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IAVI Report

Contents

Treatment as prevention

Researchers are studying the use of licensed antiretrovirals to prevent—rather than treat—HIV infection

By Kristen Jill Kresge

When HIV began spreading rapidly mostly among gay men in the US there were no therapies available. As scores of people started dying from AIDS, members of these affected communities staged protests and organized die-ins, demanding that the government and the nation's massive pharmaceutical industry put its weighty scientific resources into developing treatments for this lethal virus and that community members have a say in the process.

This became the model for patient advocacy and changed the way companies and licensing agencies approach the development of new therapies for many diseases. Now 25 years after the first AIDS cases in the US were described, there are more than 20 licensed drugs for the treatment of HIV infection.

A similar story does not exist for HIV prevention, where activism is a much harder sell. Instead of fighting to keep infected people alive, prevention activists are lobbying for healthy people to alter their behaviors. Almost five million new HIV infections occurred last year alone, yet still there are no newly demonstrated options for stopping transmission of the virus. Speaking recently at the closing ceremony of the Microbicides 2006 conference in Cape Town, renowned activist Zackie Achmat of South Africa's Treatment Action Campaign said, "Everyone present realizes that there is a local and global crisis of HIV prevention."

Many novel approaches to HIV prevention are currently in testing but to some, research seems to be crawling along. "Prevention studies are always a tough sell," says Kenneth Mayer, a prevention researcher at Brown University, who points to the lack of funding and interest from many of the pharmaceutical companies that successfully developed antiretrovirals (ARVs).

One novel idea that is gaining momentum is to give these effective ARVs to non-infected, healthy individuals who are considered at high risk of exposure to HIV. Much like travelers headed to endemic countries who swallow anti-malarials, it is hoped that

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Perspective

Natural killer cells: Bridging innate and adaptive immunity?

New findings indicate that natural killer cells can respond to target cells in a peptide-specific manner and could be involved in the memory response to specific antigens, suggesting that their role in antiviral immunity should be reassessed

By Galit Alter and Marcus Altfeld

All organisms, ranging from the simplest unicellular ones to humans, are subject to relentless attack by pathogens[1] and so have consequently evolved the means to defend themselves by dedicating a large number of genes to ensuring their survival[1,2]. In higher animals, starting with the jawed fish, these defense mechanisms have resulted in the development of two elaborate but critical arms of the immune system, innate and adaptive immunity.

Innate immunity is the most ancient and can be found in relatively simple organisms as diverse as plants and

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taking ARVs could prevent the establishment of HIV infection. This idea is known as pre-exposure prophylaxis, or PrEP, and is being tested in five ongoing clinical trials (Table 1). "We urgently need new types of prevention tools and PrEP is one of many promising strategies, like microbicides and vaccines," says Albert Liu, director of one of the PrEP trials at the San Francisco Department of Health.

Several animal studies provided the preliminary evidence that PrEP might be an effective approach but the complexities of conducting clinical trials to test the idea has put them at the forefront of debate. Some researchers harbor concerns that giving drugs that are known to be effective for treating the disease will encourage people to increase their risk behaviors, a phenomenon known as behavioral disinhibition, but others insist that these placebo-controlled trials are designed to prevent this from happening. And PrEP may have the greatest benefit for people who can't negotiate the use of traditional barrier methods, leaving them with few options when it comes to HIV prevention. "We desperately need PrEP to protect women in resource poor settings," says Joep Lange of the University of Amsterdam (*PLoS Med.* **2**, e248, 2005).

If the idea of healthy people popping pills to stay HIV free is borne out in clinical trials, many other questions may arise about implementing this strategy on a global basis. Researchers will confront issues of drug toxicity, selection of drug-resistant virus in people on PrEP drugs who become HIV infected anyway, drug pricing, and the need to link PrEP availability to community outreach and educational campaigns to ensure compliance with daily dosing and to minimize disinhibition. "PrEP is not a universal panacea," says Lange, who calls an AIDS vaccine an "an absolute priority" since its impact will be far greater.

We urgently need new types of prevention tools and PrEP is one of many promising strategies, like microbicides and vaccines

Albert Liu

Birth of PrEP

The idea behind PrEP is not altogether new. "The concept of using an antiretroviral as a preventive has been tested and proven successful in preventing mother-to-child transmission of HIV," says Jim Rooney of Gilead Sciences, a company that manufactures both of the drugs currently being tested in PrEP trials. Over the last 12 years countless children have been spared from HIV infection because mothers and babies received ARVs during labor or for a short while following birth (see *New strides in protecting infants from HIV*, *IAVI Report* **9**, 2, 2005).

Administering ARVs to laboratory or healthcare workers immediately after accidental exposure to HIV is also a common practice, known as post-exposure prophylaxis (PEP). But in both of these situations the potentially infectious event is known and healthy individuals are only exposed to ARVs for a limited time. The premise of PrEP is that ARVs could be taken on a daily (possibly less frequent) basis for years in order to protect against the potential of multiple exposures to the virus either through sexual activity or injection drug

Ongoing or Planned PrEP Trials

Location	Sponsor	# of Volunteers	Population Being Studied	Drug
Botswana	CDC	1200	heterosexual men and women	tenofovir/Truvada
Ghana	FHI	800	high-risk women	tenofovir
Peru	NIH/UCSF	1400	MSM	Truvada
Thailand	CDC	1600	IDUs	tenofovir
United States	CDC	400	MSM	tenofovir

Table 1. Ongoing trials to evaluate the safety and efficacy of daily tenofovir or Truvada in preventing HIV infection. *All trial particpants are healthy, HIV-uninfected individuals.* ***FHI****: Family Health International;* ***CDC****: US Centers for Disease Control and Prevention;* ***NIH****: National Institutes of Health;* ***UCSF****: University of California, San Francisco;* ***IDUs****: injection-drug users;* ***MSM****: men who have sex with men*

use. Since the drugs are only licensed by the US Food and Drug Administration for the treatment of HIV infection, providing them to healthy people is considered off-label use. Such use has been reported anecdotally and in informal surveys among gay men in the US, but there are clear safety concerns when giving drugs to otherwise healthy people, especially long term.

The choice of drug is therefore paramount. Tenofovir, a once-daily drug licensed by Gilead for the treatment of HIV infection, was the first drug that researchers considered for PrEP. It is a nucleoside reverse transcriptase inhibitor (NRTI) that has been on the market since 2001 and, apart from some reported cases of renal toxicity or bone mineral loss in HIV-infected individuals, has a relatively good safety profile. Additionally, viral resistance mutations are not formed in response to selective pressure put on the virus by the drug as readily as they are to some other ARVs. The development of drug-resistant virus is an important concern for PrEP because no intervention is failsafe and in practice volunteers could still become HIV infected. Until their infection is discovered they would be taking a suboptimal therapy that could encourage the development of viral resistance and compromise their future response to treatment.

Recent evidence also offers a pharmacological rationale for choosing tenofovir to try to prevent sexual transmission of HIV. Researchers from Myron Cohen's group at the University of North Carolina at Chapel Hill, along with Gilead, conducted pharmacokinetic studies in HIV-infected individuals and found that tenofovir concentrations are significantly greater in the genital tract than in blood for both men and women. This data, presented in a poster at this year's Conference on Retroviruses and Opportunistic Infections (CROI; www.retroconference.org/2006; Ab# 569), shows that both the extra- and intracellular concentrations of tenofovir in the genital tract are the highest, compared to blood, of any ARV observed so far.

Monkeying around

The initial non-human primate study by Gilead looked at the efficacy of tenofovir at preventing infection when given either just before or just after exposure to simian immunodeficiency virus (SIV) (*Science* **270**, 1197, 1995). The macaques were given subcutaneous injections of tenofovir (then known as PMPA) once daily for 4 weeks starting either 48 hours before, 4 hours after, or 48 hours after intravenous challenge with SIV.

All 10 control animals had established viremia within 3 weeks, while the 15 macaques that received tenofovir prior to viral challenge remained SIV-uninfected for 56 weeks. No clinical signs of drug toxicity were observed in animals that received the treatment regimen for one month.

A subsequent study looked at the efficacy of tenofovir in preventing SIV infection in newborn macaques with a viral isolate (SIVmac055) known to have a five-fold reduced susceptibility to the drug *in vitro* (*J. Virol.* **74**, 1767, 2000). Five macaques were treated with tenofovir once daily for 4 weeks starting 24 hours prior to an oral viral challenge. Of these infant macaques, 2 had no evidence of infection and the other 3 had delayed onset of viremia, slower disease progression (immunodeficiency in 5-15 months), and enhanced antiviral antibody responses. The three untreated control monkeys developed fatal immunodeficiency within three months after viral challenge.

But these studies only tested tenofovir against a single viral challenge. At last year's CROI Tom Folks and colleagues at the US Centers for Disease Control and Prevention (CDC) presented some less encouraging data on PrEP when evaluated with a repeat-challenge model (www.retroconference.org/2005; Ab# 136 LB). His group looked at tenofovir's ability to prevent the establishment of infection in 12 rhesus macaques that received weekly inoculations of SHIV (an HIV/SIV hybrid) administered rectally at a dose similar to the level of HIV found in human semen during acute infection. This low-dose, repeat-challenge model is considered by many researchers to more closely mimic human infection.

Four macaques received tenofovir daily, four received a weekly dose, and four were untreated controls. Two of the control animals were infected after a single SHIV inoculation, while the other two were infected after 2 and 11 viral challenges each. In the weekly and daily tenofovir groups, half of the animals in each group were infected after six SHIV inoculations, with all of the remaining animals becoming infected after

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14 doses. Although daily or weekly treatment with tenofovir increased the median time to infection in macaques, it failed to completely protect any animals and disease progression was similar to that seen in the untreated controls.

Trials and tribulations

Although these findings raised concerns among researchers, the ultimate answers on the efficacy of this approach will come from studying tenofovir PrEP in humans, and clinical trials are now underway. The CDC started a Phase II safety study in February last year of daily tenofovir in 400 men who have sex with men (MSM) in the US, and two larger Phase IIb/III trials with tenofovir PrEP with 1600 injection drug users (IDUs) in Thailand and 1200 heterosexual volunteers in Botswana.

Family Health International, a US-based nonprofit public health organization, also launched a series of tenofovir PrEP trials in Malawi, Nigeria, Cameroon, Cambodia, and Ghana, with funding from the Bill & Melinda Gates Foundation, but only the Ghana trial is still ongoing. Some of the trials were stopped or suspended after protests from activists over the lack of a lifetime guarantee to treatment for volunteers who happen to become infected during the trial. Others were halted due to ethical or biological questions about these trials or the sites (*Lancet* **366**, 1499, 2005; *Science* **309**, 2170, 2005; PloS Med. **2**, e234, 2005). In Malawi the government closed the trial due to concerns that it could foster HIV resistance to tenofovir, a drug they are now using in treatment. In response to these events the International AIDS Society held a global consultation on PrEP research last year where researchers and activists discussed the issues regarding these trials (www.iasociety.org/images/upload/1025.pdf).

Another PrEP trial conducted by researchers at the University of California, San Francisco (UCSF), with support from the US National Institutes of Health (NIH), is in the process of getting approval from local institutional review boards to begin recruiting 1400 MSM in Peru. This study is expected to start later this year, according to IMPACTA, a Peruvian non-governmental organization.

But even now some question why it has taken more than a decade, since the original study in macaques showed promise, to finally test PrEP in humans. "The process is incredibly frustrating. For some reason PrEP is controversial," says Lange who doesn't see why studying this intervention should be any more complicated than microbicide trials.

Some researchers hesitated to dive into such studies because of fear that PrEP could actually encourage behavioral disinhibition in volunteers, who feel a false sense of protection from the intervention being tested and so increase their risk of HIV infection by abandoning other proven methods of prevention like condoms. "It did take a while to think about how to best design these trials," says Liu.

Others like Lange are not as concerned about disinhibition. As in any clinical trial, volunteers in PrEP trials will be tested frequently for HIV infection and counseled on how they can reduce their risk. "Usually people are better off in a clinical trial than on the outside," he says. Volunteers will also have easy access to condoms. "We want to test the efficacy of PrEP on top of what we know already works," Liu adds.

Trial volunteers may be further discouraged from risky behavior since all of the trials are placebo controlled—counseling sessions throughout the trial will remind volunteers that they may not be receiving active study drug. The trial Liu is coordinating in San Francisco is attempting to further evaluate the extent of disinhibition by providing only half of the volunteers with either tenofovir or placebo for the first nine months of the study so that researchers can compare the reported behaviors of volunteers who are taking pills with those who aren't.

Several studies have analyzed the behaviors of volunteers during prevention trials and while some indicate a reduction in HIV incidence among trial volunteers, others found evidence of disinhibition. During the Phase III AIDS vaccine trial run by VAXGEN, the risk behaviors among injection drug users did not increase (*AIDS* **18,** 295, 2004). But Mayer warns that this may not be a fair comparison. "We can't say that what happened in a vaccine trial will happen with chemoprophylaxis." Volunteers in vaccine trials may receive at most three inoculations. "It's very different taking a pill every day," he adds.

Combo-PrEP

Researchers have long speculated that a combination of ARVs for PrEP may be even better at stopping the virus in its tracks,

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just as for treatment, and data has recently emerged supporting this hypothesis (see *CROI covers advancements from start to finish*, *IAVI Report* **10**, 1, 2006). The idea for combo-PrEP relies on the drug Truvada, also manufactured by Gilead, which is a single pill containing tenofovir and another NRTI called FTC. In studies conducted at the CDC with this PrEP regimen, macaques were able to fend off 24 viral challenges in the low-dose SHIV, repeat-challenge model.

These impressive results sparked great interest among PrEP researchers and in response the NIH/UCSF trial protocol has been altered to include combo-PrEP instead of tenofovir alone. The CDC trial in Botswana has also switched to Truvada, although the 70 volunteers that are already receiving just tenofovir will continue on this regimen. Both of these trials with Truvada are expected to start around August. The CDC also has plans to add an additional site to the US safety trial where volunteers will receive Truvada rather than tenofovir.

But there are additional concerns about individuals developing FTC-resistant HIV, which occurs more rapidly than to tenofovir, in some cases occurring overnight. FTC also has activity against hepatitis B virus so intermittent use could have implications for those who are HIV/hepatitis B virus co-infected.

Non-viral challenges

Results from these trials are still several years away but some investigators are already considering the next steps. All of the current trials are testing daily ARV doses but the next round of studies will evaluate more sporadic use. "If it works when taken daily, then you back up and look to see if you can just take it closer to the exposure time," says Lynn Paxton, who is running the PrEP trials at the CDC.

Others are considering how this approach could be implemented if found effective and one of the first considerations on everyone's mind is cost. "The access question is very important to start thinking about now," says Liu. Both drugs are only available from Gilead and a year's supply costs an average of US$4800 for tenofovir and $7800 for Truvada. Gilead has provided free drugs for all of the trials, but otherwise has stayed out of PrEP research altogether.

The company does have an access program for treatment, offering the drug at no-profit pricing in 97 developing countries. But even at this drastically reduced price of around a dollar a day it is expensive for governments struggling to treat those already HIV infected and Gilead seems to recognize this. "If data suggest that tenofovir or Truvada is safe and effective in preventing transmission of HIV, we would continue to work to ensure access at the lowest feasible cost," says Rooney.

Giving drugs to those most in need would be another challenge for PrEP programs. In developing countries it may be more difficult to educate communities on PrEP and to give out drugs to healthy individuals who are at high risk for HIV infection if they aren't accustomed to seeking medical care. "This is going to have to be a team effort," says Paxton, "but there's no reason to think that it couldn't be done with proper planning."

Researchers who conduct AIDS vaccine trials are also considering how PrEP could affect their work. If PrEP is found to be effective or partially effective and is adopted as the standard of care in the community where the vaccine trial will occur, then investigators may be obligated to offer PrEP drugs to volunteers in vaccine trials, says Lange. The same may happen with male circumcision, if additional clinical trials show its protective effect. Offering these interventions in large-scale vaccine trials could drastically increase costs and may make it more difficult to tease out the efficacy of the vaccine candidate.

Regardless of these questions, researchers and activists alike eagerly await the results of the ongoing PrEP trials and the public health opportunities this prevention strategy may hold.

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>
> ***Lynn Paxton***

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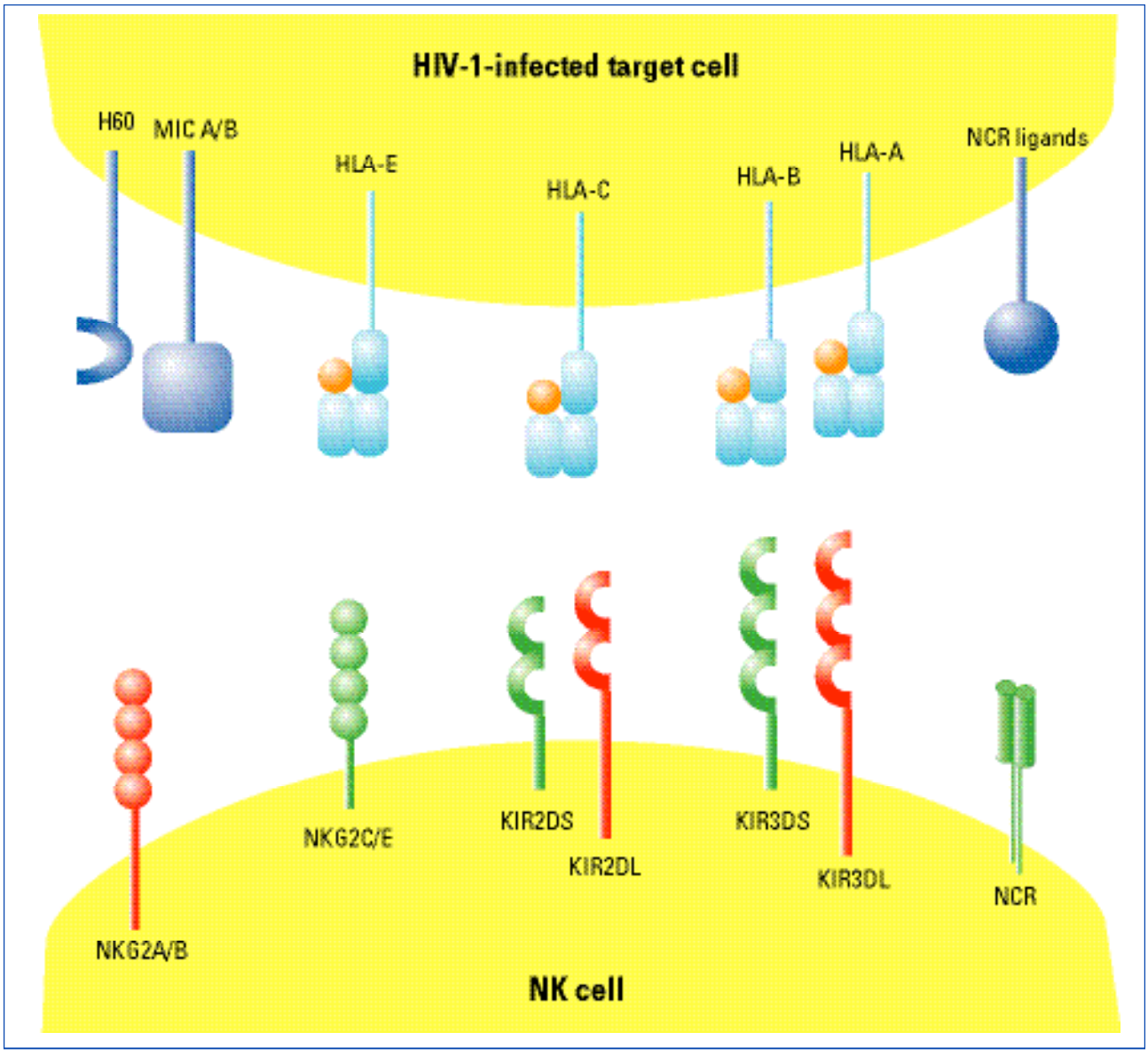

Figure 1. NK cell receptors and their ligands. *The figure shows the three major classes of NK cell receptors (bottom) and their respective ligands on an HIV-1-infected target cell (top). Inhibitory receptors are drawn in red, while activating receptors are depicted in green. NKG2A/B interact with MICA/B or H60, NKG2C/E interact with HLA-E, KIR2DL/S interact with HLA-C, KIR3DL/S interact with HLA-A or HLA-B, and the NCR interact with a still-unknown ligand expressed on infected target cells.*

Drosophila sp[1]. The innate response is induced rapidly following infection and relies on the activation of various cell subsets via specific cellular receptors[3] or pattern-recognition molecules[4] to alert these cells of the invading agents' presence. Once activated, innate immune effectors, including monocytes, macrophages, neutrophils, and natural killer (NK) cells[5] are responsible for containing and clearing the infection and secreting a number of immunomodulatory cytokines that drive the adaptive immune response[6]. This adaptive response, geared towards providing antigen-specificity and immunological memory, complements but does not replace the activity of the innate immune response[2].

Of particular interest when considering HIV-1 infection are NK cells, which represent a subset of innate effector cells that are critical for the control and clearance of a number of viral infections[6,7]. NK-cell deficiencies in humans and NK-cell depletions in mice can result in recurrent viral infections and death[7-9]. Human NK cells can be subdivided into at least three separate subpopulations[10-12]. The first subgroup includes the $CD3^{neg}CD56^{bright}CD16^{neg}$ NK cells, or "immunomodulatory" NK cells, that express little perforin but secrete large quantities of inflammatory and antiviral cytokines and chemokines. The second group is the $CD3^{neg}CD56^{dim}CD16^{pos}$, or "cytolytic" NK cells, which express high quantities of perforin and are able to medi-

ate both direct cellular cytotoxicity and antibody-dependent cellular cytotoxicity. Recently, a third group of NK cells was described that is preferentially expanded in the context of viremic HIV-1 infection, the $CD3^{neg}CD56^{neg}CD16^{pos}$ ("anergic") NK cells[10,12].

How do NK cells recognize their target cells?

Unlike B and T cells, NK cells do not express unique clonally distributed receptors for specific antigens, rather they express many different promiscuous stimulatory and inhibitory receptors that can be divided into at least four classes[13]: the killer immunoglobulin-like receptors (KIRs), the C-type lectin receptors, the natural cytotoxicity receptors (NCRs), and the toll-like receptors (TLRs) (Figure 1). Fourteen KIRs have been described to date. These receptors are activating or inhibitory in nature and their predominant ligand comprises HLA-class I molecules that are expressed on all nucleated cells[3]. The C-type lectin receptors (NKG2A-E in humans, Ly49 in mice) monitor the expression of HLA-E, HLA-G, and non-classical MHC-class I-homologs (MIC A/B)[13]. Three NCRs have been identified to date (NKp30, NKp33, NKp36) but their ligands remain largely undefined[13]. TLRs are pathogen-associated, pattern-recognition receptors involved in the non-specific recognition of infection[14]. While the NCRs are exclusively expressed on NK cells, KIRs, NKG2A, and TLRs are found on other cells of the immune system.

Effect of HIV-1 infection on NK cells

A large number of studies have assessed the impact of HIV-1 infection on the NK cell compartment. During acute HIV-1 infection, NK cells are significantly expanded[10] and activated. In particular, the cytolytic $CD3^{neg}CD56^{dim}CD16^{pos}$ NK cells are preferentially expanded, express high levels of KIR, and exhibit strong responses to MHC-devoid target cells at this early stage of the infection when the adaptive arm of the immune response is just developing. Following the emergence of virus-specific T-cell responses, the number of cytolytic $CD3^{neg}CD56^{dim}CD16^{pos}$ NK cells decreases, and is reduced in chronic viremic HIV-1 infection compared to non-infected individuals[10]. In parallel to this loss of functional $CD56^{pos}$ NK cells, a population of anergic $CD3^{neg}CD56^{neg}CD16^{pos}$ NK cells is expanded in chronic infection[10,12]. These changes in the NK-cell compartment result in an overall stable number of NK cells that respond vigorously to MHC-devoid target cells in chronic viremic infection, but also in a significant reduction in NK-cell cytolysis due to the replacement of functional NK cells by $CD56^{neg}$ anergic NK cells. This accumulation of anergic NK cells in chronic HIV-1 infection may also contribute to the overall immunodeficiency in progressive infection, making the host more susceptible to opportunistic infections and tumors. In contrast to HIV-1 infection, there is a marked preservation of functionally-active NK cells, as well as T and B cells, in the less-pathogenic HIV-2 infection[15] and in SIV-infected sooty mangabeys[16].

The precise mechanisms that lead to these differences in the functional impairment of different leukocyte subsets between HIV-1 and HIV-2 infection (as well as SIV-infected macaques and sooty mangabeys) are still largely unknown. However, data presented by Mark Feinberg at the recent Keystone meeting have begun to address the role of immune activation as the underlying difference between SIV-susceptible macaques in contrast to sooty mangabeys, which serve as the natural host of this infection. Feinberg presented provocative data demonstrating a marked reduction in the innate response to become activated by SIV via TLR in sooty mangabeys, including a reduction in IFN-α production by plasmacytoid dendritic cells potentially resulting in reduced NK-cell proliferation. In contrast, the innate response was strongly activated in SIV-infected macaques. Given the critical role of the initial innate immune response in initiating the adaptive immune response, it is likely that subdued innate immune response, both in acute as well as in chronic infection, may result in the generation of moderate NK- and T-cell responses and consequently less general immune activation in infected sooty mangabeys. These studies again emphasize the crucial interplay between the innate and adaptive immune responses in AIDS-virus infections and the potential consequences of manipulating the innate response for HIV-1 pathogenesis.

NK cells suppress HIV-1 replication

Recent observations from epidemiological studies suggest that NK cells may play a

NK cells... represent a subset of innate effector cells that are critical for the control and clearance of a number of viral infections

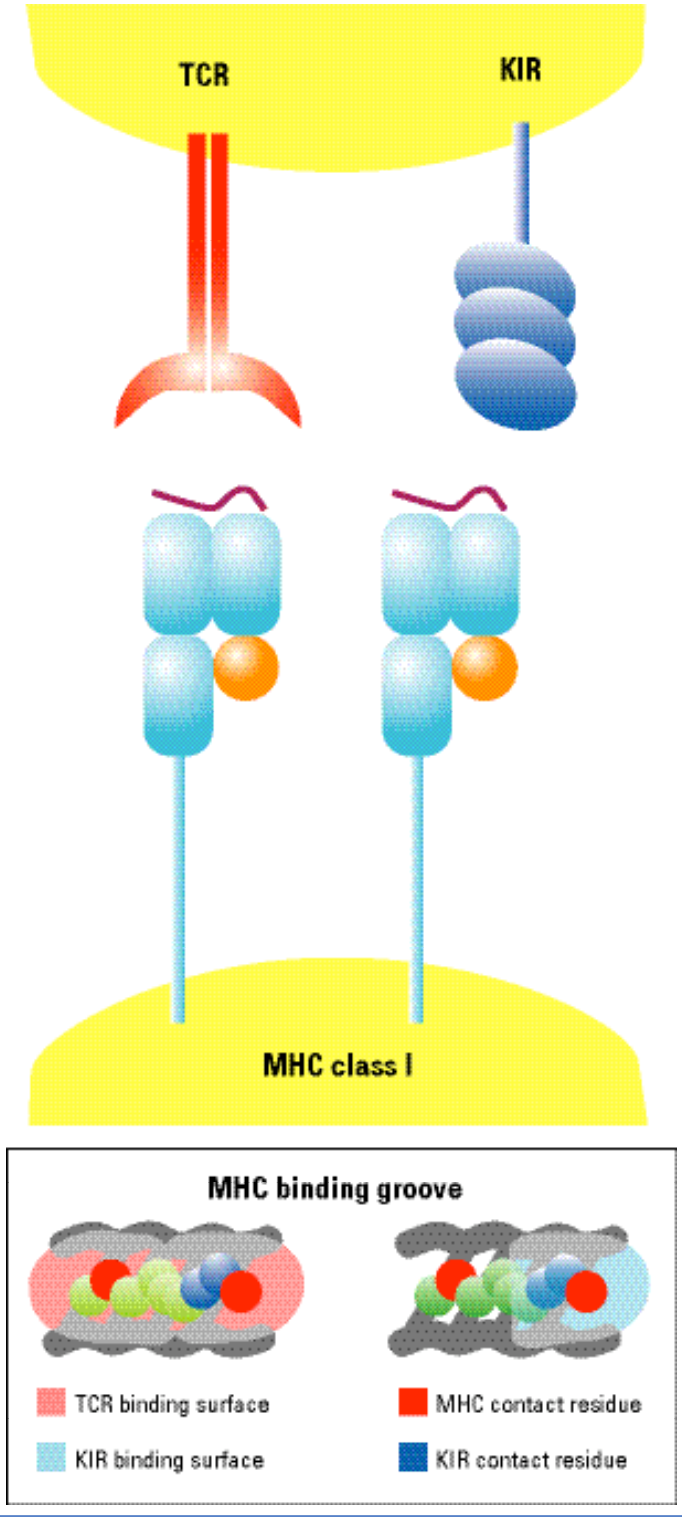

Figure 2. Comparison of the contact areas between KIR and MHC class I molecules and TCR and MHC class I molecules. *The top panel schematically demonstrates the manner in which TCR and KIR engage the MHC-peptide complex. Whereas the TCR makes contact with the full binding cleft, covering the entire MHC-binding groove, KIR interacts primarily with the C-terminal end of the MHC-peptide binding groove. The bottom panel shows the top view of the epitope residues that are critical for TCR (red) and KIR (blue) interaction as well as the TCR (left) and KIR (right) binding surface on the peptide-binding groove of the MHC molecule.*

significant role in the control of HIV-1 replication and disease progression. A strong association between the expression of a particular activating KIR, KIR3DS1, and its ligand, HLA-B Bw4 with an isoleucine at position 80 (Bw4 80I), and slower HIV-1 disease progression has been noted[17]. One potential interpretation of these data is that KIR3DS1 may play a direct role in sensing changes in the expression of its ligand on infected cells in subjects with HIV-1 infection.

More recent work from Carrington's group, presented at the last American Association of Immunologists meeting in Boston[18], has also begun to shed light on a potential protective role of the expression levels of KIR3DL1, an inhibitory receptor binding to HLA-B Bw4. KIR3DL1 is a highly polymorphic allele and different subtypes lead to different expression levels of this receptor on the surface of NK cells. Interestingly, the presence of two high-expressing KIR3DL1 alleles in the presence of HLA-B57, an HLA-B Bw4 allele already associated with slower disease progression, renders the protective effect of HLA-B57 even greater. Given the significant effect of HLA-B alleles on HIV-1 disease outcome it is quite intriguing that KIRs expressed on NK cells, and not only the TCRs of $CD8^+$ T cells, interact with HLA-B molecules and that the expression level of KIR3DL1 further modulates the effect of HLA-B alleles on HIV-1 disease progression, suggesting a potential role for NK cells in controlling HIV-1 replication.

Further evidence for NK-cell mediated immune pressure in HIV-1 infection comes from studies assessing the changes in the surface expression of HLA class I molecules on HIV-1-infected T cells. Nef has been demonstrated to selectively downmodulate expression of HLA class I A and B molecules on the surface of HIV-1-infected cells[19], which subvert the virus-specific $CD8^+$ T-cell response that recognizes viral epitopes presented by these HLA class I molecules. However this downmodulation may render these infected cells more susceptible to KIR-mediated recognition and destruction, since modulation of the levels of HLA class I molecules on the surface of either malignant or infected cells serves as an important trigger of NK cell activity.

In contrast to its effect on HLA-A and -B molecules Nef does not downregulate expression of HLA-C molecules on the sur-

face of infected cells, and several studies have actually demonstrated increased expression of HLA-C and HLA-E on HIV-1-infected cells[20]. HLA-C and HLA-E represent the major ligands for KIR2DL and NKG2 and provide a strong inhibitory signal to NK cells. These data suggest that HIV-1 Nef may be able to tip the scales in favor of $CD8^+$ T-cell evasion by downregulating HLA-A and HLA-B, while increased expression of HLA-C and HLA-E may protect these infected cells from the second subset of cytolytic effectors, the NK cells. Overall, these sophisticated methods that HIV-1 has developed to evade both T-cell and NK-cell mediated recognition provide direct evidence for the strong immune selection pressure exerted by NK cells in HIV-1 infection.

Despite these relative changes in the expression of HLA class I molecules on infected cells, functional *in vitro* studies have shown that blocking inhibitory KIR on NK cells[20], and in particular NK-cell clones bearing fewer copies of inhibitory KIRs, can lead to lysis of HIV-1-infected cells and inhibit viral replication. How can the different receptors expressed on NK cells mediate this recognition of HIV-1-infected target cells? At least two models have been hypothesized. The first proposes that HIV-1 infection induces HLA-independent changes in cell surface molecules (in addition to the changes in HLA class I molecules described above) that are subsequently recognized by activating NK-cell receptors, resulting in lysis of infected cells. An alternative model suggests that HIV-1 infection results in a change in the repertoire of peptides presented by HLA class I molecules such that these novel peptide-HLA class I complexes no longer allow for recognition by inhibitory KIRs—or, alternatively, bind more effectively to activating KIRs—and so target cells are lysed.

TCRs expressed on $CD8^+$ T cells bind to HLA-class I molecules in such a way that the TCR broadly covers the epitope-presenting HLA groove centered around position 5 of the epitope, whereas KIR binds in a different location (Figure 2). The crystal structure of an HLA/epitope/KIR complex (KIR2DL2 in complex with HLA-Cw3) has been resolved[21] and demonstrates that KIR interaction with the peptide-HLA class I complex is centered around amino acid position 7 and 8 of the epitope, close to the C-terminal portion of the peptide binding groove. This resolution of the structure of the HLA/epitope/KIR complex validated a series of previous studies demonstrating that KIRs can discriminate between peptides presented by HLA-B*2705 (KIR3DL1)[22], HLA-Cw*0304 (KIR2DL2)[23], HLA-Cw*0401 (KIR2DL1)[24], HLA-Cw7 (KIR2DL2)[25], and, more recently, HLA-A3/A11 (KIR3DL2)[26]. These studies have confirmed the critical role of the residue in position 8 on the HLA-presented epitopes in the peptide-specific interaction with KIR, as individual amino acid changes in that position determine recognition of the HLA/peptide complex by the respective KIRs. Taken together these data demonstrate that the sequence of the peptide presented by HLA class I molecules can influence the ligation by inhibitory and activating KIRs expressed on NK cells, and thereby modulate target cell recognition and lysis. Based on these structural and experimental data on the peptide-specificity of the KIR/HLA interaction, it is tempting to speculate that the recognition of HIV-1-infected target cells by NK cells is more 'specific' than previously thought.

NK cells: a reassessment?

Recent published data challenge the dogma that NK cells are simply mediators of the innate immune response, and also suggest the involvement of NK cells in a memory response to hapten-induced contact hypersensitivity (CHS)[27,28]. These studies demonstrate that hepatic $Ly49C^+$ NK cells alone, derived from a previously sensitized mouse, were able to mount detectable CHS upon adoptive transfer into a naïve animal. These data, in the SCID mouse model in the absence of B and T cells, are very exciting but preliminary, and their relevance for human NK cell biology requires further investigation. But given this potential role of NK cells in secondary memory responses and the fact that some degree of peptide specificity, in particular around residue 8 of the epitope presented by HLA class I molecules, appears to play a significant role in NK-cell mediated recognition of target cells, it is probably time that we reevaluate the role of NK cells in antiviral immunity.

Antigen specificity and memory are two distinctive features attributed to the adaptive immune response. Yet it is becoming

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increasingly apparent that adaptive and innate immunity are not necessarily mutually exclusive. NK cells are the earliest cytolytic effector cells responding to viral infection, can secrete large amounts of cytokines and chemokines that drive the subsequent adaptive immune response, and these cells have now also been implicated to some extent in both immunological memory and peptide-specificity. This strongly suggests that NK cells are involved in both innate and adaptive immunity and in bridging these arms of the immune response. If these initial provocative findings can be generalized and reconfirmed in the context of HIV-1 infection then their implications for immunotherapy and vaccine design may be profound, as they provide an opportunity to manipulate an additional arm of the immune system. Current AIDS vaccine efforts have largely neglected to exploit the important interplay and potential interface of innate and adaptive immunity that have evolved to play synergistic roles in the fight against infection. A better understanding of the innate immune receptors and mechanisms involved in the initial recognition of HIV-1 infection will hopefully help us to better understand HIV-1 pathogenesis, and to strengthen the immunogenicity and potency of future AIDS vaccine candidates.

Galit Alter is a research fellow leading the efforts at the Partners AIDS Research Center to elucidate the role of NK cells in the control of HIV-1 disease progression.

Marcus Altfeld is an Assistant Professor at Harvard Medical School and directs the Innate Immunity Program at the Partners AIDS Research Center at Massachusetts General Hospital.

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Current AIDS vaccine efforts have largely neglected to exploit the important interplay and potential interface of innate and adaptive immunity

An Interview with

Anthony S. Fauci

25 Years of AIDS

Tony Fauci, MD, has been at the forefront of the global response to HIV/AIDS ever since the first cases were described 25 years ago. Among many achievements related to the pandemic, he was responsible for the early expansion of the US National Institutes of Health's (NIH) HIV research capacity and won plaudits from many for engaging with HIV treatment activists and fostering dialog with them in the mid 1980s.

After gaining his MD degree from Cornell University, New York, in 1966, Fauci has spent almost his entire career at the National Institute of Allergy and Infectious Diseases at the NIH. He has been director there since 1984 and currently oversees an annual budget of US$4.4 billion that is applied to HIV/AIDS, influenza, tuberculosis, malaria, and other infectious diseases. He is also a key advisor to the White House and Department of Health and Human Services on global AIDS issues and other emerging infectious disease threats such as pandemic influenza.

Despite these prominent leadership roles Fauci continues to run a productive research program as chief of the Laboratory of Immunoregulation. His work has contributed fundamental insights into how the human immune system interacts with the replication cycle of HIV, and his laboratory continues to focus on elucidating the nature of the immunopathogenic mechanisms of HIV infection. Fauci has also contributed significantly in other areas of human immunology, including developing effective therapies for the previously fatal diseases polyarteritis nodosa, Wegener's granulomatosis, and lymphomatoid granulomatosis.

Fauci spoke recently to* IAVI Report *Editor Simon Noble about 25 years of the pandemic and the outlook for AIDS vaccines.

This is the 25th year since HIV/AIDS was first medically recognized, and you've been closely involved since the very early days. How has this pandemic changed the public health landscape?

The implications and the impact of HIV/AIDS over the last 25 years have just been absolutely enormous—certainly, in the history of how diseases change paradigms, HIV/AIDS over the last 25 years stands very high among them. It has touched so many of the components of global public health—how we appreciate and respond to emerging new infections subsequent to HIV/AIDS, the role of constituency groups and how the research and public health agenda is set, the relationship between resources that are put into a particular arena and the results that one gets out of them. I'm referring specifically to the large infusion of research resources that have led to such striking advances, despite the fact that 25 years later we still have a long way to go with regard to the epidemic.

You've acknowledged that treatment activists were hugely important in the development of effective antiretrovirals and gaining attention for the epidemic generally. Why do you think there is not now an analogous constituency actively advocating for HIV prevention efforts?

HIV/AIDS was unique, because for all the great killers in society—heart disease, lung disease, cancer—there have been a number of therapeutic interventions for a long time, and research and clinical trials in those diseases are aimed at developing improved therapies. The unique nature of the activism and the response of the research, public health, and regulatory community was that at the time there was virtually no recourse for HIV-infected individuals. There was no therapy. That was a very unique situation, to have a disease evolving while you're trying to do clinical trials and trying to get people to pay attention to the development of therapeutics at a time when there was no therapy.

So there had to be a delicate balance between doing a proper clinical trial to get the data required at the same time that you're sensitive to the needs of the community and people who are afflicted with the disease. That led to a number of things, including the parallel track that I was involved with, to allow clinical trials to proceed in a very well organized, precise way to get data at the same time as you make the drug available to those who, for one reason or other, cannot get into a clinical trial. So the compelling, urgent nature of the situation was very obvious to everyone when you have a deadly disease with no treatment.

Now, the situation with prevention is just as compelling, because it's just as important to prevent people from getting infected as it is to treat people who are infected; they should go hand in hand. And yet we have not seen as uniform a move towards the prevention measures that are necessary. Activists are involved in prevention, but it's such a complex issue because there are so many societal, traditional, cultural issues that go into the kind of prevention that will work for a particular region of the world. Take Sub-Saharan Africa: the whole issue of prevention is

There's a big gap between educating people and changing their behavior. For instance, traditionally, over centuries, women have been disempowered—how do you change that, and quickly?

not just, 'avoid exposure to infection,' the situation there is very much steeped in the lack of empowerment for women and their inability to take into their own hands what needs to be done to protect themselves. That's very different from getting a gay population in a Western, developed nation to realize that despite the fact that there are adequate, even very good, therapies right now, it's still a very bad thing to get infected. In some segments of society in which the infection rate was going down and down, now it's starting to rebound, and the likely reason for that is the general impression—very, very incorrect—that to get infected is not really a big deal. It is a big deal.

It's also much tougher to get people galvanized about prevention when they're not infected. During the early days when activism was born, predominantly among the gay population, it was people who were fighting for their own lives. If you're not infected, you don't perceive it as fighting for your own life.

So beside the scientific challenge of developing a vaccine, one of the biggest challenges that we still face is that it's equally as compelling and difficult to implement the other preventive measures that we need.

So is more sociological research required?

I think that we certainly could use more but it's a very frustrating field, because very knowledgeable and scholarly people in the area of sociology and behavior have emphasized that there's a big gap between educating people and changing their behavior. For instance, traditionally, over centuries, women have been disempowered—how do you change that, and quickly? The only hope for that in my mind is real, strong leadership from national politicians, be that the presidents, the prime ministers, the health ministers. Obviously, you need both grassroots and leadership from the top, but the leadership needs to step up to the plate and really make it very clear to their citizens that there needs to be some dramatic changes in how their society looks upon the relationship between men and women.

CHAVI was granted recently. How was CHAVI initially envisaged within the NIH, and what are your hopes for it?

Despite the fact that there are a lot of very smart people and a lot of resources applied, the obstacle to an HIV vaccine is still a scientific one. There are some unique characteristics of HIV that relate directly to our inability, at this point, to develop a vaccine. The most important is that we do not know what a protective immune response would be because, astoundingly, there isn't a single documented case of anybody who has developed an established HIV infection and then spontaneously eradicated the virus. That tells us something very ominous. But the only way to determine the correlates of protective immunity is to specifically dissect out the exact nature of the immune response, what the holes are, what the stumbling blocks are, and what we think we can do to induce an adequate immune response with a vaccine.

As part of the Vaccine Enterprise, the NIH felt that we would take the first step towards encouraging others, because in the document that Rick Klausner, I and others wrote in *Science* (**300**, 2036, 2003) we said we need several centers of excellence in vaccine research, each covering a particular component that is a major gap in our knowledge regarding an HIV vaccine. Clearly, one of those is immunology.

So I decided to put out a request for proposals for what we would call a center for HIV/AIDS vaccine immunology, hence CHAVI. It was a very robust competition. The principal investigator who scored highest in the competition is Barton Haynes, from Duke University, and his team is putting together a program that we hope will answer some of those very important questions related to the immune system and its response to HIV/AIDS. They have some very good people involved, and I think that will get us another step closer to developing an HIV vaccine.

So is CHAVI more a matter of extra funding, or will it be a truly new way of conducting research?

It'll be both. It's certainly a significant increase in resources, because the first-year funding was something like US$15 million and then it stands to get up to $40-50 million per year subsequently, but it's also a new way of looking at it, modeled on multiple groups synergizing with each other to answer some specific questions. It has a critical mass phenomenon.

So do you think the NIH can do it all?

No, I don't think the NIH can or should do it all. There needs to be a lot of input from industry, a lot of input from NGOs, a lot of input from other nations who fund through their own mechanisms, the nations in the Western world and in the developing world. A resounding no to the question of NIH doing it all alone. And I think one of the major partners in the development of a vaccine is industry, obviously.

So how can the biopharmaceutical industry be encouraged to engage more fully in AIDS vaccine research? Do you think advance market commitments and other such financial incentives will be useful?

Yes, those are mechanisms that can help. In discussions that I've had with some of my colleagues in the pharmaceutical industry, particularly some of the CEOs who will remain unnamed, they've said, 'All you have to do is just give us a wedge, open up a scientific window for us, and we'll jump all over it.' Some companies, like Merck and others, they're committed and in for the duration, from both scientific and developmental standpoints. But we really need to continue to engage them and, as scientists, clarify and elucidate some of those scientific barriers that still exist; then it will be easier for the pharmaceutical companies to come in when they see enough scientific basis to take the risk to get involved in the vaccine field.

Do you think that organizations will continue with their traditional roles—fundamental research done by academia and government institutes, and the development process taken over by the biopharmaceutical sector?

No, I think it's going to be more of a sharing of both, because we're seeing companies like Merck and others doing research as well as the developmental process, and then other models, particularly the NIAID Vaccine Research Center (VRC) with Gary Nabel, its director, getting into the actual vaccine development process. With the VRC we wanted to have, under one roof and within one organizational structure, the capability of doing everything, from fundamental research to the development of pilot lots and the institution and execution of a clinical trial. So I don't think there's a further Balkanization of roles, I think it's more of a sharing of roles now.

So do you subscribe to this idea of the industrial research model?

You've got to be careful when you say "industrial research" because industry, with few exceptions, doesn't do undifferentiated, fundamental research. They have a concept that's already proven and then they develop something from that. That is very important to get to where we want to be with a vaccine but we've got to be real careful that we don't declare a basic science victory and say, 'Now we just need to develop.' There are still fundamental questions that we really need to address, and that may not be able to be done in an industrial, pharmaceutical company environment.

If it's all we can achieve with a first generation AIDS vaccine, do you think that a partially effective vaccine that protects from disease rather than infection will be acceptable in different countries?

That is an important issue that doesn't have a clear-cut answer. It really is going to depend on where we are. I think the acceptance of a partially effective vaccine will be much greater in those countries that are just being devastated by infection, where you're seeing the very fiber of society crumbling under the burden of HIV.

At this point in the scientific endeavor, which do you think will advance the AIDS vaccine field fastest: increased clinical trial capacity to test vaccine candidates in humans, or more fundamental research capacity, particularly nonhuman primate model research?

I think there needs to be a balance between fundamental research and empiricism, and I've been saying that for so many years. You don't want to go overboard on the empiricism; there are enough scientific concepts that need to be elucidated. I think you will ultimately have to bite the bullet and empirically go into a clinical trial once you have a concept that's firm enough to warrant it; however, we have a lot of mileage to go in understanding some of the fundamental pathogenesis and correlates of immunity in HIV infection. You cannot jump into a clinical trial with virtually any candidate that happens to come your way.

So what do you make of the current heightened interest in innate immunity and the links with adaptive immunity?

I think that's terrific. It's the way the discipline of immunology was going anyway, and there's now a very fertile arena in HIV/AIDS research. There's a lot of funding going into innate immunity now, more over the last couple of years than in the previous decade.

Do you think innate immunity has most potential for manipulation by a vaccine directly, or in an adjuvant capacity?

I think both but, practically speaking, in the adjuvant capacity. HIV research is helping us explain what we never knew about adjuvants and how they work, to understand the molecular mechanisms of how innate immunity leads into adaptive immunity, and how perhaps we can actually amplify the immune response by utilizing some of the molecules, mechanisms, and signaling pathways that innate immunity uses. Now that we've got

We have a lot of mileage to go in understanding some of the fundamental pathogenesis and correlates of immunity in HIV infection

that off the ground, we need to design adjuvants to actually utilize what we now know about that exquisite interaction between innate and adaptive immunity.

There's also now a renewed interest in mucosal immunity since it's likely a vaccine will have to be effective there. Mucosal surfaces aren't easy to work with; sampling, particularly in humans, is difficult, time consuming, and costly, and it's difficult to recruit volunteers for such studies. How do you think we can work with that?

I remember meetings I had 15 to 20 years ago with mucosal immunologists, trying to get them involved in HIV research. Logistically, it's just a tough area of study. I think that's one of the big gaps that we have not adequately filled and I'm disappointed in that. We've really got to get them more involved.

So how can new talent, particularly established experts in other fields, be encouraged to enter into AIDS vaccine research?

By continuing to do what we're doing, namely having conferences and workshops that try to bring people together. Obviously, funding is the greatest way to encourage people. We've done that and there has been disproportionately greater funding for HIV/AIDS compared to other areas, which I think was appropriate. We haven't succeeded as much as we wanted but we've made some steps in getting those people involved. The problem is now, with the funding crunches at the NIH, with everything essentially being flat, there's not a lot of new money to put into new areas to stimulate things, so that's going to make it more difficult from a funding standpoint.

How can young investigators be encouraged to enter into AIDS vaccine research?

We've just got to encourage them, and emphasize that it's a very interesting field and the payoff is big—we're dealing with a global public health catastrophe of enormous proportions; therefore, not only is it exciting science but look at what you can accomplish.

Are you disappointed in the progress we've made with the antibody component of a vaccine?

Yes, of course. We have good people working on it and we are now finally defining the conformational issues involved in the induction of antibodies that would be cross-reacting and neutralizing against primary isolates. But we are certainly not where we want to be and this is still one of the big enigmas of HIV vaccinology. There's a lot of focus on cell-mediated immunity, T-cell based vaccines, but we will not prevent HIV infection unless we get a vaccine that will elicit cross-reacting neutralizing antibody against multiple isolates. We may get a vaccine that blunts the progression of disease, but we will not have a vaccine that prevents infection unless we crack the antibody enigma.

We will not prevent HIV infection unless we get a vaccine that will elicit cross-reacting neutralizing antibody against multiple isolates

You've been director of NIAID since 1984, at the very beginning of the HIV/AIDS pandemic. How has your role changed in that time?

It's been a very interesting and important evolution in my own life, professionally and personally. I was a host-defense, infectious-disease immunologist in 1981 and I had already been a senior investigator at the NIH for nine years, dissecting out the immune system. My lab, long before HIV, was called the Laboratory of Immunoregulation.

When HIV came along I essentially completely turned my lab around and started studying the small group of gay men with GRIDS, gay-related immunodeficiency syndrome, as it was known then. Literally, from the very first reading of that June 5th, 1981 *Morbidity and Mortality Weekly Report*, I was fascinated by the disease I've been studying ever since. I was asked to be the director of NIAID in 1984 and one of the reasons I took the position was that I felt I could have a much broader impact on a number of fields, including HIV. One of the first things I did was to ask for an extraordinarily large increase in our funding, and I took the chance of getting into all the difficulties you do when you ask for funding, that is they'll tell you to implement your expanded program and they won't give you the money.

Fortunately for me, the Administration and the Congress did actually give us a considerable amount of new money. What I've seen evolve over the last 22 years is really one of the most extraordinary scientific odysseys imaginable. We've seen the evolution of a brand new disease that has already taken its place in the history of civilization as one of the most catastrophic public health events ever. For that to be evolving right before you as you're trying to do something about it is, in some respects, a frightening concept, but is also a phenomenal opportunity to do good for society.

That's the way I look at it—being in a certain place at a certain time in history, my whole career training in infectious diseases and immunology, and then all of a sudden along comes a disease that's an infectious disease of the immune system, that has already infected 60 million people and killed 25 million more people. To me, that's pretty heavy stuff.

So what is the secret to your longevity as director of this prominent NIH institute?

Be consistent, be honest, and work hard. You've got to stick by your principles. I've been through a lot of battles—with Congress, with Administrations, with constituencies. I think if you're just honest and consistent and fair, and let the science and the public health issues drive what you do, then it works. I think that's really the secret of it.

Cloudy with a chance of prevention: Demand forecasts and assessments

New efforts are trying to gauge future demand for healthcare products, especially vaccines for neglected diseases

By Catherine Zandonella

Preventive vaccines can alleviate needless suffering and deaths and save millions of dollars in health expenditures. For a variety of reasons, however, countries do not always rapidly adopt them. The reasons include inadequate health care infrastructure, country-level immunization policies, inability to pay, and individual and community attitudes towards the acceptance of the product.

As a result, the public health *need* for a vaccine is not the same as the *demand*. Need is determined through epidemiological estimates of the number of doses required to protect the at-risk population. Demand is a more complex concept that includes consideration of the country-specific issues mentioned above as well as vaccine characteristics, product availability, and funding. "Needs forecasting is extremely dangerous," says Stephen Jarrett, deputy director of UNICEF's vaccine supply division. "Anybody can come up with those figures; the real question is, what is the fundable forecast?"

The multinational pharmaceutical sector is experienced at estimating demand in resource-rich markets, but this expertise is not always applied to developing country markets, both because of the perceived lack of viable markets and the paucity of data to use in modeling.

Recently, public private partnerships (PPPs) and non-governmental organizations (NGOs) have stepped in to undertake demand assessments for vaccines and therapeutics as part of wider efforts to increase industry engagement in research and development (R&D) into diseases prevalent in developing countries. These organizations are developing the data sets and capacity needed to create demand forecasts with the goal of maximizing global access to these products.

To help with the task of creating these forecasts, PPPs and NGOs are seeking the advice of economists, industry forecasters, and consulting groups. IAVI, the Accelerated Development and Introduction Plans (ADIPs) for pneumococcal and rotavirus vaccines (PneumoADIP and RotaADIP, respectively) coordinated by the Global Alliance for Vaccines and Immunization, and the Program for Appropriate Technology in Health's (PATH's) Malaria Vaccine Initiative are each currently developing (or have recently conducted) strategic demand assessment research. And the Center for Global Development (CGD) is in the process of holding a series of stakeholder workshops over the course of this year to gain consensus on how best to share data, techniques, and principles for demand assessments of vaccines and other medicines.

> **Needs forecasting is extremely dangerous ...the real question is, what is the fundable forecast?**
>
> **Stephen Jarrett**

Some of these assessments are for products already available, such as antiretroviral drugs, while others are for products like the preventive pneumococcal vaccine that are just beginning to be introduced. Still other assessments are for products such as an AIDS vaccine that are still very much in the R&D phase. The accuracy of the resulting forecast will necessarily depend on whether a product already exists, is just being introduced, or is still in development. There are outstanding questions about almost all of the determinants of demand for products farther from launch. In these instances, researchers must identify what they believe will be the main drivers of demand and envision plausible scenarios within which to frame and analyze potential demand.

Looking at the extremes of the spectrum, a short-range demand forecast might be based on previous consumption levels, how many people need the drug, how many new infections are expected, the price, how many doses can be purchased given available funding, and whether people come forward to be treated and remain on treatment. Since many of these parameters are not known for very long-range assessments, the endeavor is more about creating a set of demand scenarios, according to Wendy Woods of the Boston Consulting Group, which is working with IAVI to develop an AIDS vaccine demand assessment. "We never use the word forecast," she says, "because frankly we don't think we can pinpoint scenarios for a product that will be launched years from now."

An important impetus behind these demand forecasts and scenario specifications is having the conversation itself and taking into account the viewpoints of all constituents to consider the relevant issues. These constituents include national health offi-

A credible demand forecast ...could provide incentives for industry to enter markets in developing countries

cials, outreach organizations that understand the challenges of delivering vaccines and healthcare products, donor organizations that give grants for healthcare products or directly supply them, and developers or producers that research, develop, and/or manufacture the vaccines.

If demand forecasting and scenario building are done correctly they can act as tools that stakeholders can use to help make decisions. For example, country leaders and policymakers can evaluate required infrastructure investments, decide how to allocate funds for procurement, and potentially offset the costs of treating a disease through preventive efforts. Donors can map out multi-year finance strategies and ensure that funds are used appropriately. Outreach organizations can educate the community so that individuals are willing to come forward to be vaccinated, and PPPs can use the framework to undertake access planning.

Forecasts for existing products

Accurate forecasting for existing healthcare products is essential to ensure that appropriate quantities can be ordered from manufacturers, which in turn contract with downstream suppliers.

Inaccurate forecasts may result in oversupply, as was the case by the end of 2005 with the production of artemisinin-based combination therapies (ACTs) for the treatment of malaria. In response to estimates that demand would reach 50 million treatments, Novartis scaled up production of its ACT drug Coartem to 30 million treatments. This involved placing orders with downstream suppliers, including farmers who grow the agricultural product from which artemisinin is extracted. Real demand for Coartem, however, came in closer to 14 million treatments, resulting in an oversupply, says Hans Rietveld, global access and marketing director at Novartis' malaria initiative. "Long-term forecasts have proven to be very unreliable," says Rietveld, "because of uncertainties around the availability of donor funding and the absorption capacity of countries to implement new first-line drug policies. It is especially challenging for a product such as Coartem which originates as an agricultural compound and requires a 14-month production process."

In addition to helping manufacturers allocate resources, forecasts can reassure national decision makers about funding commitments and encourage policies that scale up adoption of available treatment or preventive interventions. Such forecasts may be helpful to national finance ministers, for example, who may be reluctant to make commitments even when donors pledge to provide funding for the first five years. "The finance ministers are worried about year six," says Jarrett.

Forecasts for emerging products

Demand forecasts for products that will be ready for introduction into developing markets in the near future can help developers/manufacturers decide to enter a market that they might not have previously considered. Without confidence in demand, vaccine developers and manufacturers may not attempt to market their products in developing nations. Even if they do, a lack of confidence may foster caution and production of fewer doses, which will then command higher prices. The result is a "vicious cycle" of demand uncertainty, inadequate supply, and high prices.

A credible demand forecast, however, could provide incentives for industry to enter markets in developing countries, which historically have implemented vaccinations 10-20 years after their introduction to wealthy nations. With this goal in mind, PneumoADIP created a near-term forecast based on the number of children that could be vaccinated in a few select countries using the existing public health infrastructure. While hundreds of millions of children are in need of pneumococcal vaccines in developing countries, PneumoADIP arrived at a demand forecast of about one to three million doses of the existing version of the vaccine that could be delivered over the next three years. "For the manufacturer, that was doable, whereas a needs forecast of 300 million doses was not," says Angeline Nanni, director of vaccine finance and supply for the PneumoADIP.

Starting small, Nanni hopes, will enable the demonstration of the health impact of a pneumococcal vaccine and lay the foundation for the introduction of future vaccines with broader serotype coverage. "If we can accomplish this," says Nanni, "we will have accelerated the introduction of a new vaccine in developing countries by 7-10 years and saved lives sooner."

Long-term vision

While demand forecasts clearly provide benefits for the introduction into developing

markets of existing products like Coartem and emerging products like the pneumococcal vaccine, they can also benefit health care products that are in research or early development.

Demand forecasting and scenario building require epidemiological information as a starting point but additionally require identifying potential target populations, estimating the likelihood that each group will use the product based on its actual or likely characteristics, surveying the availability of funding and the projected or available amount of the product, and finally, calculating the number of doses needed at a given time and for a given country or region. In the case of scenario building, researchers must specify potential future states of the world to analyze how demand might change given a particular set of assumption about the future.

For these health care products, demand assessment and scenario building allow one to identify and pre-empt potential barriers to future demand. From the perspective of public-private partnerships like IAVI and PATH, the goals are to produce health technologies that are appropriate for their intended settings, promote policies that accelerate R&D, and maximize the breadth and speed of access to forthcoming vaccines and pharmaceuticals.

Demand scenario specification can be used to assist development of an 'access strategy' for new health care products. By identifying the most significant drivers of demand, these organizations can plan to influence these drivers to maximize access to new vaccines for those who need them most.

Through consultation with national stakeholders and individuals working in developing countries, demand scenario assessments can uncover potential social and psychological barriers to seeking or receiving a vaccine or other health intervention due to mis-information, cultural stigmas, or beliefs. Having revealed such issues, PPPs and outreach organizations can plan educational and social marketing interventions that might facilitate vaccine or pharmaceutical uptake.

Understanding the possible scenarios for the uptake of a new vaccine or other healthcare product can aid in advocacy as well. PPPs and NGOs can use the demand forecast to advocate about the need for the vaccine to policymakers and donors. "It allows us to talk about the level of investment required and the social impact the vaccine will have," says Patricia Roberts, senior officer for commercialization and corporate partnerships at PATH's Malaria Vaccine Initiative.

This is important for products that are already on the market, but even more important when considering long-range planning for products in early stages of development, such as an AIDS vaccine. "I don't think [a demand forecast] will give you particularly robust market values but I do think that it can help identify key drivers of demand," says Saul Walker, executive director for global public policy at the International Partnership for Microbicides.

Understanding key drivers of demand and possible future scenarios serves as a basis for program planning aimed at accelerating both the development and introduction of long-range vaccines because it will identify barriers to delivery and enable organizations to target needed areas, whether those be technical assistance, training, education, communication, or improving access to health care.

A developer perspective

For all these reasons, the efforts by PPPs and NGOs are promising. Forecasts and assessments for long-range products will contain more uncertainty than near-term ones but as vaccines move closer to market that variability will decrease. "Slowly we are closing the gap towards something that was shaky 10 years ago to a forecast that makes sense," says Rudi Daems, executive director of policy and corporate affairs at Chiron Vaccines. "This is credible, feasible, this is something that will match reality."

Understanding demand drivers in developing country markets can assist researchers in managing the portfolio of research projects. For example, if a robust demand assessment illustrated that demand would be high even for a vaccine with a low duration of protection in important markets, then this will have profound implications for decisions of those conducting and managing research.

A realistic estimate of future market may also spur producers/manufacturers to enter new markets and make investment decisions based on the size of the market opportunity. "One of the things that pharmaceutical companies cite as a reason for their reluctance to serve the developing country markets is the risk associated with poor demand forecasts," says Ruth Levine, director of programs and a

> ***[A demand forecast] allows us to talk about the level of investment required and the social impact the vaccine will have***
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Ruth Levine

senior fellow at the CGD.

Mark Feinberg, vice president for policy, public health and medical affairs at Merck Vaccines, thinks that vaccine demand scenario analyses conducted by PPPs and NGOs are useful in raising the level of discussion of how to implement vaccines in developing countries. "The demand assessment is an essential component of the development process. If these are done in an expert way, a transparent way, they can provide valuable information. They are not necessarily a substitute for an analysis that industry would develop itself, but they would benefit industry by gathering information on how product profiles would influence demand."

AIDS vaccine scenario building

For an AIDS vaccine, some of the specific variables will be the target population—which could be a certain age group or vulnerable populations such as intravenous drug users (IDUs) or commercial sex workers—and vaccine characteristics, including efficacy, number of inoculations needed to achieve protection, duration of protection, and price.

Three global demand assessments have been conducted for preventive AIDS vaccines, each with differing assumptions about the vaccine and its uptake. The latest, which was conducted by the WHO, UNAIDS, and IAVI, found that while the need was potentially 700 million doses, uptake of the vaccine would be only 20% for a vaccine with low- to moderate-efficacy and 40% for a highly effective vaccine. "A vaccine with low- to medium-efficacy will be acceptable in countries with high incidence and prevalence, and will be used to target specific populations," says Saladin Osmanov, coordinator of the joint WHO-UNAIDS HIV Vaccine Initiative.

How each country will adopt a preventive vaccine will hinge on the pattern of HIV incidence and prevalence in each country amongst many other factors. In Brazil the epidemic is concentrated in men who have sex with men and IDUs, so any vaccine would probably be deployed within such populations first. "To ensure that the vaccine has most effect," says Osmanov, "each country will have to develop its own vaccine strategy."

The WHO-UNAIDS-IAVI demand assessment was accomplished by staging workshops that brought together groups of stakeholders from various regions around the world. The stakeholders were asked, given a set of hypothetical vaccine characteristics, how widely they would adopt such a vaccine. With current vaccines now in clinical trials uncertainties exist around the level of efficacy, the number of doses required per course, the price, and the delivery cost. Demand will be sensitive to all of these factors.

IAVI is now taking a more in-depth look at demand assessments. In consultation with Boston Consulting Group IAVI is developing a flexible and dynamic framework that can be continually updated. "As the state of AIDS vaccine research progresses, data input quality will improve and, correspondingly, our understanding of the determinants of demand will evolve," says Gian Gandhi, manager of policy research and analysis at IAVI. "It is not a one-off answer or number that we want to generate but an ongoing process of scenario building and refinement."

The new effort will place a greater emphasis on how stakeholder preferences change in relation to the drivers of demand than did the WHO-UNAIDS-IAVI effort, which focused mainly on country needs. To better understand patterns of vaccine adoption across countries, IAVI will look at previous rollouts of vaccines such as for hepatitis B virus. The recently approved human papilloma virus (HPV) vaccine may also offer lessons on rolling out a vaccine targeted at adolescents and adults, assuming this would be the population in which an AIDS vaccine would be used. Another proxy measure of adoption might be the levels of coverage that have been achieved by countries involved in the WHO's 3 x 5 initiative. IAVI will also look at how well individual countries are able to deliver existing AIDS programs and whether any countries are conducting AIDS-related clinical trials since adoption is often quickest in regions where trials have been conducted.

By identifying the factors that influence introduction in each country IAVI hopes to learn which of these can be influenced to facilitate more rapid adoption. "We are using our best guesses for what a future vaccine will look like to predict how the world might respond to its availability," says Gandhi.

Catherine Zandonella, MPH, is a freelance writer whose work has appeared in Nature *and* New Scientist.

IAVI Report

Two preventive AIDS vaccine trials begin

GeoVax, a US-based biotechnology company, recently began enrolling volunteers for a Phase I trial to evaluate the safety and immunogenicity of the company's AIDS vaccine candidates at four US sites in conjunction with the HIV Vaccine Trials Network (HVTN).

The vaccine utilizes a prime-boost immunization strategy to first deliver two doses of a DNA plasmid vaccine comprised of *gag*, *pro*, RT, *env*, *tat*, *rev*, and *vpu* genes from clade B HIV. This will be followed by two booster immunizations with a modified vaccinia Ankara (MVA) vector carrying clade B *gag*, *pol*, and *env* genes. These candidates were developed by Harriet Robinson at Emory University's Yerkes National Primate Research Center in Atlanta, who is also a co-founder of GeoVax, in collaboration with researchers at the US National Institutes of Health (NIH) and the US Centers for Disease Control and Prevention (CDC). The DNA candidate was tested in a previous safety trial in three US cities.

The trial will be conducted in multiple phases in order to determine the optimal dose and schedule for delivery of the prime-boost vaccinations, beginning with a dose-escalation study in only 12 volunteers. Once safety and immunogenicity data for this group are reviewed, a higher dose of the two candidates will be administered to 36 volunteers. Subsequently a larger group of 72 volunteers will then receive the higher dose in a study intended to optimize the dosing schedule.

A second trial also began recently in Zambia to evaluate the safety and immunogenicity of tgAAC09, an AIDS vaccine candidate that uses an adeno-associated virus (AAV) vector carrying genes for clades A and C HIV. This Phase II trial is the first AIDS vaccine trial to take place in the country and is being conducted by IAVI in collaboration with the Zambia Emory HIV Research Project.

The vaccine candidate was developed by US-based biotechnology company Targeted Genetics in collaboration with IAVI and the Children's Hospital in Columbus, Ohio. Safety and immunogenicity data from a series of Phase I trials using a lower dose of tgAAC09 in Belgium, Germany, and India should be available later this year. This Phase II study is a multi-center trial and volunteers are also being enrolled at sites in South Africa and Uganda.

Vaccine against human papillomavirus receives US approval

The first vaccine capable of preventing cervical cancer recently received approval and licensure by the US Food and Drug Administration (FDA) for use in females ages 9-26. Gardasil, the quadrivalent vaccine manufactured by Merck, also prevents the development of precancerous genital lesions and genital warts caused by four types of the human papillomavirus (HPV), which is the most common sexually-transmitted infection in the US according to the CDC and is responsible for 3700 deaths each year (see *Cervical Cancer Vaccines*, *IAVI Report* **9**, 5, 2005).

The efficacy of the vaccine, administered through 3 immunizations over a period of 6 months, was illustrated in 4 Phase III trials conducted in 21,000 women in several countries. At the end of June the CDC's Advisory Committee on Immunization Practices will consider recommending vaccination with Gardasil. This recommendation greatly influences whether or not the vaccine is routinely used and will open the possibility that the high cost of vaccination will be covered by national health insurance programs in the US.

However, the greatest need for the vaccine lies in developing countries, where the majority of the 250,000 deaths from cervical cancer occur each year. On June 5, the Bill & Melinda Gates Foundation awarded the Seattle-based not-for-profit organization Program for Appropriate Technology in Health (PATH) a US$27.8 million grant to conduct a five-year effort to ensure that this vaccine is made available to women and girls in developing countries. PATH is collaborating with Merck and GlaxoSmithKline, which also manufactures a cervical cancer vaccine, as well as officials in Peru, India, Uganda, and Vietnam to establish mechanisms for financing purchase of these vaccines and to ease introduction efforts.

Editor
Simon Noble, PhD

Science Writer
Kristen Jill Kresge

Production Manager
Nicole Sender

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The International AIDS Vaccine Initiative (IAVI) is a global not-for-profit organization whose mission is to ensure the development of safe, effective, accessible, preventive HIV vaccines for use throughout the world. Founded in 1996 and operational in 23 countries, IAVI focuses on four key areas: Accelerating scientific progress; education and advocacy; ensuring vaccine access; and creating a more supportive environment for industrial involvement in HIV vaccine development. IAVI's financial and in-kind supporters include the Bill & Melinda Gates Foundation, the New York Community Trust, the Rockefeller Foundation, the Starr Foundation; the Governments of the Basque Country, Canada, Denmark, the European Union, Ireland, the Netherlands, Norway, Sweden, the United Kingdom, and the United States; multilateral organizations such as the World Bank; corporate donors including BD (Becton, Dickinson & Co.), Continental Airlines, DHL and Pfizer; leading AIDS charities such as Broadway Cares/Equity Fights AIDS, Crusaid, Deutsche AIDS Stiftung, and the Until There's A Cure Foundation; and other private donors such as the Haas Charitable Trusts. For more information, go to www.iavi.org.

Vaccine Briefs

World AIDS Vaccine Day commemorated

On May 18 communities around the world held events to commemorate an annual day dedicated to the development of a safe and effective AIDS vaccine. Activities in Uganda were organized by several of the organizations conducting vaccine trials or preparatory work in the country, including IAVI, the Uganda Virus Research Institute, Makerere University, Walter Reed Army Institute of Research, and US-based Johns Hopkins University. Several local AIDS groups and non-governmental organizations held a march through the city and the AIDS Information Center provided free voluntary counseling and testing for HIV. It is estimated that 6000 people attended these events.

In collaboration with the Vaccine Support Networks and the Ministry of Health's National sub-committee on AIDS vaccines, IAVI sponsored several events in five provinces throughout Kenya. The US National Institutes of Health also sponsored several community events throughout the US. Other activities were held in India, as well as many other countries around the world, to raise awareness and highlight advances in the field.

This day was chosen as a reminder of the urgent need for an AIDS vaccine after US President Bill Clinton called for a renewed commitment toward the development of a vaccine by saying "only a truly effective, preventive HIV vaccine can limit and eventually eliminate the threat of AIDS."

United Nations convenes annual meeting on AIDS to adopt an updated political declaration

Just days before researchers and activists around the world marked the 25th year of battling the HIV epidemic, the United Nations General Assembly Special Session (UNGASS) on HIV/AIDS convened in New York City to revise the "declaration of commitment" on AIDS, which was created at the first meeting of this kind held five years ago. This high-level event, held from May 31 to June 2, was attended by more than 10 heads of state and leaders from more than 140 UN member states, as well as over 1000 representatives from activist groups and other civil society organizations.

Although few of the goals laid out in the 2001 declaration adopted by the General Assembly were achieved, the total expenditure on AIDS in developing countries, which reached $8.3 billion last year, did fall within the target range of $7-10 billion set in the initial document. This money has in part provided treatment for the 1.3 million people now receiving antiretrovirals (ARVs), up from just 240,000 in 2001, and helped to quadruple the number of people accessing voluntary HIV counseling and testing services.

But now the Joint United Nations Programme on HIV/AIDS (UNAIDS) estimates that $20-23 billion will be needed each year until 2010 to control the spread of AIDS and provide ARV treatment, care, and prevention services. The record number of civil society groups involved in the meeting pushed for the assembly to endorse a new target of providing ARVs to 80% of HIV-infected individuals in need and to an equal number of HIV-infected pregnant women to prevent them from transmitting the virus to their infants. However after extensive negotiations many of the organizations involved, including the International AIDS Society and the International Council of AIDS Service Organizations, were disappointed with the final declaration.

Many said that it failed to set concrete goals for the future by which progress could be measured. Instead much of the wrangling during the three-day meeting was centered on language as several countries and organizations became embroiled in the use of terms like "vulnerable groups" to describe men who have sex with men, transactional sex workers, or injection drug users, who are at increased risk of HIV infection.

Prior to the meeting IAVI and its partners worked to ensure that the UN leaders recognized how research into new prevention technologies, like vaccines and microbicides, could play an important role in combating the epidemic in the future and in the final declaration AIDS vaccines were acknowledged as crucial to global public health.

Just before UNGASS took place, UNAIDS released the 2006 Report on the global AIDS epidemic (www.unaids.org/en/HIV_data/2006GlobalReport/default.asp). This report highlighted the accomplishments of the last five years, while also pointing out that few of the countries fulfilled their commitments based on the 2001 declaration. The report cited a slowdown in the global epidemic for the first time, highlighted by a decline in HIV prevalence in Kenya, Zimbabwe, Burkina Faso, Haiti, and other countries in the Caribbean. But even as infection rates are dropping in some areas, the overall number of people dying from AIDS or AIDS-related illnesses continues to rise. Increasing HIV prevalence was reported in several countries, including China, Indonesia, Papua New Guinea, and Vietnam, and there is evidence of possible "HIV outbreaks" in Bangladesh and Pakistan, according to UNAIDS.

This report also declared India as the nation with the highest number of HIV-infected individuals at 5.7 million, surpassing South Africa, which still has the greatest prevalence owing to its much smaller population. While HIV prevalence is declining in four Indian states where efforts have been focused on improving access to prevention services, the epidemic in South Africa shows no evidence of decline.

Vaccine Briefs *written by Kristen Jill Kresge*