-attenuated HIV vaccines are likely not feasible because of safety concerns and there are no candidate Jennerian vaccines, live viral vectors are the next best option in terms of immunogenicity.

Parks then described some of the qualities sought in replication-competent viral vectors: replication and spread of the vector should occur such that it generates abundant and sustained antigen expression, but the replication should be sufficiently restricted so that disease does not result; the antigen expression should induce durable immune responses (antibodies, polyfunctional T cells with a balanced Th1/Th2 response) and immunologic memory; and ideally, the vector would be sufficiently immunogenic to be efficacious without recall to complex heterologous prime-boost regimens.

Additionally, it may well be desirable that a replicating vector has cell and tissue tropism that leads to induction of mucosal immunity, and that any pre-existing immunity issues are minimal.

Parks, formerly of Wyeth Vaccines, recounted the story of vesicular stomatitis virus (VSV), one of the leading replicating viral vectors currently being advanced to the clinic by the manufacturer. Prototype VSV vectors developed in John Rose’s lab at Yale University showed promising results—NHPs vaccinated with the vectors were protected from disease after challenge with pathogenic simian-human immunodeficiency virus (SHIV), and no adverse events were seen. Wyeth in collaboration with the National Institute of Allergy and Infectious Diseases (NIAID) at the NIH had planned to go into clinical trials but first cautiously conducted a pilot neurovirulence test in NHPs; research from as early as the 1930s had indicated that VSV could be neuroviral in neonate mice. The results from the the pilot NHP study indicated that the prototype vector did cause inflammation and tissue necrosis when inoculated intrathalamically. This result subsequently initiated years of further research that resulted in development of a highly attenuated vector that was far less neuroviral and retained its immunogenicity. Wyeth and NIAID are now advancing this candidate into clinical trials.

The VSV tale, Parks noted, exemplifies the need to balance safety and efficacy. But he also asked whether it should be necessary to test neurovirulence by direct inoculation into the brain of a vector that will be administered intramuscularly, and said that these questions around safety testing standards need to be discussed. Equally vexing is the issue of the public’s evolving view of acceptable risk with regard to vaccination, and Parks contrasted the relative risks associated with some vaccines over the past 60 years or so; smallpox vaccination was associated with notable complications, ranging from erythematous urticaria to death, but was still widely accepted by the general public, whereas the current measles, mumps, and rubella (MMR) combination vaccine has drawn suspicion due to well-publicized but unsupported claims that the vaccine is associated with autism and gastrointestinal disease.

This decreasing tolerance for risk has consequences. Pharmaceutical companies now must demonstrate that a vaccine is nearly ‘risk-free’ and this is extremely complex, lengthy, and costly. It also increases the probability of vaccine candidate failure during development, and developers are mindful that rare adverse events post-licensure will be costly to both economics and reputation. Parks ended by saying that development of risk-free live vaccines is probably unachievable, which makes replicating vectors less appealing to vaccine developers.

Regulatory affairs

The regulatory experts presenting at the satellite session emphasized many of the same practical aspects highlighted above. Jim Ackland of Global BioSolutions, a regulatory consultancy based in Australia, pointed out that it’s not just the potential for reversion to virulence and adverse events that concerns regulators with regard to replicating viral vectors. He pointed out that, as with any novel product, there are both predictable and unpredictable potential risks, and the latter can only be assessed through careful clinical development. Predictable risks can often be assessed during preclinical development, and these include manufacturing and the potential for contamination with adventitious agents, genetic stability of the vector and its antigenic insert, toxicity, virulence, transmission, and recombination with wild type or other viruses that might facilitate reversion to virulence.
Ackland also noted that there are only limited guidelines available for viral vaccines from regulatory authorities and these tend to be generic in nature, and emphasized the need to engage regulatory authorities in the development process and appreciate their challenges and expectations. Good regulation is based on good science—for regulators “it’s not publish or perish, it’s document or perish,” he said—and it’s important to be familiar with the existing guidelines and identify precedent.

Keith Peden of the FDA indicated that replicating virus vectors presented authorities with a potential regulatory nightmare since they can evolve rapidly in the human host, which he summed up as “predicting, and regulating, the unpredictable.” He reiterated the importance of involving regulatory authorities early and throughout the development process. As to the choice of the vector, he emphasized the importance of virus tropism in the selection process, cautioning that neurotropic or cardiotropic viruses should be avoided. He also counseled to choose prudently an acceptable cell substrate on which to grow the vector. He stated that the use of recombinant DNA virus clones is preferable to generate the vaccine virus, as this eliminates concerns due to adventitious agents in any original virus vector and provides a constant source of the vaccine. Generation of the vaccine virus from a recombinant DNA clone in qualified cells under cGMP would simplify the regulatory path. Peden did illustrate that regulatory positions do change when data are provided by reference to the recent discussions at the FDA regarding the use of continuous cell lines for vaccine production, saying that products manufactured in continuous cell lines (e.g., PER.C6 cells and Madin-Darby canine kidney cells) have been permitted to enter clinical trials.

Gary Grohman of the Therapeutic Goods Administration, the Australian Government regulatory body, concurred with Peden and said that “it’s obvious we are moving beyond the conventional.” He stressed the need for public acceptance of replicating viral vectors, and asked if there was the need for a communication forum specifically for this purpose. Gust made the point that regulatory authorities traditionally reflect the feeling of a community.

The regulatory context and challenges in developing countries were discussed by Helen Rees of the University of Witwatersrand, Johannesburg, and she began by pointing out that the risk-benefit analysis of any AIDS vaccine approach will differ by region. She pointed out that some of the inequalities in global health research greatly affect attitudes in developing countries, including biomedical regulatory attitudes, such as the “10/90” gap that sums up the fact that only 10% of global investment in health research is committed to solving 90% of the world’s health problems, and that there are more African researchers in the US than there are in the whole of Africa. Also, Rees said that “the ‘guinea pig’ concern continues to overshadow all other considerations” and affects political attitudes toward research and clinical trials conducted in developing countries.

There has been a huge increase in the number of clinical trials being conducted in developing countries—from fewer than 1000 trials in 1990-92 to more than 5000 in 1996-98—but clinical trial review is still an emerging area of expertise and therefore these countries are still largely reliant on the FDA and European Agency for the Evaluation of Medicinal Products (EMEA) as the default standard. Similarly, the capacity to evaluate trial ethics is still emerging, so it must be made clear that trials are being done in the context of sufficient ethical and regulatory oversight. Recently there have been moves to streamline regulation in East, Southern, and West African regions, “but it will be catch up, if a replicating vector is developed then it will have to be considered and ways to evaluate it developed.”

In discussion after the presentations, Rees said that the media also needs education so they can communicate difficult concepts in simple language. She also said that many in developing countries look toward the World Health Organization (WHO) as a reputable, independent, and trustworthy body that can facilitate acceptance of new concepts, and strongly recommended that the WHO be engaged in an expert consultation to continue the discussion about the acceptance of replicating viral vectors. Koff agreed that now is the time to begin this dialogue since the VSV vector should be ready to go into clinical trials in 12 to 18 months and other replicating vectors are under active consideration.
Microsoft, Bill Gates, and coffee—for many, these themes are synonymous with the city of Seattle, and all of them were pertinent to the AIDS Vaccine 2007 Conference held there August 20–23. More than 900 AIDS vaccine researchers from over 50 countries convened at this year’s meeting, the first to be held under the auspices of the Global HIV Vaccine Enterprise.

At the opening session Tachi Yamada of the Bill and Melinda Gates Foundation began his presentation with a quote from Nobel laureate neuroscientist Roger Sperry, on what would have been his 94th birthday. Sperry worked on the split-brain conundrum and showed that specific areas of the brain were associated with particular cognitive abilities, in the process solving some of the perceived dichotomy between the human brain’s capacities for reason and emotion. In his 1981 Nobel lecture he said, “where there used to be a chasm and irreconcilable conflict between the scientific and the traditional humanistic views of man and the world, we now perceive a continuum. A unifying new interpretative framework emerges with far reaching impact not only for science but for those ultimate value-belief guidelines by which mankind has tried to live and find meaning.” Yamada said that this holistic view of science integrated with humanistic goals encapsulated the ethos that drives the work of the Gates Foundation.

He then changed his theme to innovation. There are around 30 AIDS vaccine candidates currently in various stages of clinical trial, but all focus on eliciting cell-mediated immune responses, and Yamada asked whether the field should be looking more closely at antibodies, including non-neutralizing ones. He said the field of AIDS vaccines needed more innovation, and to some amusement he brought up former US Secretary of Defense Donald Rumsfeld’s infamous quote about “unknown unknowns,” saying that we need to take aim at the things we don’t know.

To illustrate, he admitted that in his long career as a gastroenterologist he was once part of the “Acid Mafia” that was unshakable in its belief that gastric acid was the cause of peptic ulcers. When Barry Marshall and colleagues of Royal Perth Hospital, Australia advanced their idea that peptic ulcers were caused by bacterial infection, they were widely ignored; almost all their peers were skeptical that any microbe could exist in the extremely acid pH of the stomach. It took Marshall to drink a culture of Helicobacter pylori and then cure the ensuing gastritis with antibiotics before he caught the attention of the gastroenterological community. He then went on to win a Nobel Prize.

Yamada said he had seen for himself how innovative funding can spur new research paths, and in a previous role as a grant administrator he provided seed money to seemingly off-the-wall projects in neuroplasticity and malaria vector engineering that either proved successful or now receive major funding from more mainstream sources.

He said the field should explore “the unorthodox path” and pursue “ideas that would not stand up to peer review, for, after all, novel ideas are without peer.” He then talked about IAVI’s Innovation Fund, a new $10 million finance mechanism that will seek to nurture new technologies that might not otherwise be funded; half of the funding will come from the Gates Foundation. Research is currently structured to foster incremental advances and these can be vitally important, but Yamada wants to supplement that and “create an innovation ecosystem” and “reach out to colleagues in other fields to solve problems.”

Tony Fauci of the National Institute of Allergy and Infectious Diseases (NIAID) at the US National Institutes of Health (NIH) revisited his theme from the International AIDS Society’s Sydney conference (see HIV Down Under, page 1) of “much accomplished, much to do.” He began by discussing US government funding levels and revealed that although through 2003-2007 there had been a flat budget for the NIH overall and specifically HIV/AIDS research at the NIH, AIDS vaccine funding had increased year on year from 1997 through 2006; the budget for 2007 remained unchanged from the previous year. Fauci picked out the HIV Vaccine Trials Network (HVTN), Partnership for AIDS Vaccine Evaluation (PAVE), Vaccine Research Center (VRC), and Center for HIV-AIDS Vaccine Immunology...
(CHAVI) as examples of new initiatives that the NIH has rolled out in recent years, while emphasizing the continued commitment to individual investigators through the R01 grant mechanism. He made reference to the balance and tension that exists between R01’s and some of the huge initiatives like CHAVI, and acknowledged that the NIH will “have to prove, and I think we will, that the initiatives implemented are well worth the investment.”

In terms of research, Fauci said that AIDS vaccine researchers have had to accept that they are in a “different ballgame... we thought we were in the same playing arena as previous vaccinologists working on other diseases” but the field may have to accept a new paradigm—a less-than-perfect vaccine that will control acute viremia and lower viral setpoint. Although good progress has been made with T cell-based vaccines, Fauci emphasized that ways to elicit broadly-neutralizing antibodies must be pursued. He warned that with AIDS vaccines we still have more to do than we have accomplished but the field should take heart from the progress in the treatment arena, saying “we’re at the AZT phase.” He finished by warning that regardless of the degree of efficacy achieved with future vaccines it will still have to be used in the context of a comprehensive HIV prevention toolbox.

Not neutralizing

On the topic of protective antibodies, Robin Weiss of University College London began by stating that his main message would be “neutralization is very important, but it ain’t everything.” Weiss’ early work (Nature 316, 69, 1985) showed that HIV elicits antibodies during infection but they have low neutralizing activity. Nobel laureate Rolf Zinkernagel has said that all effective vaccines induce neutralizing antibody (NAbs), and Weiss agreed that indeed effective vaccines like those against yellow fever, rabies, and smallpox do elicit NAbs. But he said that is what is measured because it’s easy to do, and not necessarily what is important in protection.

In the case of influenza virus, Weiss said, antibodies against the neuraminidase protein are protective, but they don’t show up in a typical neutralization assay. That’s because they don’t neutralize mature particles but rather they neutralize an enzyme activity and block maturation; Weiss called them “the equivalent of a protease inhibitor.” Also, typical neutralization assays don’t consider the contribution of complement, which he considers makes them a very artificial test.

Weiss said there can be antibody enhancement whereby low-affinity, poorly-neutralizing antibody can attach to virus and then tether to Fc receptors. He cited a recent paper from Dennis Burton and colleagues that indicates that the broadly NAb b12 is much more potent against pathogenic virus challenge in nonhuman primates (NHPs) if the Fc portion is intact, indicating that antibody-complexed infected cells interact with Fc receptors on effector cells to reduce viral loads (Nature 449, 101, 2007). Weiss speculated that with non-neutralizing antibody, complement effector activity might be similarly important.

Weiss ended with a picture of Emil von Behring, winner of the first Nobel Prize for Medicine for the diphtheria vaccine he developed. The diphtheria vaccine doesn’t prevent infection—it elicits antibody against diphtheria toxin—and Weiss asked if we should aim for something similar for HIV, saying “I don’t know the answer to that.”

Bacterial vector

Tom Dubensky of Cerus Corporation is developing *Listeria monocytogenes* as a vaccine vector platform and is currently testing it in Phase I trials. *L. monocytogenes* is a Gram-positive bacterium that has a facultative intracellular lifecycle. It multiplies within phagocytic cells, including dendritic cells and macrophages, and as well as inducing robust CD4+ and CD8+ T-cell responses it is a potent activator of innate effectors. This immunogenicity profile make *L. monocytogenes* an attractive prospect as a vaccine vector, but it is ubiquitous in nature and can be a food-borne pathogen that can cause serious disease, so novel approaches to attenuate its pathogenicity whilst retaining its immunogenicity are required for use in humans.

Dubensky’s team is developing two different vectors. The first, Δ*actAΔinlB*, is a live-attenuated mutant that has two virulence factors deleted, Internalin B and ActA; the former is important in infection of non-phagocytic cells, the latter induces reorganization of the actin-based cytoskeleton that is crucial to intracellular movement and cell-to-cell spread (Proc. Natl. Acad. Sci. 101, 13832, 2004).

Dubensky showed that *Lm ΔactAΔinlB* exhibited greatly reduced hepatotoxicity compared to wild-type in mice and non-human primates (NHPs), and induced robust CD8+ T-cell responses in mice, including to HIV immunogens, even in the face of pre-existing immunity. He also presented data indicating that *Lm ΔactAΔinlB* could break tolerance against mesothelin, a human tumor antigen, in NHPs. They are going ahead with two Phase I trials of the *Lm ΔactA ΔinlB*-mesothelin vaccine candidate in patients with carcinoma, hepatic metastases, and other malignancies and early indications are encouraging.

Dubensky’s second vaccine approach is a wholly novel platform termed a killed but metabolically active (KBMA) mutant that attempts to preserve the potency of live vaccines while acquiring the safety of killed vaccines (Nature Medicine 11, 855, 2005). Genes required for nucleotide excision repair (uvrAB) have been deleted from *L. monocytogenes*, rendering the bacterial vector exquisitely sensitive to psoralen and ultraviolet light photochemical inactivation. The lack of nucleotide excision repair pathway means that only one essential gene has to be cross-linked for an individual microbe to be inactivated, but on a population level all genes are expressed and proteins synthesized and secreted. Dubensky showed data indicating that KBMA vaccines are almost as immunogenic as live *L. monocytogenes*. One potential problem as
an AIDS vaccine vector that was raised in questions after the presentation is that *L. monocytogenes* is most often associated with pathogenesis in immunocompromised individuals.

**Clinical progress**

There were many oral and poster presentations from ongoing and recently completed AIDS vaccine clinical trials. Sandhya Vasan of the Aaron Diamond AIDS Research Center gave an update on the modified vaccinia Ankara (MVA) candidate they are developing. This is a distinct MVA from the lab of Bernie Moss at the NIH, and the vaccine candidate contains *nef-tat, env*, and *gag-pol* genes as immunogens from a clade C/B HIV isolate that is dominant in Yunnan province, China. The dose-escalating Phase I trial (C002) tested 1x10^7, 5x10^7, and 2.5x10^8 pfu doses in 12 vaccinees per group inoculated at weeks 0, 4, and 24, with follow up for 18 months. The last visit by the last volunteer was just a week prior to the presentation, so some data analysis was still ongoing. The candidate was safe and well tolerated and induced T-cell responses in 25%, 42%, and 62% of vaccinees with respect to escalating dose, as measured by interferon (IFN)-γ ELISPOT. Also, by escalating dose there were anti-gp120 binding antibodies in 62%, 50%, and 77% of vaccinees. The researchers now hope to proceed with further testing in a prime-boost regimen.

Another Phase I trial (D001) of a recombinant MVA-vectored vaccine candidate called TBC-M4 containing HIV clade C *env, gag, tat-rev*, and *nef-RT* genes was described in a poster by Vadakkumpattu Ramanathan of the Tuberculosis Research Center, Chetput, India, and colleagues. In a dose-escalation trial of either 5x10^7 or 2.5x10^8 pfu in 12 volunteers per group inoculated at 0, 4, and 24 weeks, no serious adverse events were reported. Dose-dependent HIV-specific T-cell responses were detected by IFN-γ ELISPOT in 67% and 92% of vaccinees after two injections, and in 75% and 100% of the vaccinees after the third. Overall, most responses were directed to *gag* and *env* epitopes, and although the magnitude of the responses were moderate (39 to 430 spot-forming cells (SFC)/10^6 peripheral blood mononuclear cells (PBMC)) they were persistent over the time-points sampled. HIV-specific antibodies were measured in ELISA; even though the data is still blinded, it seems that after three inoculations of the high dose all vaccinees have an antibody response. These encouraging results will be followed up with trials of TBC-M4 in prime-boost regimens with DNA and possibly other viral vector-based vaccines.

DNA vaccines present a number of advantages—they’re simple, safe, and not prone to pre-existing immunity—but a longstanding question has been how to augment their immunogenicity. Many trials employ a boost with a recombinant viral vector to enhance the immune responses induced, but Ray Dolin of Harvard Medical School and colleagues have used co-administration of the immunostimulatory cytokine interleukin (IL)-2. Previous data indicated that the efficacy of a DNA vaccine encoding SIV Gag and HIV Env was substantially augmented in NHPs against simian-human immunodeficiency virus (SHIV) challenge by the administration of IL-2; potent CD8+ and CD4+ T-cell responses, stable CD4+ T-cell counts, low or undetectable set-point viral loads, and no sign of clinical disease or mortality were all great improvements on animals given the DNA vaccine without IL-2 (*Science* 290, 486, 2000). But IL-2 has a short half-life, and use of plasmid encoding the cytokine and administration after the DNA vaccination seems to further improve responses. Dolin and colleagues conducted a Phase I trial (HVTN 044) of the VRC-HIVDNA vaccine that contains *gag-pol-nef*-multiclude *env* in a complex trial design that escalated dose of IL-2. Vaccine was administered to groups of 10 volunteers at week 0, 4, 8, and 24, along with concurrent administration of 0, 0.1, 0.5, 1.5, or 4.0 mg of IL-2 plasmid. A further group of volunteers were given vaccine on the same schedule and then 2 days after each vaccination an inoculation of 4.0 mg of the IL-2 plasmid. All regimens were well tolerated and no anti-IL-2 antibodies were detected.

The volunteers given the IL-2 plasmid 2 days after their DNA vaccinations showed substantially higher T-cell responses 2 weeks after the third vaccination. By IL-2 ELISPOT, 40%, 40%, and 80% of volunteers given DNA vaccine alone, DNA + IL-2(4 mg), and DNA + IL-2(4 mg + 2 days) respectively, were responders; 80%, 50%, and 100% respectively were responders by IFN-γ ELISPOT. The magnitude of median responses was also enhanced, 103 versus 380 SFC/10^6 PBMC in the DNA + IL-2(4 mg) and DNA + IL-2(4 mg +2 days) groups respectively. Dolin conceded that it is a “very com-
plex inoculation protocol giving adjuvant 2 days later, but it will be interesting to see if DNA plus IL-2 two days later can act as a prime to be boosted. He speculated that the IL-2 could be injected simultaneously with the DNA vaccine in a time-release mechanism.

Walter Jaako of the Kenya AIDS Vaccine Initiative gave an update on a Phase I trial (V001) of VRC-HIVADV, a recombinant adenovirus serotype 5 (rAd5) that contains gag, pol, and multiclade env, alone or in combination with VRC-HIVDNA, at sites in Kigali, Rwanda, and Nairobi, Kenya. Volunteers were inoculated with either rAd5 at 10^10 or 10^11 pfu at week 0 (rAd5 alone), or 4 mg DNA at weeks 0, 4, and 8, followed by rAd5 10^10 or 10^11 pfu at week 24 (DNA + rAd5). DNA was administered by Biojector.

All vaccinations were safe and well tolerated with no serious adverse events related to vaccination. HIV-specific T-cell responses were measured by IFN-γ ELISPOT, and in vaccinees given rAd5 alone there were 46% and 54% responders with respect to escalating dose. In the other groups, after the third DNA inoculation there were cumulatively 45% responders. But after these volunteers were given their rAd5 boost, the number of responders rose to 73% and 69% with respect to escalating dose, suggesting that the heterologous prime-boost regimen did substantially augment immunogenicity over either the rAd5 or DNA component alone.

Overall, T-cell responses in volunteers were skewed towards Env-specific responses. In DNA + Ad5 vaccinated volunteers, these responses were persistent up to week 48, the longest follow up to date. When vaccinees were sorted by pre-existing anti-Ad5 antibody titer there was at most only a modest curtailment of HIV-specific T-cell responses. Jaako said that future trials will focus on larger sample sizes and those at higher risk for HIV infection.

Michael Keefer followed this with an update on a Phase IIa trial (HVTN 204) testing the same VRC-HIVDNA and VRC-HIVADV candidates. In total the trial has enrolled 480 volunteers, 240 in the Americas (US, Brazil, Haiti, and Jamaica) and 240 in South Africa, who were randomized and placebo-controlled, with equal numbers being given vaccine and placebo. Keefer said the data he was presenting were only from participants at the sites in the US and South Africa.

Volunteers were given DNA intramuscularly by Biojector at week 0, 4, and 8, followed by rAd5 (10^10 pfu) at week 24. Vaccination was safe and well tolerated, with only one serious adverse event that was possibly vaccine related. HIV-specific immune responses were measured by IFN-γ ELISPOT and multiparameter intracellular cytokine staining (ICS). Overall, at day 210 (6 weeks after the rAd5 administration) 74% of vaccinees had HIV-specific immune responses by IFN-γ ELISPOT, mostly against Env and Gag in volunteers at both sites. As to breadth of response, 69% and 59% of vaccinees at the US and South Africa sites respectively showed responses to two or more proteins. ICS analysis is still ongoing.

With regard to pre-existing immunity, after stratification according to anti-Ad5 antibody titer there was a clear “step down” in responders at the US sites (titer <12, 86% responders; 12-1000, 83%; 1000-5000, 57%; >5000, 47%) that was not seen in responders at the South African sites (67% to 72% across all anti-Ad5 titers). While pending further data collection and analysis with respect to the remaining sites and the ICS assays, Keefer concluded by suggesting that the immunogenicity data so far would warrant efficacy evaluation of the DNA + Ad5 regimen.
Balancing act

Regulatory T cells suppress immune responses and researchers are now working to determine precisely how, but their role in HIV pathogenesis is still unclear

by Andreas von Bubnoff

What goes up must come down. That’s not only true for everyday life but also for complex biological systems like the immune system: Where there is activation, there must also be suppression. Such homeostasis is a tenet of biology, but finding the mechanism that prevents the immune system from spiralling out of control was not easy. After following many blind alleys, it would take until 1995 to identify T cells that suppress the immune system.

Today, it is clear that these regulatory T cells—or Tregs—are indeed crucial to keeping the immune system in check, although the exact mechanism as to how they suppress their target cells has not yet been resolved. Tregs are important in suppressing autoimmune disease in that they suppress T cells directed against self-antigens. They also appear to play an important role in HIV pathology, although it’s an open question whether they make things better or worse. Already, some researchers are thinking about modifying Tregs to help boost the response to AIDS vaccine candidates, but potential side effects such as autoimmune disease could make this a difficult balancing act.

Road to discovery

Immunologists thought they had discovered T cells that suppress the immune response over 30 years ago. In the early 1970s, Richard Gershon of Yale University coined the term “suppressor T cell” after experiments suggested that a certain type of T cell suppressed immune responses through a soluble factor and not through direct interaction. In the following years, many prominent groups tried to characterize these suppressing factors. With 1300 journal citations during 1981, “this was a tremendously active field,” says Ethan Shevach of the US National Institutes of Health (NIH).

But the genes for the postulated suppressing factors couldn’t be found, and the field shrank dramatically. In 1987 there were only 150 papers published on suppressor T cells. “Nobody could find how they were working,” says Claire Chougnet of the Children’s Hospital Medical Center in Cincinnati, Ohio. “So they became this thing that nobody wanted to touch. Some people started to doubt that they were real.”

But not everyone was on the wrong track. In 1969 a Japanese group reported that removing the thymus during a certain period of mouse development resulted in autoimmune disease (Science 166, 753, 1969). This could be prevented, however, by transferring a normal thymus back into the mice. It appeared, then, that the mouse thymus produces T cells that suppress the action of T cells recognizing self-antigens, and thereby prevent autoimmune disease. For the most part, though, immunologists ignored these early studies. “They were more interested in these soluble suppressor factors that the boys at Harvard or Yale were working on,” Shevach says.

The renaissance of the field came in 1995, when Shimon Sakaguchi, now at Kyoto University, showed that removing a subset of CD4+ T cells that express a marker called CD25 resulted in severe autoimmune disease in mice (J. Immunol. 155, 1151, 1995). The condition resembled the one described in the 1969 study. This time, Sakaguchi called the cells regulatory T cells. Suppressor T cells had become the “s-word,” Shevach says, “based on 10-15 years of terrible studies which are basically regarded as fundamental artifacts.”

Still, the earlier name probably describes their function better: These Tregs suppress immune responses, including the activation of CD4+ and CD8+ T cells and also dendritic cells. They are important because, for example, not all T cells against self-antigens are deleted in the thymus, and Tregs are thought to suppress the activity of those that escape. They can also suppress T-cell activation in response to pathogens.

Defining Tregs

The 1995 study made it possible for the first time to identify Tregs at a molecular level. Then, in 2001, several groups identified a similar population of Tregs in cultured human cells. One problem was that CD25 is expressed not only by Tregs but also by CD4+ and CD8+ T cells once they are activated. But David Hafler’s group at Harvard Medical School showed that Tregs could be better defined as the ones that express the highest levels of CD25 (J. Immunol. 167, 1245, 2001). Still, what’s high is in the eye of the beholder. “How you define high CD25 is highly subjective,” Chougnet says.

Then several papers added one more marker to the molecular definition of Tregs: The transcription factor Foxp3. A mutant mouse strain called scurfy with a severe autoimmune condition proved to have a mutation in Foxp3 (Nature Genetics 27, 68, 2001). Similarly, humans with an autoimmune condition called IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome also had mutations in Foxp3 (Nature Genetics 27, 20, 2001). What’s more, Sakaguchi’s group showed that Foxp3 could convert naive T cells into Tregs (Science 299, 1057, 2003).
"[Foxp3] really established the Tregs as something real," says Derya Unutmaz of New York University, “because now you have a molecular program that you can turn on and show that you develop suppression or autoimmunity.” But like CD25, human Foxp3 expression is not specific to Tregs. Recently-activated CD4 T cells express it as well. “It’s not clearcut in humans as to how specific [Foxp3] is,” Chougnet says.

**Suppression, but how?**

These and other markers have made it possible to better study Tregs. Studies suggest that, just like CD4 and CD8 T cells, Tregs differentiate in the thymus from bone marrow-derived precursors. Like other T cells, they are also specific to millions of different antigens. In peripheral lymphoid tissues, particularly in the gut, Tregs can also differentiate from naive CD4 T cells, Shevach says.

Tregs have now been implicated in suppressing many parts of the immune system, but precisely how they do it remains unclear. “There is no consensus on that,” Chougnet says. Several mechanisms have been proposed that mediate the suppressive effects of Tregs. They involve soluble factors Tregs make to suppress target cells, for example the cytokines TGF-β and interleukin (IL)-10, which inhibit the activation of T cells and dendritic cells. There are also data that Tregs suppress target cells by cell-cell contact. “There are some people, including me, who still believe that there are some unknown cell surface molecules involved in mediating the suppressive effects,” Shevach says.

Tregs have several options to suppress target cells but may only use some of them depending on how much suppression is required. ‘They] pick various items from the menu, depending on how fancy the restaurant is,” Shevach says. Tregs don’t use IL-10, for example, to prevent autoimmune gastritis in the stomach, where bacteria are not involved, Shevach says. But they do use it to prevent inflammatory bowel disease, where bacteria are involved.

**Checks and balances**

In the past few years researchers have also started to look into the role of Tregs in infectious diseases. One early study found that inhibiting Tregs leads to clearance of infection with the parasite *Leishmania major* in mice (*Nature* 420, 502, 2002). Intuitively that’s a good thing, says Lishan Su of the University of North Carolina at Chapel Hill. “But the bad thing is that you also lose immunity against secondary infection with *Leishmania*,” Su says. This implicates Tregs in keeping immunity to secondary infection intact by preventing complete clearance. “It’s a perfect balance,” he says.

In HIV infection it’s still unclear if Tregs are beneficial or detrimental. The area is still only a few years and about two dozen papers old, Unutmaz says. The first studies suggested that Tregs suppress the immune response to HIV. In 2004 several groups removed CD25 Tregs with magnetic beads from blood-derived, *ex vivo* cells of HIV-infected individuals and showed that depletion of Tregs increased the T-cell response to HIV antigens *in vitro*. “That suggested that Tregs play a role by decreasing the response to HIV,” Chougnet says, indicating perhaps that Tregs are a “bad” thing because they suppress cellular immune responses to HIV.

But other studies have suggested that Tregs could be a “good” thing because they also suppress chronic immune activation, which often correlates with progression of HIV to AIDS. For example, SIV-infected African Sooty Mangabeys don’t develop disease or chronic activation of the immune system. And a recent study showed that they also maintain more Tregs than SIV-infected rhesus macaques, which do develop disease and show chronic immune activation (*J. Virol.*, 81, 4445, 2007).

It’s possible that Tregs are “bad” early in infection but “good” later on, Unutmaz says. Early in infection, or when giving a vaccine, a
We have to be very careful with modulating the immune system. You may accelerate the disease

Lishan Su

potent HIV-specific immune response is a good thing, so suppressive Tregs are “bad.” Later on, Tregs are “good” as they suppress the chronic activation of the immune response. “They are a double-edged sword,” Unutmaz says. “You don’t want to mess with it too much, it could work both ways.”

**Getting the story straight**

In HIV infection, Tregs appear to behave differently in different sites in the body. They disappear from the blood, some studies have found, but accumulate in the lymphoid tissues where most of the HIV infection occurs.

But just how Tregs disappear from the blood after HIV infection is unclear. One possibility is that HIV infects and kills the circulating Tregs, and Unutmaz has found that this can happen with cultured human Tregs. If HIV does deplete Tregs in vitro, this could contribute to the chronic activation of the immune system that’s observed in HIV-infected individuals. “The more immune activation, the quicker they will develop AIDS and disease,” Unutmaz says.

Intuitively, that doesn’t fit with Choungnet’s observation that there are many Tregs and also many HIV particles in the lymphoid tissues. The Tregs may migrate from the blood to the tissues where most HIV replication occurs, and HIV may actually promote accumulation and perhaps even survival of Tregs, which then further suppress the immune response. “Tregs may be one way that HIV uses to limit the capacity of the immune system,” Choungnet says.

In a field this young, it’s perhaps not all that unexpected that there are contradictory hypotheses. “The field has turned into quite a mess,” Unutmaz says. “It is a bit like the story of blind scientists trying to figure out the elephant by touching different body parts.”

**Manipulating Tregs**

Since it’s unclear whether Tregs in HIV-infected patients are a good or a bad thing, Choungnet says, “it’s difficult to predict how we can manipulate them in a clinical setting.” Genoveffa Franchini’s lab at the National Cancer Institute, with Choungnet, Israel Lowy of Medarex and others, has been trying to inhibit Tregs in monkeys. They used an antibody to block CTLA-4 (cytotoxic T lymphocyte antigen 4), a receptor that, among other things, inhibits activation and proliferation of T cells. Franchini says the antibody modestly boosted the immune response in SIV-infected macaques that were treated with antiretroviral therapy after they were infected with SIV (*Blood* **108**, 3834, 2006). Franchini’s lab has also combined the CTLA-4 antibody with a therapeutic SIV vaccine in macaques, but did not observe an increase in T-cell response.

Medarex has also used the CTLA-4 antibody in a Phase I clinical trial in HIV-infected patients and shown that it was safe and well tolerated, says Lowy, the lead physician for that trial. But Lowy and Shevach say there is little evidence that the CTLA-4 antibody actually affects Tregs and not effector T cells, which also express CTLA-4.

With experimental tumor vaccines, studies in humans have shown that a drug called ONTAK, which binds CD25 and kills Tregs, can enhance the response to the vaccines. And CTLA-4 antibodies can lead to remission but also autoimmune side effects in some patients. However, Shevach says, the precise specificity of ONTAK is unclear, so they may not only affect Tregs but other T cells that also express CD25.

For now, it’s still up in the air what the implications of the continuing research on Tregs will be for AIDS vaccines. Su cautions that preventive vaccination together with an anti-Treg treatment could exacerbate the chronic immune activation in HIV-infected people. “We have to be very careful with modulating the immune system,” Su says. “You may accelerate the disease.”

**Hindsight is 20/20**

But when it comes to the way Tregs were discovered, one thing is clear: “When one looks back, one can find what’s a right and what’s a wrong experiment 20 years later,” Shevach says. “Some [of the experiments] people paid little attention to in the end proved to be correct.”

In a paper describing the CD25 antibody in 1983, Shevach had also found that even in uninfected mice, 8% of the normal T cells expressed CD25—a similar fraction to the Tregs described by Sakaguchi. But at the time, he was not thinking about Tregs, he says. CD25 was thought to be only expressed by activated T cells. So he thought he was looking at regular T cells that expressed CD25 because they had become activated in the animal facility which was quite dirty. “[I] didn’t pay any attention to that,” he says.

So how did Sakaguchi have the idea to look at CD25 as a marker? “I asked him that,” Shevach says, laughing. “He wouldn’t tell me.”
Lasker awardees announced

The winners of this year’s Albert Lasker Medical Research Award were announced on September 17 in advance of the awards ceremony, which will take place on September 28 in New York City. This year’s awardees include two scientists—Ralph Steinman and Tony Fauci—who have contributed substantially to the fields of HIV/AIDS and immunology.

Ralph Steinman of Rockefeller University was awarded the Lasker for Basic Medical Research for his seminal work on the discovery of dendritic cells (DCs), a principal subset of immune cells that control the body’s response to pathogens. His discovery of DCs opened up the entire field of T-cell activation and has led researchers to study the therapeutic use of these cells for cancerous tumors and the development of dendritic cell-based vaccines for several viral infections, including HIV.

Steinman first discovered that it was dendritic cells that stimulated the immune system and propelled other T cells into action by studying cells derived from a mouse spleen. He noticed a novel type of cells, which had long branch-like projections, and thereafter called them dendritic cells. These cells were found to induce T-cell replication and bolster the ability of T cells to kill pathogen-infected cells with a far greater proficiency—more than 100-fold—than that of B cells.

In later studies Steinman showed that dendritic cells harbor HIV and can transmit the virus to T cells, helping to spread the infection to other immune cells. This suggests that dendritic cells will play an important role in the development of preventive AIDS vaccines.

This year’s Lasker Award for public service was given to Tony Fauci, director of the National Institute of Allergy and Infectious Diseases (NIAID), a division of the NIH, for his development of two public health programs in the US. Fauci was instrumental in helping to develop the US President’s Emergency Plan for AIDS Relief (PEPFAR), which is a US$15 billion program to sponsor AIDS treatment and prevention programs in targeted developing countries. He also played a key role in Project Bioshield, which is a program designed to accelerate research into medical countermeasures to biological, chemical, or nuclear agents, such as a vaccine against anthrax.

Fauci has been director of NIAID since 1984 and was awarded the National Medal of Science earlier this year for his research on the pathogenesis of HIV (see An Interview with Tony Fauci, IAVI Report May-June, 2006).

The Lasker awards, often referred to as “America’s Nobels,” were established in 1946 and are awarded to scientists, physicians, and public servants whose accomplishments help alleviate major disease. Since 1962, 71 of the Lasker awardees have gone on to also receive the Nobel Prize.

AVAC receives large grant to advocate for HIV prevention research

The AIDS Vaccine Advocacy Coalition (AVAC) recently received a five-year, US$14 million grant from the Bill & Melinda Gates Foundation to support the organization’s international advocacy efforts. This new funding will expand AVAC’s focus beyond AIDS vaccines to include the broader field of HIV prevention research. AVAC now plans to step up efforts to advocate for several interventions that are currently being tested in clinical trials, including microbicides and pre-exposure prophylaxis (PrEP), which involves the use of antiretrovirals to prevent HIV transmission.

There are currently several ongoing Phase III efficacy trials that are separately testing both microbicides and PrEP, and AVAC plans to work with the communities that are involved in and affected by this research to help prepare them for the results of these trials. The organization, which is based in New York City, will also work to ensure that any benefits of this research become available globally.
Researchers establish new enrollment criteria for African volunteers

Researchers from IAVI, the US Military HIV Research Program (USMHRP), and the US Centers for Disease Control and Prevention (CDC) recently presented research at the AIDS Vaccine 2007 meeting in Seattle (see Seattle sound, page 12) that suggests that a new set of medical criteria should be adopted to screen potential volunteers for AIDS vaccine trials in East and Southern African populations.

Healthy individuals who want to enroll in a preventive AIDS vaccine trial undergo routine medical screening to assess general health; blood chemistry and hematology are tested. The values are compared against a standardized reference range, typically one that has been established for populations in North America and Europe. Potential volunteers with lab values that fall outside the norms are excluded from trial participation.

Altogether, the research studies were conducted over two years and involved approximately 5500 healthy individuals from Uganda, Kenya, Rwanda, and Zambia. Researchers evaluated the blood chemistry and hematology parameters of healthy, HIV-uninfected individuals across seven different research sites to evaluate the kidney, liver, and immunological health of the potential volunteers.

For some of the clinical parameters there was a clear difference between what would be considered a normal result in a healthy African individual. Some of the most marked differences were in some of the baseline immune cell counts, including neutrophils, CD4+ T cells, and eosinophils. There was also a high percentage of Africans who had values for amylase, creatine phosphokinase, bilirubin, and hemoglobin outside of accepted North American/European ranges.

Establishing reference ranges that are relevant to local populations could help improve the enrollment process for clinical trials, including those of AIDS vaccine candidates, by reducing unnecessary exclusion. This could drastically improve the speed and ease of enrolling volunteers. In an AIDS vaccine trial previously conducted by USMHRP in Uganda, 58% of potential volunteers were unable to participate because their laboratory results were outside of the established reference ranges. When a second trial was conducted by USMHRP at the same site using the newly-established reference range, researchers excluded only 23%. Local reference ranges will also help researchers differentiate naturally-occurring laboratory abnormalities from any possible side-effects caused by the vaccine candidate or other intervention being tested.

IAVI and DNAVEC partner to evaluate Sendai virus vector

IAVI recently announced a collaboration with the Japanese biotechnology company DNAVEC to develop and test a new AIDS vaccine candidate based on DNAVEC’s Sendai virus (SeV) vector technology. This is the first time the SeV will be tested as an AIDS vaccine vector and the candidate developed by IAVI and DNAVEC will be designed to stimulate mucosal immune responses, which are thought to be critical for the development of a preventive AIDS vaccine.

SeV is an RNA virus that replicates in the respiratory system but does not cause disease in humans. The SeV vector is unique because it is a replicating vector, which researchers think may help improve its immunogenicity of the vaccine candidates. Preclinical studies of the SeV vector carrying genes from simian immunodeficiency virus (SIV) indicated that the candidate was able to protect non-human primates against infection with SIV. These studies were conducted by DNAVEC and the Japanese National Institute of Infectious Diseases.

The collaboration between DNAVEC and IAVI includes further preclinical testing of the candidate to collect more safety and immunogenicity data in non-human primates prior to conducting a Phase I clinical trial. Both IAVI and DNAVEC intend to advance the SeV-based candidate into human testing within the next three years.