At the pivotal United Nations General Assembly Special Session on HIV (UNGASS) meeting in New York in June 2001, there was an urgent call to action regarding the expanding HIV pandemic, including increased treatment access for HIV-infected persons worldwide. Since then over two million individuals in low- and middle-income countries have gained access to antiretroviral (ARV) therapy through expanded global treatment programs, such as the Global Fund to Fight AIDS, Tuberculosis and Malaria, the US President's Emergency Plan for AIDS Relief, as well as increasing national treatment programs.

On an individual level, treatment results have been spectacular, showing that ARV therapy can be effective even in the most resource-constrained environments. Even so, the overall effort to contain the epidemic is actually losing ground. In some areas more persons are in need of therapy now than before these programs were started and issues about the long-term sustainability of these programs in the face of daunting medical needs remain to be
the incidence,” says Omu Anzala of the Kenyan AIDS Vaccine Initiative (KAVI) in Nairobi.

But accurately determining HIV incidence can be difficult because of the substantial lag time between when a person is first infected and when they develop symptoms of the disease. Measuring HIV incidence requires testing people who may be without signs or symptoms of infection, typically in a research study that follows uninfected volunteers over time. There are also several logistical challenges to determining incidence rates; these longitudinal cohort studies are expensive and time consuming, incidence rates can often fluctuate, and many of the shortcut methods that have been developed to estimate incidence by detecting recently HIV-infected individuals fail to work universally. There are also questions about how far in advance of efficacy trials to start studying incidence. Still, most researchers agree that conducting longitudinal incidence studies are critical. “The feasibility studies to determine true HIV incidence are extremely important,” says Gita Ramjee of the Medical Research Council in South Africa. “They allow you to build capacity so that your Phase III trials are successful.”

**The “power” of incidence**

There are many reasons to study HIV prevalence and incidence in different populations. Prevalence refers to the number of people in a population infected with HIV at any given time. While HIV prevalence can be determined more easily, this doesn’t provide a clear picture of the current dynamics of an epidemic. Even as incidence drops, HIV prevalence may continue to rise due to the lapse between initial HIV infection and mortality. Some experts contend that the dramatic drop in HIV prevalence in countries like Uganda was due at least partly to increasing death rates of those infected early on in the epidemic.

Incidence measurements have several advantages. They can show how the epidemic is changing within certain groups, the speed at which HIV is spreading in light of current sexual or drug-use behaviors, and the effectiveness of available HIV prevention technologies.

Accurate estimates of HIV incidence are also indispensable to the design of HIV prevention trials. Statisticians “power” a study based on the number of people they predict will become HIV-infected during the course of the study, and this prediction is based on the HIV incidence in that population. This determines, among other parameters, how many volunteers must be included in the trial. If the actual incidence ends up being much lower than predicted, it can profoundly affect the outcome of the trial.

Benôit Masse of the Statistical Center for HIV/AIDS Research & Prevention (SCHARP) in Seattle says that even small differences between the predicted and observed HIV incidence can have an enormous impact. He presented an example at an IOM meeting showing that in a trial where statisticians assume an HIV incidence rate of 5% and a rate of only 4% is observed, the total number of volunteers would have to be increased by 25% to preserve the statistical power of the trial. This affects the length and cost of the trial. In the worst case, if the HIV incidence is much lower than expected the trial may be determined futile because it would be unable to provide conclusive results on the efficacy of the intervention and, depending on their charter, could be stopped by the DSB.

“If you underestimate, that’s OK. You just don’t want to overestimate,” says Zeda Rosenberg, chief executive officer of the International Partnership for Microbicides (IPM), a non-profit microbicide research and advocacy group.

**Measuring incidence**

For this reason it is critical to start a trial with the most accurate incidence estimates possible within the specific population where a study will occur. Most often incidence data is reported from antenatal clinics because almost all pregnant women are tested for HIV infection so that health officials can intervene to protect infants. This is the only setting where semi-mandatory HIV testing occurs. But this data fails to capture HIV incidence in other high-risk groups, including injection drug users (IDUs), men who have sex with men (MSM), and commercial sex workers.

Collecting incidence rates among the general adult population and within certain subgroups is substantially more complicated. The gold standard method is the prospective cohort study where researchers follow large groups of uninfected individuals for long periods of time, testing them regularly—typically at three-month intervals—for HIV infection to determine the rate of seroconversion. Incidence rates are usually reported as the
percentage of people infected in a single year.

But prospective studies are time-consuming, labor-intensive, and expensive, and add substantially to the already complex process of running a clinical trial. Consequently some sponsors may rely on previously published incidence data when designing their study. But this approach can be risky. CONRAD, a US-based reproductive health and HIV/AIDS prevention organization, and Family Health International (FHI), a non-profit organization in North Carolina, based their Phase III efficacy trial of the microbicide candidate cellulose sulfate in Nigeria on incidence data collected several years prior to the start of their study. Although this data suggested a 4% annual incidence in the population they intended to enroll—women who were considered at high risk of heterosexual HIV transmission—the actual observed incidence during the study was half that. As a result the DSMB advised investigators that they would have to increase recruitment significantly. When researchers determined this wasn’t feasible because of difficulty working in the country, they had to close the Nigerian trial sites and start anew in South Africa where incidence rates are much higher. The discrepancy between predicted and actual incidence illuminates a conundrum facing HIV prevention researchers. “We don’t want people to get infected, but without [infection] events you can’t make any conclusion about the product,” says Doug Taylor, director of biostatistics at FHI.

Another Phase III trial of the microbicide candidate SAVVY conducted by FHI, which was powered based on older published incidence data, was stopped prematurely by the DSMB in Ghana because of lower-than-expected HIV incidence. The other branch of this trial in Nigeria was stopped a year later, also for futility.

We don’t want people to get infected, but without [infection] events you can’t make any conclusion about the product.

Doug Taylor

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Figure 1. Estimated incidence rates for 8 African countries over a 10-year period. Incidence estimates provided by the US Census Bureau and are based on unpublished data consistent with population projections presented in the April 2005 release of the International Data Base, available at www.census.gov/ipc/www. These figures are modeled from HIV prevalence data collected from sentinel surveillance sites and prevalence studies conducted in these countries. This data was also included in the article, “Has global HIV incidence peaked?” (The Lancet 368, 1120, 2006).
Putting the BED to bed

The BED immunoassay seemed to provide promising results when originally tested in the US in a cohort of women in Atlanta. Researchers reported there was “excellent agreement when estimating incidence,” based on this approach (J. Acquir. Immune. Defic. Syndr. 44, 196, 2007). Samples collected during the AIDSVAX AIDS vaccine trial were also evaluated with the BED assay and there was strong agreement between the actual and BED-estimated incidence (AIDS Res. Hum. Retroviruses 22, 945, 2006).

But when this assay was tested in Uganda, Kenya, Zamb ia, and Rwanda, researchers found that the BED assay drastically overestimated the HIV incidence. In Masaka, Uganda the incidence as determined by BED was 6%, while prospective data from that same population showed an incidence of 1.7% the year before the BED assay was used and 1.4% the year after (AIDS 21, 403, 2007). Similar findings were also reported by researchers working in Cote D’Ivoire (J. Acquir. Immune. Defic. Syndr. 45, 115, 2007). In this study the authors tested both the “detuned” and the BED assay methods and concluded that neither could be used routinely to estimate HIV incidence in the country.

One problem with the BED assay is that it can’t distinguish a recently infected individual from someone in late-stage HIV infection whose immune system has begun to fail, and therefore has waning levels of HIV-specific antibodies. In many cases the BED assay was incorrectly classifying individuals who had already met the clinical diagnosis of AIDS and were taking antiretroviral therapy as recent infections. “We found that our BED estimates were so bad you might as well guess,” says Matt Price, clinical program manager at IAVI. The BED assay was tested by IAVI with several collaborators, including the Uganda Virus Research Institute, the Kenya AIDS Vaccine Initiative, the Zambia Emory HIV Research Project, and Project San Francisco, and they found that “it hugely overestimates incidence,” says Price.

Where there’s a will

Several other methods also exist for estimating HIV incidence. One method is to use mathematical models to predict incidence based on existing prevalence data, but the accuracy of this approach is dubious.

Over the past 10 years several immunological assays have also been developed to differentiate between recent and chronic HIV infections in an effort to estimate incidence. By sampling a large number of individuals researchers can extrapolate the HIV incidence based on the number of people surveyed who appear to be recently infected. This eliminates the need to repeatedly test people over time and relies on the presence of certain antigens or antibodies that appear within a defined window period of acute HIV infection.

One approach is to use a test that detects the plasma levels of p24, the HIV capsid protein that reaches peak titers during acute HIV infection. Once HIV-specific antibodies are induced they bind the p24 antigen, thereby making it undetectable. This assay, however, is not sufficiently sensitive to reliably detect the narrow window period between p24 antigen positivity and the presence of antibodies and therefore isn’t useful for calculating incidence.

Another approach is to use a combination of two ELISA tests of differing sensitivity for HIV-specific antibodies, known as the Serological Testing Algorithm for Recent HIV Seroconversion (STARHS) or “detuned” assay. If antibodies are detectable by the more-sensitive assay, the ELISA is repeated with a purposely less-sensitive assay to see if antibodies are still detectable. The theory is that only chronically HIV-infected individuals who have developed a much more robust and polyclonal immune response to the virus will have a high enough titer of antibodies to still be detectable. But in practice the assay doesn’t work so well. “Our finding was that this was just not true,” says Salim Karim, director of the Centre for the AIDS Programme of Research in South Africa. He found that STARHS substantially underestimated HIV incidence at sites in South Africa.

A third method for detecting recent infection is known as the BED capture enzyme immunoassay, so-named by its developers at the US Centers for Disease Control and Prevention (CDC) because it was originally developed based on the B, E, and D clades of HIV. The premise of the BED assay is that the immune system ramps up production of HIV-specific antibodies over time and these responses, IgG in particular, evolve from having a weaker to a stronger avidity for HIV. The BED approach uses an ELISA
The missing piece

A handful of countries around the world have aggressively monitored HIV incidence for many years as a way to track their own epidemic’s progress. Thailand, a country lauded for its early and progressive response to HIV/AIDS, began a national surveillance program in 1984 and has been determining annual incidence rates ever since. Early on in the epidemic there was also a national effort in Thailand to determine new cases of HIV infection among particularly high-risk groups, like commercial sex workers, injection drug users, and men who have sex with men. This allowed Thai officials to detect the first wave of the epidemic in these individuals, says Kerks-Ngarm. “Knowing what the real situation was like was the most important thing we could do to solve the problem,” he says. This led to the requirement that all of the country’s sex workers use condoms to limit the spread of HIV.

In Uganda, another place where early HIV prevention efforts were credited with stunting an exploding HIV/AIDS epidemic, public health officials started collecting HIV incidence data in 1989. From 1990 until around 2000, the HIV incidence in the general population hovered around 1%, says Kamali. “This is good, reliable data on incidence,” he adds. This relatively stable incidence level, compared to other African countries, was attributed to the government’s endorsement of the ABC approach (abstinence, be faithful, use condoms). But since 2000, there seems to be a slight increase in HIV incidence within the general population, according to Kamali.

In many other countries there is very little current data on HIV incidence. Throughout Asia, for example, reliable HIV incidence data are scarce. Recently India revised its estimates on the number of HIV-infected people in the country based on declines in HIV prevalence among commercial sex workers and within the general population in some of the southern regions of the country (see Vaccine Briefs, page 16). Although there is very limited incidence data in the country, UNAIDS concludes that based on the revised prevalence data there is probably also a decline in the number of new HIV infections.

Even in South Africa, which is home to the world’s largest HIV/AIDS epidemic, incidence data is limited. In 2005 researchers from the Human Sciences Research Council determined incidence rates in 16,000 South Africans and found that the total number of new infections during the year was 571,000 (South African Medical Journal 97, 194, 2007). The highest incidence rate of 5.6% was observed in women between the ages of 20 and 29. But since the BED immunoassay (see Putting the BED to bed) was used to collect this national incidence data, Karim warns that the absolute numbers should be “regarded as tentative” (South African Medical Journal 97, 190, 2007).

assay to measure the proportion of the total IgG levels that are HIV-specific. This ratio is then compared with a set of predefined parameters to determine if an infection is classified as recent or not.

With the BED assay, incidence estimates could be calculated after conducting a survey of only around 1000 people. “But the assay has tended to overestimate HIV incidence in African situations,” says Anatoli Kamali of the Medical Research Council in Entebbe, Uganda (see box, Putting the BED to bed).

Peripheral benefits

Even if immunological assays could more accurately and cheaply estimate HIV incidence, they fail to replicate the conditions of a clinical trial, where individuals are receiving regular counseling, education on their risk behaviors and HIV prevention, and continuous access to condoms. “They look at incidence in populations that are not exposed to behavioral interventions, which could, and likely will, lower HIV incidence,” says Price, whereas all participants in a longitudinal study to determine HIV incidence receive risk-reduction counseling, just like their counterparts in clinical trials.

HIV incidence tends to be even lower among volunteers in clinical trials than in the general population. “Every time you start working in a community the incidence drops,” says Anzala, highlighting another conundrum of this work—it is impossible to measure incidence without affecting it.

Incidence studies prior to a clinical trial also provide an opportunity for researchers to cultivate relationships with the community members and leaders, to start educational programs that will aid enrollment in future trials, and help establish both the infrastructure and technical know-how among people working at the clinical trial site. “There’s no point undertaking a clinical trial in an area where you have no community support,” Ramjee says.

In the process of conducting longitudinal HIV incidence studies at several sites in South and East Africa, investigators are able to refine both their recruitment and retention skills, other aspects that will be critical to the success of a future trial. “The traditional way of looking at incidence lets you decide if it is really a suitable community for doing a vaccine trial,” says Anzala.

There is also valuable social science research that can be conducted during incidence studies. Researchers can study trends in sexual behaviors within a population, what is putting individuals most at risk for HIV infection, and pregnancy rates among female volunteers that can help determine condom use. “Invariably you obtain a lot of scientific data,” says Kamali.

Beware of falling incidence

Another complicating factor is that HIV incidence can change rapidly, often declining due to effective prevention campaigns, the recent proliferation of antiretroviral (ARV) treatment programs in some developing countries, and also due to more accurate methodologies (Figure 1). Ronald Brookmeyer, a biostatistician from Johns Hopkins University who was a member of the IOM panel evaluating HIV prevention trials, referred to incidence rates as a “moving target”.

Thailand once had one of the most rapidly expanding epidemics in the world, but now HIV incidence seems to have trailed off outside high-risk groups. When the first AIDS vaccine efficacy trial with the AIDSVAX candidate was conducted in Thailand, preparative cohort studies showed incidence rates of as high as 6%, but during the trial incidence was only 3.4%. Since then incidence has dropped even further.

The only other Phase III AIDS vaccine trial, which is evaluating the efficacy of a
high number of new HIV infections occurring globally—last year alone 4.3 million people were newly infected. Researchers are now focusing more on pockets or sub-groups of individuals where HIV transmission rates still tend to be very high. “You can go anywhere and if you find the right populations, you can have a high enough incidence,” says Karim. But the problem with working in exclusively high-risk populations is first identifying them and then working to recruit and retain them in long-term prevention studies. Many research groups are gaining experience in these areas by conducting prospective incidence studies in these high-risk populations in preparation for AIDS vaccine efficacy trials.

Kamali and others in several African countries are now working with cohorts of HIV discordant couples, where only one partner is HIV infected. In Uganda, Kamali’s group in cooperation with IAVI has established a cohort of about 500 discordant couples and has observed an incidence rate of around 4%, nearly four times that seen in the general population. Susan Allen, an HIV/AIDS researcher from Emory University in Atlanta, was one of the pioneers of working with discordant couples. At three sites in Zambia affiliated with her program, the Rwanda Zambia HIV Research Group, the transmission rates among discordant couples range from 3% to 9%, even with access to the best behavioral interventions.

“We are not just watching people get infected,” says Kamali. “We are giving them everything that is available for HIV prevention and even with that comprehensive package we still observe, unfortunately, a high HIV incidence.”

Anzala is conducting an HIV incidence study in Kangemi, Kenya involving 701 individuals, including discordant couples, commercial sex workers, and MSM. This work is also part of IAVI’s efforts to establish incidence information at trial sites in preparation for large-scale AIDS vaccine trials. Both this cohort and Kamali’s discordant couple cohort in Uganda will be participating in the upcoming Phase IIb AIDS vaccine trial known as PAVE 100. This trial will evaluate the safety and preliminary efficacy of the combination of DNA and adenovirus serotype 5 vaccine candidates developed by the Vaccine Research Center at the National Institutes of Allergy and Infectious Diseases.

Other groups including the US Military HIV Research Program are conducting incidence studies in preparation for AIDS vaccine trials. According to Rosenberg, IPM plans to conduct their own incidence studies before starting efficacy trials with microbiode candidates in women who are at high-risk for HIV infection.

In South Africa, which has the largest number of HIV-infected individuals, the HIV prevalence and incidence are generally so high that it is often unnecessary to recruit only high-risk volunteers. “I’m not saying that all the work should be done in South Africa, but you put out the fire where the fire is raging,” says Ramjee.

Lingering questions

One question still puzzling researchers is when is the right time to start incidence studies. This information is obviously important to have before beginning Phase IIb proof-of-concept trials, but if it is still years before an AIDS vaccine candidate is ready for a Phase III efficacy trial, it is likely that the work will need to be repeated. “This is a Catch 22,” says Anzala. “If you start too early, you risk that the community will be burned out. But if you wait until a vaccine is available it takes a lot of time. When you start Phase I trials you should to some extent start to prepare for Phase III,” he adds.

Also as male circumcision, improved access to ARVs, and possibly other yet unproven HIV prevention modalities become available, incidence may decline even in high-risk populations. Although this would be an outstanding accomplishment, it will make it even more difficult to conduct efficacy trials in the future. “It’s a trade-off,” says Ramjee.
addressed. Prevention efforts, including behavioral and vaccine efforts, have not kept abreast with need and the pandemic continues to expand in developed and developing countries.

Clearly the only global solution to the AIDS epidemic is an effective vaccine, yet six years after the UNGASS meeting and almost a quarter-century since the viral etiology of AIDS was defined, an effective preventive vaccine still eludes our grasp. Indeed, in the interim, additional insights into the challenges posed in generating broadly-neutralizing antibodies have resulted in the rather stark realization that a fully preventive vaccine—one that prevents infection from occurring through sterilizing immunity—is not a realistic immediate goal. Instead vaccine efforts are currently focused on a hypothesis that has shown promise in animal models—namely a vaccine that will keep the virus in check to such a degree that disease progression will not occur if a vaccinated individual does become infected, and that maintains viral load at such low levels that the likelihood of transmission will be markedly reduced.

Cohort studies have shown that viral load is directly linked to both disease progression and transmission and the likelihood of either of these outcomes markedly diminishes at viral loads below 2000 HIV RNA copies/ml plasma. Although on an individual level persons with viral loads this low can still transmit virus and/or undergo disease progression, on a population level these events would diminish to the point that the epidemic would be considerably curtailed if this level of control could be achieved with a vaccine. There is reason to be optimistic, given that infected individuals who represent precisely this desired phenotype have been identified. These persons, who have been termed HIV controllers, are able to maintain suppression of viremia and stable CD4+ T-cell counts without ARV medication, some now for close to 30 years (S. Deeks and B. Walker, manuscript submitted). The most dramatic examples are those who maintain viral loads below the level of detection by the most sensitive commercial assays presently in use.

For the purposes of this article we will focus on two groups of HIV controllers: elite controllers, who maintain viral loads of <50 HIV RNA copies/ml plasma in the absence of therapy, and viremic controllers, who maintain viral loads between 50 and 2000 RNA copies/ml. This article seeks to draw attention to recent international efforts to focus research efforts on these individuals, and to use advances from the Human Genome Project to dissect the mechanisms behind these remarkable outcomes, and thereby guide efforts to recreate this phenotype through an AIDS vaccine.

**Long-term nonprogressors and HIV controllers**

Some of the earliest data showing dramatic differences in long-term outcome of HIV infection came from the San Francisco City Clinic, where blood samples stored during a hepatitis B vaccine study conducted in the late 1970s enabled precise determination of the timing of HIV infection as the epidemic spread through that population. As the extent of the HIV epidemic became clearer in the 1990s, there was a remarkable realization that some individuals in this cohort were remaining healthy (as defined by stable CD4+ T-cell counts) despite never being treated with the ARVs that were becoming increasingly available. These persons were detected in this and other cohort studies with sufficient frequency that the US National Institutes of Health sponsored a meeting on what were to be termed long-term nonprogressors (LTNP) in the early 1990s. A subset of these LTNP with even more remarkable characteristics was then defined as sensitive viral load testing became available: those who had plasma viral loads below the limits of detection by the most sensitive assays (initially <400 RNA copies/ml and later <50 RNA copies/ml). Here was a group of persons who appeared to be able to contain HIV as is typically seen for many other chronic virus infections—such as Epstein-Barr virus and varicella zoster virus—that are effectively held in check despite the continued presence of infectious virus.

From an AIDS vaccine standpoint, understanding this subset of infected individuals, defined by the ability to control viral load rather than by CD4+ T-cell count or duration of infection, may be most relevant in terms of the rationale for a vaccine to prevent disease progression. Cohort studies suggest that elite controllers occur at a frequency of about one in 300 HIV-infected persons. A larger subgroup of HIV-infected persons called viremic controllers consists of those who maintain viral loads between 50 and 2000 RNA copies/ml plasma without treatment. At a viral load of less than 2000 copies, two important needs for a vaccine would likely be met: there would be less likelihood of progression in persons infected, and, since these viral loads are associated with delayed progression and reduced transmissibility, such individuals are also likely to provide key insights into the factors involved in successful viral containment.
**Reasons for HIV control**

Understanding the ability to maintain viral loads at levels that would diminish the likelihood of both disease progression and transmission is key to current vaccine development efforts. Multiple factors have been implicated in contributing to this outcome, including viral, host genetic, and immunologic factors, but the bottom line is that we still lack a fundamental understanding of the pathways leading to this remarkable and particularly relevant outcome of natural infection.

**Viral factors:** At the time LTNPs were first being defined, the identification of a group of transfusion recipients in Australia who had all received blood products from the same donor and all had slowly progressing diseases suggested that some persons were simply infected with less pathogenic viruses. These persons ultimately progressed, and although additional studies have shown that gross defects in HIV genes can attenuate viral pathogenicity, such deletions are not a common feature of improved outcome. Some studies demonstrated that virus isolated from LTNPs are attenuated, but replication competent viruses have clearly been isolated from persons maintaining viral loads below 50 RNA copies/ml. Even minor differences in viral fitness may have a long-term impact over the typical 10-year course from HIV infection to AIDS, and enhanced methods for discriminating fitness differences are needed to address this issue. Insights into viral factors contributing to this phenotype are also likely to come from detailed characterization of viruses from persons who establish elite control and then subsequently lose this control.

**Host genetics:** Although large host genetic studies focused solely on elite controllers are yet to be done, associations between host genetic variation and susceptibility to HIV infection and disease progression have been studied extensively across the entire spectrum of viral loads. Some examples are chemokine co-receptor polymorphisms such as CCR5Δ32 and CCR2Δ64I, CCR5 promoter polymorphisms, RANTES-28G→403A, SDF-1 3’A, IL-4 589T, and others. Variation in host factors that restrict viral replication such as APOBEC3G and Trim5α may also influence disease, but are yet to be studied in detail in HIV controllers. Except for resistance to HIV-1 infection of persons homozygous for CCR5Δ32, it is unclear how many of these factors would remain statistically significant after multivariate analysis taking into account all reported gene variation. A recent study of over 4000 HIV-infected and uninfected individuals demonstrated that combination of CCL3L1 gene (that encodes MIP-1α) copy number and CCR5 haplotype is associated with HIV/AIDS susceptibility and disease progression. As more host factors are discovered and included in these studies, greater numbers of patients will be required to perform multivariate analyses and to define true associations. Without question the strongest genetic association thus far with elite control is the expression of certain HLA class I alleles. Since these surface molecules present viral peptides to the immune system for recognition, they suggest a link between host genetics and the adaptive arm of the immune responses, particularly suggesting a key role of virus-specific CD8+ T cells.

**Immunologic:** HIV controllers are particularly relevant to AIDS vaccine design if it actually is the immune system in these persons that controls HIV replication. Although by no means conclusive, current data suggest that this is indeed the case. Perhaps the strongest indicator that the immune system influences outcome is the high frequency of certain HLA class 1 alleles among elite controllers, such as HLA B27 and HLA B57. These cell-surface molecules complexed with a viral protein indicate to the immune system that the cell is infected and should be eliminated, so the enrichment for certain HLA alleles implicates the host immune response rather than the virus itself. But the issues are far from resolved—quantitatively, using the interferon (IFN) ELISPOT assay to detect responses, there is no difference in magnitude or breadth of HIV-specific CD8+ T-cell responses and viral load. Instead it may be functional qualities of these cells, such as the ability to proliferate or to avoid exhaustion, or to directly inhibit virus replication in vitro, that play a central role in outcome. Whether these are simply associations or causative will remain very difficult to determine. Recent data indicate that even in elite controllers there is evidence of ongoing immune selection pressure on the virus, as indicated by mutations arising within targeted epitopes, suggesting ongoing functional significance of these responses. Moreover, depletion of CD8+ T cells in an animal model of elite control led to increased viremia, again indicating active containment of the virus by these cells.

Another strong association between immunologic function and disease outcome derives from studies of CD4+ T-cell function, particularly the ability to secrete multiple cytokines, including TNFα, IFNγ, and IL-2. In the largest study to date involving 30 HIV controllers, polyfunctional HIV-specific CD4+ T cells expressing IFNγ and IL-2 were the single most consistent correlate of control, but there was still a substantial proportion of subjects who did not demonstrate such responses. Another critical host defense that may impact adaptive immune responses in general is innate immunity. Recent data link particular natural killer cell receptors to enhanced control of viremia, supporting a potential role for innate immunity in chronic infection. There are almost no data describing the earliest innate immune events following an acute infection that lead to elite control.
In terms of neutralizing antibodies (NAbs), studies of elite controllers indicate generally low-level responses\textsuperscript{37}. In a comprehensive examination of NAb responses in persons with undetectable viremia (from whom pseudotyped viruses were constructed from autologous Env) there was a lack of strong responses to autologous virus\textsuperscript{37}, suggesting that there is little ongoing exposure to virus and that NAbs are unlikely to play a major role in durable containment of viremia in these persons.

**Unbiased approach: whole genome association scan**

It is fair to say that there have been insufficient numbers of elite controllers studied to date to draw firm conclusions, but the data available indicate that none of the current parameters we use to assess virus-host interactions provides high predictability for the observed elite control phenotype. In part we may be constrained by working within existing scientific paradigms of how the host responds to viruses. Now major progress in human genetics allows for a different and largely unbiased approach to defining the molecular basis for disease outcome—high throughput sequencing of the human genome to find the genotype that predicts a particular disease phenotype\textsuperscript{41}. Until recently such studies could not be contemplated since there was no practical way to sequence the entire three billion nucleotides in the human genome. However, the combination of practical shortcuts as well as reduced sequencing costs now make this a realistic approach and something we believe absolutely needs to be applied to HIV/AIDS.

The goal of whole genome association scans (WGAS) is to define the genetic variability within the host genome that is directly linked to a particular phenotype such as elite control. Fortunately there is limited variability within the human genome, which usually involves single nucleotide polymorphisms (SNPs) that occur with a frequency of about 1 in every 300 base pairs. If one only focuses on the regions of variability, this greatly reduces the amount of sequencing required, and is further diminished because of linkage disequilibrium (Figure 1). Closely adjacent SNPs are often transmitted together, so that the presence of one SNP is highly predictive of the presence of a second specific SNP, resulting in what is called a particular haplotype. By selecting one SNP, called a tagSNP, one can often obtain highly reliable information on the surrounding SNPs, greatly reducing the amount of sequencing required. Currently available commercial products allow for the rapid automated sequencing of 650,000 or more SNPs per patient, meaning that large population studies are now possible. With as few as 100 subjects this kind of approach has defined the genetic basis for diseases such as age-related macular degeneration. A recent review summarizes the approach and the power of this type of assay\textsuperscript{32}.

**Figure 1. Whole genome association scan.** A) Variability within the human genome is defined by a discrete number of single nucleotide polymorphisms. B) Many of these SNPs are in linkage disequilibrium, such that C) definition of one SNP within a group in linkage disequilibrium can define the entire group (haplotype), allowing for more limited genetic sequencing to define genomic variability.
Recently a global research consortium, The International HIV Controller Consortium, has been established with the goal of recruiting at least 1000 elite controllers and 1000 viremic controllers, as well as up to 3000 progressor controls, in order to perform a WGAS to define the genetic profile that accounts for elite control. These numbers are the minimum likely to be required to provide appropriate power for such analyses. More is always better given the multiple comparisons being made, but an approximation for this sort of approach is 1000-2000 or more persons with a particular disease phenotype. If the underlying etiology is a single nucleotide change, which for example is the cause of sickle cell disease, then this approach would generate statistical significance with very few subjects compared to controls. For more complex traits or disease states that have multiple different etiologies, the associations can be much more difficult to define in the sea of background noise. There are other potential limitations to this approach, including the possibility that elite control may be defined by some regulatory element which can be more difficult to detect, or by gene duplications, which are also harder to quantitate. Through this international consortium a total of over 600 HIV controllers have already been recruited, of whom 50% are elite and 50% viremic, and sequencing has commenced on these initial subjects. Whether this approach will yield new insights should be known soon.

Conclusion

Despite extensive research for over two decades we are still a long way from development of a preventive AIDS vaccine. In contrast, a cellular immunity-based vaccine which controls HIV-1 replication and significantly delays disease progression seems to be a realistic goal for the near future. Extremely skewed distribution of HLA class I alleles amongst elite controllers and increase in viremia after CD8+ T-cell depletion in SIV-infected elite controller monkeys strongly suggest that cellular immunity is playing a major role in containing viral replication in elite controllers. It would be essential for cytotoxic T-lymphocyte vaccine development to dissect the mechanisms behind this strict viremia control. Although conventional approaches have been unsuccessful so far, we are hopeful that novel approaches using a whole genome association scan will provide new key insights to define the correlates of immune protection and guide effective vaccine development. The probability of success of this approach increases with increasing numbers of subjects studied, and readers are encouraged to join in this broad collaborative global effort to recruit the necessary numbers of HIV controllers to allow for the mechanisms of control to be dissected (www.elitecontrollers.org), with the goal of using the information gained to guide both prophylactic and therapeutic vaccine development.

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One-two combination

Heterologous prime-boost is currently the regimen of choice for many AIDS vaccine approaches, even though exactly how it works is still far from clear

by Andreas von Bubnoff

We all use things every day even though we have little appreciation of how they work. Cars, cell phones, and computers come to mind. Still, we know that someone somewhere knows how they work so we can ask them how to fix them when they don’t work as they should.

Yet, that’s not always the case with AIDS vaccine candidates that are in development. One vaccination regimen, for example, dubbed prime-boost, is currently used in many AIDS vaccine trials. But surprisingly little is known about precisely how it works.

Intuitively, the rationale is simple: The first administration—the prime—generates a pool of memory T or B lymphocytes that allows for a quicker and stronger immune response to the second administration—the boost. And it works. “Essentially, most vaccination strategies are primes and boosts,” says Larry Corey who leads the HIV Vaccine Trials Network (HVTN) in Seattle. Giving a second dose of the same vaccine is common practice for many vaccines, such as those against chickenpox and measles.

Now heterologous prime-boost—using different vaccines for the prime and boost—is the regimen of choice for many AIDS vaccine candidates that are in clinical trials. The hope is that the regimen will lead to increased immunogenicity and could also result in a broader immune response because each vaccine component might stimulate a different type of immune response. In addition, using a different vector for the boost also ensures that there is no antivector immunity generated by the priming immunization that interferes with the boost.

Indeed, Hildegund Ertl of the Wistar Institute in Philadelphia thinks a successful AIDS vaccine is likely going to be a heterologous prime-boost regimen. “That’s the approach where I’d put my money right now,” she says. Tomas Hanke of the University of Oxford even goes as far as to suggest that, for now, it’s better to try combinations of the existing vaccines and vectors in clinical trials than develop new ones. “I don’t think we need more vaccines, more vectors,” he says.

Typically, the regimens combine DNA and replication-deficient or attenuated viral vectors such as adenovirus (Ad) or modified vaccinia Ankara (MVA) to deliver HIV proteins to antigen-presenting cells. Indeed, many different combinations have been tried and more are planned, and about half of the 30 or so ongoing AIDS vaccine trials use such approaches. Even so, there is surprisingly little data that resolves the immunological mechanism behind the relative robustness of prime-boost, and just why some combinations work better than others remains mysterious.

**Try it and see what happens, but there is some thought behind it**

**Peggy Johnston**

**Trial and error**

That’s why finding the right combination often boils down to trial and error. In 1998, for example, Hanke, then in Andrew McMichael’s group at the University of Oxford, published the first study that used DNA as prime and MVA as boost for an AIDS vaccine candidate (Vaccine 16, 439, 1998). Hanke says they chose DNA and MVA because they were readily available for use in humans at the time. DNA is also attractive because it is itself inert and doesn’t elicit an immune response to anything other than the HIV immunogens it encodes.

However, the choice of which to use as the prime and which for the boost was very much empirical, Hanke says. “We wanted to try their combinations, first without really thinking why we should use this one first as opposed to the other,” he says. “We tried all combinations of DNA/DNA, MVA/MVA, DNA/MVA and MVA/DNA, and found that DNA/MVA was by far the best.” The mouse study showed that the DNA/MVA combination resulted in the strongest CD8+ T-cell immunogenicity. The work was done in collaboration with Adrian Hill’s lab, who showed around the same time that the approach also worked for an experimental malaria vaccine (Nature Medicine 4, 397, 1998).

Even today, deciding which vectors to use for prime and boost still involves a lot of “educated empiricism,” Corey says. Peggy Johnston of the Division of AIDS at the National Institute of Allergy and Infectious Diseases (NIAID) prefers to call the approach “thoughtful empiricism.” “Try it and see what happens, but there is some thought behind it,” she says. Empiricism sometimes does have a particular advantage: the outcome can come as a surprise. In primates, for example, two groups have reported that Ad serotype 35 (Ad35) followed by Ad5 gives a better overall response than vice versa, Corey says.

Safety is an overriding factor when it comes to choosing vectors and is one reason that many trials use MVA, which is a modified version of the smallpox vaccine that has been used for hundreds of years. Similarly, one reason that Ad vectors are used is that their safety profile is well known, Johnston says.

**Lost in translation**

Demonstrating that a prime-boost regimen works in animal models doesn’t mean that it’s going to work in humans. Hanke and...
McMichael’s DNA/MVA regimen, for example, also worked well in monkeys, but not so well in humans; a Phase I/IIa trial showed that fewer than 15% of the vaccinees had an immune response detectable by an interferon (IFNγ) ELISPOT assay. A smaller trial later showed a response in half of the vaccinees, but, according to McMichael, it was a weak response compared with other vectors such as Ad.

Hanke thinks that one reason for that was the limited DNA delivery attainable in humans; it’s simply not possible to get the same high doses of DNA per weight into humans as into animals. To give humans an equivalent dose to that used in animal experiments, one would have to use something like 70 mg. “We use a gene gun for mice that covers half their body. You don’t use a cannon to go into humans,” Ertl says. “Imagine injecting a pint of material,” adds McMichael. Both Hanke and McMichael have now turned away from using DNA as a prime.

Other researchers still see value in DNA. David Ho of the Aaron Diamond AIDS Research Center in New York is using a DNA/MVA prime-boost regimen with five HIV clade C genes. In mice and rabbits the combination works about 10 times better than each component by itself, and Phase I trials have shown that each alone is safe and immunogenic. Ho will check the DNA/MVA combination in Phase II trials next and anticipates good results because of technical improvements; for example, he is adapting the DNA vaccine’s GC content in the HIV gene inserts so that the mammalian host can better express them.

Ho is also working with a company called Ichor to develop electroporation as an improved DNA delivery method, which uses electric current to temporarily poke holes into cell membranes that enables the DNA to pass through. Data in mice and rabbits suggest that electroporation improves humoral immune responses 100-fold and cellular responses 10-fold, and there is no drop in delivery efficiency between rodents and monkeys, suggesting that delivery in humans will be efficient as well. Ho plans to test electroporation in a Phase I safety trial later this year.

To circumvent the problem of DNA delivery, other groups are working on heterologous prime-boost regimens that combine different viral vectors. Dan Barouch at Harvard University is testing different Ad serotypes in rhesus macaques and says that a heterologous Ad26/Ad5 approach proved 8-fold more immunogenic than Ad5/Ad5, presumably at least partly because the heterologous boost avoids anti-vector immunity generated by the priming immunization.

One potential issue with using Ad5 as a vector is that a majority of some developing-country populations, particularly in sub-Saharan Africa, have preexisting immunity to the wild type virus that can dampen the vaccine-induced HIV-specific immune response.

To circumvent this problem, Barouch has made a chimeric Ad5 vector, Ad5HVR48, that has the surface loops of its major capsid protein replaced with ones from a less-prevalent serotype, Ad48. These loops are dominant targets of Ad5-specific neutralizing antibodies. Barouch says one particularly potent combination in animal experiments is Ad26 prime followed by a boost with either the regular Ad5 or with Ad5HVR48. He plans to bring Ad26 and Ad5HVR48 into Phase I clinical trials to test their safety and immunogenicity.

**Early results in humans**

Most of the evidence that heterologous prime-boost works better than homologous comes from animal experiments, but some regimens have already proven safe in humans and appear to show some immunogenicity in Phase I trials. Giuseppe Pantaleo of the University Hospital in Lausanne, Switzerland, is one of the coordinators of a European Phase I trial that uses a DNA/NYVAC poxvirus combination that resulted in cellular immunity in 90% of vaccinees, whereas NYVAC alone induced responses in only 40% of vaccinees. What’s more, DNA/NYVAC showed a 5-fold greater and more durable response than NYVAC/NYVAC. A Phase II trial has already started enrollment.

Another regimen that appears to work in humans uses DNA as a prime and Ad5 as a boost. In monkeys, the DNA/Ad5 candidates developed by NIAID’s Vaccine Research Center (VRC) are more immunogenic than Ad5/Ad5 or either vector alone. According to Corey, early returns in Phase II studies with the VRC DNA/Ad5 vaccine suggest that more than 70% of the recipients develop HIV-specific T-cell responses. The regimen will be tested soon in a Phase Ib efficacy trial called PAVE 100.
Corey says that additional human trial results with heterologous prime-boost regimens will be presented at the upcoming AIDS Vaccine 2007 conference in Seattle in August; for example, a small Phase I trial has indicated that a combination of MVA and fowlpox vectors results in better immunogenicity than repeated doses of MVA.

These preliminary human results look encouraging. But not everyone has observed that heterologous prime-boost regimens work better in humans than using the same vaccine repeatedly. “Heterologous prime-boost has not worked in people in our hands in fairly large Phase I studies,” says John Shiver of Merck Research Laboratories. “We haven’t found anything that shows that prime-boost adds a synergistic effect in people, and we have tested probably more things than anybody else. I know of some that work in monkeys but not any that work in people.” For example, he says, Ad5 prime and canarypox boost does not work better than Ad5/Ad5. What’s more, Merck did not see any difference between DNA/Ad5 and Ad5/Ad5 regimens in humans. This is why the company is currently conducting Phase IIb trials that use repeated injections of Ad5. “The immune responses we get with the adenovector [alone] are actually pretty good,” Shiver says.

**Mechanism**

Given that, in many cases, heterologous prime-boost appears to induce stronger immune responses, the question is how. The approach makes sense because it circumvents antivector immunity, but there also appears to be a degree of synergy because heterologous vectors induce more robust responses, and this may include a distinct type of response. But there is a dearth of experimental data to demonstrate how this happens. “I don’t think people know,” Johnston says. “It could be that each vector will potentially enter different cell types.”

“Exactly why it’s better, I don’t think anybody knows,” says Rockefeller University’s Sarah Schlesinger, who collaborates with Ho on the DNA/MVA trials. She thinks that one problem is that it’s difficult to directly measure priming.

As for why the DNA/NYVAC regimen works better than NYVAC alone, Pantaleo thinks that what DNA does from an immunological standpoint is very different from what poxvirus does. “DNA goes into different cells and the way it is presented is different,” he says, adding that very little is known about the mechanism. He thinks that one problem is that it’s hard to study in vitro what happens in humans in vivo. He has his own hunch as to why priming with DNA results in a longer lasting and more powerful T-cell response, and he suggests there could be large deposits of DNA in muscle cells at the site of injection. “But I don’t have experimental evidence for that,” Pantaleo says.

There is, however, some evidence that using heterologous prime-boost results in a more diversified immune response than using the same vaccine repeatedly. Several groups have observed that different vaccines induce a different type of cellular response when used as a prime or boost; both Hanke and Pantaleo say that DNA appears to cause a better CD4+ T-cell response than viral vectors.

A mouse study comparing a DNA/Ad5 regimen with either DNA or Ad5 alone showed that DNA induced more of a balanced CD4+ and CD8+ T-cell response (J. Virol. 79, 8024, 2005), whereas Ad5 induced more of a CD8-biased response, according to Gary Nabel, the director of the VRC who led the study. What’s more, DNA/Ad5 induced a broader CD4+ T-cell response and a stronger CD8+ T-cell response than either DNA or Ad5 alone. “It’s a quantitative increase in the CD8s, it’s both a quantitative and a qualitative increase in the CD4s,” Nabel says, adding that there aren’t many analyses that do this kind of comparison. “I can’t say I know of any other papers that do this kind of analysis,” he says. “We don’t know a lot about the mechanism of how DNA is immunogenic.”

Together, these observations may help explain why some but not all heterologous prime-boost combinations work. Using DNA as prime followed by Ad boost works very well, says Rick Koup of the VRC. “But if you do it the other way around, with Ad first and then with DNA, it doesn’t seem to work.” Similarly, Merck’s Shiver says that in monkeys, Ad prime followed by poxvirus boost works well, but not the other way around. “We don’t know [why],” he says. Koup thinks that in most combinations that work well, the prime generates more of a CD4+ response and the boost more of a CD8+ response. “It may be that having a good CD4+ T-helper cell response really helps the boost,” Koup says. Still, he adds, “that’s a theory.”
But just why it is that some vaccines target more of a CD4 response, and others more of a CD8 response, one can only speculate, Koup says. He believes the difference may have to do with how different vectors affect the antigen-presenting cells, for example the specific profile of chemokines or cytokines that they induce the antigen-presenting cells to secrete.

**Apples and oranges**

One obstacle to understanding which regimens work better than others is that it's often difficult to compare the results of different studies since they use slightly different vectors and HIV gene inserts. "I think there is a false assumption that a DNA is a DNA and an MVA is an MVA," Johnston says. "That's just not true."

This could in part account for different results of studies that use similar prime-boost regimens, such as DNA/Ad5 or DNA/MVA. Hanke says the use of different HIV gene inserts could explain why his DNA/MVA regimen showed immunogenicity in fewer people than the similar DNA/NYVAC trial. DNA/NYVAC uses HIV genes including env, whereas the DNA/MVA regimen used only gag. Hanke thinks that immunogenicity to Env, however, may not be very helpful. "Some data suggest that immune responses to Env do not correlate with better control to the virus," Hanke says (Nature Medicine 13, 46, 2007). What's more, he suggests the Env-specific responses are immunodominant and could suppress other relevant responses, for example against Gag. Different inserts can also differ in expression levels of HIV immunogens and in their ability to cross prime—that is, to make non-dendritic cells express antigen that is then taken up and presented by dendritic cells.

Such differences are the reason why Nabel and others have initiated studies looking at a standardized insert—such as the env gene from HIV clade A—as an immunogen in the context of different vectors. "We are trying to organize efforts to look at a common insert," Nabel says; a Phase I trial (HVTN 072) that just began uses this insert in DNA, Ad5, and Ad35 in various combinations. "I think that's going to be a very worthwhile set of studies," he says. Barouch's group will also use this insert in upcoming clinical trials.

Meanwhile, Pantaleo says a lack of understanding as to why some prime-boost regimens work better than others shouldn't delay clinical trials. "If you are trying to develop a vaccine, you need to go fast," he says.

Still, none of this may be relevant if cellular immunity shows no degree of protection in the first place. Merck's Ad5/Ad5 trial is widely anticipated to demonstrate whether it does or not. "If it doesn't work, there is no prime-boost or other modality of making a T-cell response that's going to work," says Shiver.

Even if a heterologous prime-boost regimen works one day, the cost and logistics of administering it will likely be higher than using just a single vaccine component. There is currently no licensed vaccine that's a heterologous prime-boost regimen, Schlesinger says. "Ideally you would have a single product," she says. "The only reason we are doing [heterologous] prime-boost is that we don't."
First clinical trial with a novel adenovirus vector begins

The Vaccine Research Center (VRC) at the US National Institute of Allergy and Infectious Diseases (NIAID) in partnership with GenVec recently began a Phase I clinical trial to evaluate the safety and immunogenicity of a novel adenovirus serotype 35 (Ad35)-based AIDS vaccine candidate in 15 volunteers. Vaccine candidates based on the more commonly circulating serotype Ad5 are now being tested in two large Phase Iib trials by the US-based company Merck in North and South America, the Caribbean, Australia, and South Africa and in a series of Phase II trials by the VRC. The VRC is also preparing to begin a Phase Iib trial with their DNA and Ad5 candidates in a prime-boost strategy through the Partnership for AIDS Vaccine Evaluation (PAVE).

One possible drawback to using Ad5 as a vector is the high prevalence of the naturally-circulating virus, especially in developing countries. People previously infected with Ad5 may have pre-existing immunity to the viral vector that could hinder their immune responses to the AIDS vaccine candidate. Ad35 has a much lower prevalence globally. Using a different serotype also opens up the possibility of using two different adenovirus vectors in a prime-boost strategy (see One-two combination, page 11). Previous studies have shown that immune responses are often blunted when non-human primates are given multiple immunizations with candidates that used Ad5 vectors.

The VRC’s two-part trial will evaluate the safety of an intramuscular injection of the vaccine candidate at three different doses. Once the safety data is reviewed researchers will evaluate the safety and immunogenicity of the candidate when administered in combination with the VRC’s Ad5 candidate.

Other groups, including IAVI and Dan Barouch of Beth Israel Deaconess Medical Center in Boston, are also investigating alternate serotypes of adenovirus for use as AIDS vaccine vectors. Barouch and colleagues expect to begin Phase I clinical trials later this year with two candidates—one using an Ad26 vector, which appeared more immunogenic than Ad35 in their non-human primate studies, and another with an Ad5/Ad48 chimeric vaccine vector.

UN Secretary General appoints new AIDS envoy for Africa

Elizabeth Mataka, formerly the executive director of the Zambia National AIDS Network and the vice-chair of the board for the Global Fund to Fight AIDS, Tuberculosis, and Malaria, was recently appointed to the position of Special Envoy for AIDS in Africa by Ban Ki-moon, the Secretary General of the United Nations (UN). Mataka is a native of Botswana and has been involved in HIV/AIDS prevention, treatment, and care for the past 16 years. She succeeds Stephen Lewis, who left the post after five years when previous Secretary General Kofi Annan retired at the end of 2006.

During his time as Special Envoy Lewis spoke passionately about the devastation that HIV/AIDS is causing in Africa and he became one of the most outspoke and well-known advocates for the rights of women on the continent (see An Interview with Stephen Lewis, IAVI Report 9, 3, 2005). The appointment of Mataka fulfills Lewis’s request that his replacement be an African woman. She is the first African to hold the position of Special Envoy at the UN and as she assumes the post, the situation facing African women has never been more dire. As HIV continues to spread in sub-Saharan Africa, women are increasingly becoming infected. It is estimated that 4.6% of young women in sub-Saharan Africa are currently living with HIV, compared to 1.7% of young men. The most recent incidence study conducted in South Africa found the highest rates of new infections were occurring in young women between the ages of 20 and 29 (see Moving target, page 1).
Female contraceptive diaphragm shows no HIV prevention benefit

The recently completed study of the female contraceptive diaphragm indicates that the cervical barrier does not provide any additional benefit over already available prevention strategies in reducing HIV transmission in women. This first randomized controlled trial of the latex diaphragm was conducted by researchers at the University of California, San Francisco (UCSF) Women’s Global Health Imperative and involved nearly 5000 female volunteers in Durban and Johannesburg, South Africa and Harare, Zimbabwe. Results of the trial showed that HIV incidence rates among women in the control group who only received condoms and counseling were nearly identical—at around 4%—to those seen in women who also received a diaphragm and lubricating gel. During the 18-month study, 158 new HIV infections occurred in the group of women who received the diaphragm, with 151 occurring in the control group (Lancet 370, 251, 2007).

Nancy Padian, principal investigator of the trial, says these results do not support adding the diaphragm to the current list of HIV prevention strategies. She promoted the idea of testing the diaphragm, which shields the cervix during sex, as a way to prevent HIV transmission after research initially conducted at UCSF suggested that the cervix may be a potential hot-spot for HIV infection (see Capping infection, LAVI Report 10, 4, 2006). The cervix is considered more vulnerable because it has a thinner cellular lining than the vaginal tract and has a high density of lymphocytes, one of the virus’ primary targets.

Padian advocated testing the diaphragm as an HIV prevention strategy for many years before finally receiving US$77 million from the Bill & Melinda Gates Foundation. Prior to starting the efficacy trial, Padian conducted several acceptability studies to determine if African women were willing to use a diaphragm; as with many HIV prevention methods, excluding vaccines, compliance is crucial to the success of the intervention. In this study, women who received diaphragms reported using them during only 70% of their sexual acts. These women reported that condoms were used 54% of the time, while women in the control group who were not using the diaphragm reported that their partners used condoms 85% of the time.

Since condom use was lower in the diaphragm group yet the number of new infections was equivalent, it is possible that the diaphragm contributed to protection. However because the trial was not designed to compare the protective effects of the diaphragm to condoms, researchers are unable to draw any firm conclusions. The authors of the Lancet article did suggest the “observation that lower condom use in women provided with diaphragms did not result in increased infection merits further research.”

This is the second HIV prevention trial this year to end with disappointing results. In January a study of the microbicidal gel cellulose sulfamate showed that the product may have possibly increased women’s risk of contracting HIV. A final study analysis was presented at the 4th International AIDS Society Conference on HIV Pathogenesis, Treatment, and Prevention in Sydney (www.ias2007.org).

India revises their HIV/AIDS prevalence estimates

The National AIDS Control Organization (NACO) in India recently revised their national HIV prevalence estimates, drastically reducing them from 0.9% to 0.36% in a country with a population of 1.1 billion. This puts the estimated number of HIV-infected people in the country at 2.5 million (with a range of 2.0-3.1 million), less than half of the figure previously projected by the Joint United Nations Programme on HIV/AIDS (UNAIDS). Since 2005 India was thought to have even more HIV-infected individuals than South Africa, based on previous surveillance data collected from antenatal clinics and from mainly high-risk individuals, but the new figures suggest this is not the case.

The new prevalence data in India reflects the country’s efforts to expand their national HIV/AIDS surveillance system. Last year alone the government added 400 new HIV/AIDS testing sites and also conducted a population-based survey that tested 102,000 individuals for HIV infection. This, along with enhanced methodology, provided a much different estimate of the HIV prevalence within the general population. These new figures are endorsed by both UNAIDS and the World Health Organization (WHO) and were calculated with the help of the UN and the United States Agency for International Development (USAID).

The additional surveillance shows that in some of the southern states, including Tamil Nadu, the HIV prevalence has started to either stabilize or decline. This is promising news since HIV prevention has been a focus in these regions for several years. But Indian officials warn against assuming the country's HIV epidemic is sharply declining. Surveillance data from 2006 suggests that HIV infection rates among groups at high-risk of HIV infection, including injection-drug users and men who have sex with men, are actually increasing, especially in urban centers.

India’s Health Minister, Anbumani Ramadoss, also announced a US$2.8 billion national program to provide free antiretroviral treatment to already infected individuals and to improve existing HIV prevention programs.