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Go forth and multiply

AIDS vaccine researchers are turning their focus from replication-deficient viral vectors to potentially more efficacious replication-competent approaches

by Andreas von Bubnoff

Just like politics can swing back and forth between conservative and progressive approaches, so can approaches to AIDS vaccine development. Early strategies focused on the traditional methods for developing a vaccine—using a weakened version of the pathogen that the vaccine is designed to protect against. For HIV, however, this strategy was shown to be unsafe.

As a result, many researchers focused on more conservative approaches, including using replication-deficient viral vectors to deliver HIV immunogens. But after Merck's AIDS vaccine candidate that used a replication-incompetent adenovirus serotype 5 (Ad5) vector failed to provide any protection in the STEP trial, this approach came under mounting scrutiny. Several researchers have started to focus more recently on replication-competent viral vectors, hoping they will generate a robust and durable immune response against HIV and mimic the protection seen with a live-attenuated vaccine approach. "Today we know that replication-deficient vectors are not giving the kind of immune response that we are looking for," says Eddy Sayeed of IAVI.

Exploring live-attenuated vaccines

In the early 1990s, researchers began tackling AIDS vaccine development with an historically effective, yet aggressive approach: evaluating the efficacy of live-attenuated simian immunodeficiency virus (SIV) vaccines in nonhuman primate (NHP) studies. In 1992, Ronald Desrosiers' group at Harvard Medical School showed that vaccination of adult rhesus macaques with a live-attenuated SIV—replication competent but at a reduced level compared to the wild-type virus—could protect against SIV challenge (*Science* **258**, 1938, 1992). The experiment was conducted with SIVmac239Δ*nef*, which had a 182 base pair deletion in the *nef* gene, according to Matt Reynolds, who is currently studying this strain as a member of David Watkins' laboratory at the University of Wisconsin-Madison.

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A static epidemic

New estimates show that public health efforts in the US have had little success controlling the number of new HIV infections over time

by Regina McEnery

Twenty-seven years after the US Centers for Disease Control and Prevention (CDC) published a report about a mysterious cluster of *pneumocystis carinii* pneumonia cases among five gay men in Los Angeles, the number of people living with HIV/AIDS in the United States has grown to an estimated 1.2 million, according to the most recent figures (see www.cdc.gov). According to the updated prevalence estimate, derived from an improved methodology, of the 1.2 million HIV-infected individuals in the US today, an estimated 34% have AIDS, 42% have not yet progressed to AIDS, and nearly 25% are unaware of their HIV infection (*Public Health Rep.*, **122**, 63, 2007).

Morbidity and mortality associated with HIV/AIDS have waned dramatically since the days when AIDS was a virtual death sentence, contributing to the ballooning HIV prevalence. HIV-related deaths in the US

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"[Desrosiers] made the first observation that taking out the *nef* gene seemingly converts a pathogenic virus into a live-attenuated vaccine," says Ruth Ruprecht of the Dana Farber Cancer Institute. The response in the field at the time was quite enthusiastic, with many thinking, "this is the breakthrough that we have needed," recalls Ashley Haase of the University of Minnesota. The *Washington Post* wrote that scientists at the time called the study "one of the most impressive achievements to date in the search for an AIDS vaccine."

And it wasn't the only good news: A study of the so-called Sydney blood bank cohort, a group of eight people who had accidentally been infected, as it later turned out, with *nef*-deficient HIV through a blood transfusion, showed no apparent signs of disease progression (*Lancet* **340**, 863, 1992; *Science* **270**, 988, 1995). This corroborated Desrosiers' finding in humans. "At that time there was a lot of optimism for the use of live-attenuated viruses," says Paul Gorry of the Macfarlane Burnet Institute in Melbourne, who studies the Sydney blood bank cohort.

However, the optimism inspired by these observations was short lived. Longer-term studies showed that using live-attenuated HIV and SIV as vaccines was unsafe. The first evidence of this came from Ruprecht's group. When they orally vaccinated newborn rhesus macaques with SIVmac239Δ3, which is more attenuated than Δ*nef*, the viral load in the first vaccinated animal didn't decline for months. "We said, gee, there is something funny going on," Ruprecht recalls. The result was the same after vaccinating two additional newborn animals, Ruprecht says. The animals couldn't clear the attenuated virus and although they grew normally and gained weight at first, their $CD4^+$ T-cell levels declined and all animals vaccinated as infants eventually developed AIDS. "This was a big shocker," Ruprecht says. When the results were published, she says it "hit like lightning" (*Science* **267**, 1820, 1995).

Today we know that replication-deficient vectors are not giving the kind of immune response that we are looking for

Eddy Sayeed

Initially, some thought the infant macaques became ill because their immune systems were immature, but later Ruprecht showed that adult macaques also got sick when vaccinated with this live-attenuated SIV vaccine (*Nature Medicine* **5**, 194, 1999; *AIDS* **17**, 157, 2003). "Live-attenuated [SIV] is largely pathogenic, period," Ruprecht concludes, adding that to this day, some researchers are still unaware of that. "Unfortunately even now some people still cite that the live-attenuated virus is pathogenic in newborns [only]. That's really only part of the story."

In other sobering news, the HIV strain that had infected the people in the Sydney blood bank cohort also turned out to be pathogenic despite the *nef* deletion. "Some of them are progressing," Gorry says. "Their $CD4^+$ [T cells] are actually declining." The donor, and two of the eight blood recipients eventually developed a reduced $CD4^+$ T-cell count, although all of the progressors had low-level viremia (*N. Engl. J. Med.* **340**, 1715, 1999; *J. Infect. Dis.* **190**, 2181, 2004; *J. Acquir. Immune Defic. Syndr.* **46**, 390, 2007). "The take-home message is that even persistent very low-level replication places these individuals at risk of progression," Gorry says. He classified low-level viremia as about 3,000-5,000 copies of HIV/ml of blood, compared with 60,000-100,000 HIV copies/ml of blood in a typical progressor not on antiretroviral (ARV) therapy. The Sydney blood bank cohort is "probably the best evidence that Δ*nef* in human beings is certainly not safe enough," concludes Ben Berkhout of the University of Amsterdam.

Other researchers experimented with a different attenuated strain called SIVmacC8, which has a 12 base pair deletion in the *nef* gene. While C8's efficacy is "pretty impressive," Ruprecht says, safety was also a problem (*J. Virol.* **73**, 2790, 1999).

Deciphering the dangers

After these discoveries, researchers set out to unravel just how live-attenuated SIV can still cause disease. Additional research showed that these live-attenuated viruses eventually become pathogenic because of their ability to mutate. "The virus evolves," Berkhout says. "There is some evidence that over time it will become pathogenic." And there are several possible mechanisms that allow the virus to regain its pathogenicity. For example, Berkhout's group showed that in cultured cells, SIVΔ3 virus, which has deletions in the long terminal repeat (LTR) promoter and in the *nef* and *vpr* genes, replicates more quickly after adding binding sites at its LTR promoter for a transcription factor called Sp1 (*J. Virol.* **73**, 1138, 1999).

Duplicated NF-κB and Sp1 binding sites in combination with additional deletions in the *nef* gene might also account for the increased replication capacity of the HIV that eventually became pathogenic in some members of the Sydney blood bank cohort (*Retrovirology* **4**, 66, 2007). "The virus is really deleting out all of the unnecessary material," Gorry says. Still, he says, host differences probably also play a role in the Sydney cohort because some individuals with similar viral mutations eventually had progressive disease, while others didn't.

Ruprecht also observed that the SIVΔ3 in macaques showing signs of disease had additional deletions. "The virus actually shrunk," Ruprecht says. Her research group injected other macaques with DNA encoding these mutant virus strains and found that they actually caused much faster disease progression than the original strain.

Playing it safe

Together, these observations made it clear that live-attenuated HIV vaccines are not safe enough to test in humans. "I don't think Δ*nef* will ever be used in humans," says Reynolds. But others aren't as quick to dismiss the live-attenuated vaccine approach. Haase says

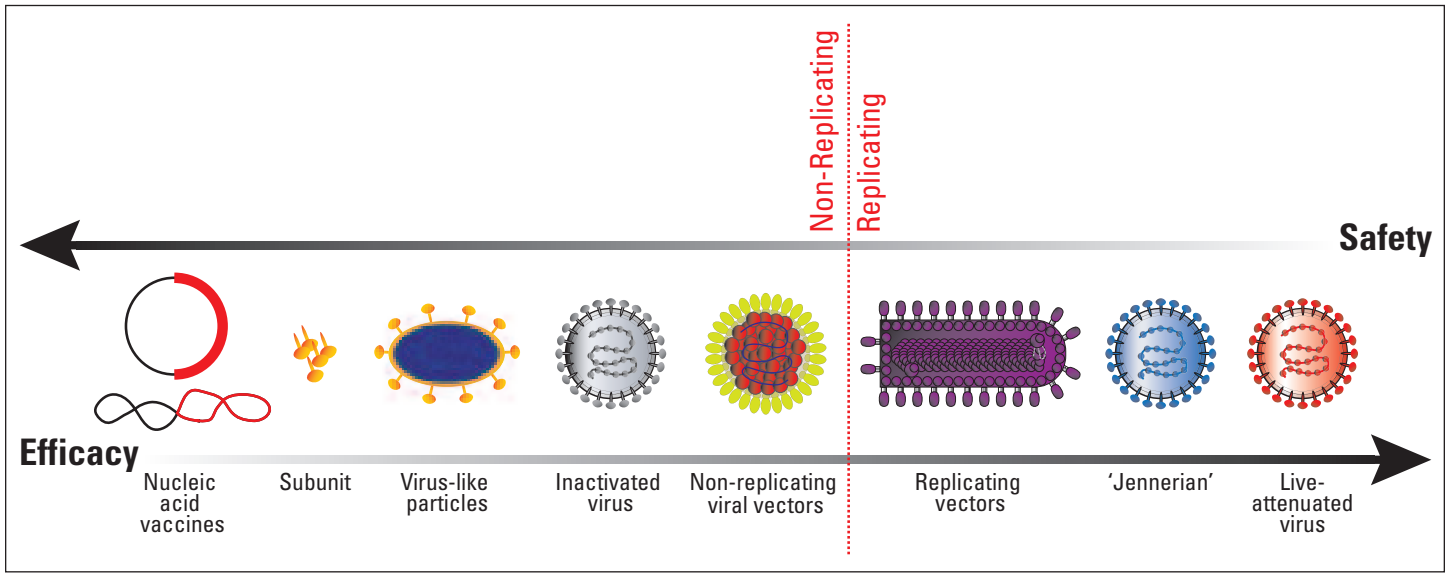

Figure 1. Safety and efficacy of replicating and non-replicating vaccine approaches. *There are a number of distinct technologies used to develop viral vaccines. Some of the most efficacious vaccines in use today, such as the measles, mumps, and rubella vaccines, are based on live-attenuated viruses. The smallpox vaccine, which was used to eradicate the disease, is also a live vaccine. It is frequently called a 'Jennerian' vaccine because Edward Jenner discovered that a related live animal virus (cowpox virus) could be used to vaccinate humans against smallpox. Although live viral vaccines are highly efficacious, there is also potentially a greater risk involved with these vaccines because they are replication-competent. Vaccines that are not based on replication-competent viruses are perceived to be safer and have been used to develop a number of important vaccines such as Hepatitis B virus (subunit), HPV (virus-like particle), and inactivated poliovirus vaccine (inactivated virus). Numerous AIDS vaccine candidates have been developed using the non-replicating strategies, notably Merck's Ad5 vaccine candidate, but so far none have been effective. This image was adapted from one provided by Wayne Koff and Chris Parks of IAVI.*

a live-attenuated vaccine in humans may be justified in some cases. "[In] a population with a very high [HIV] prevalence and incidence, it's a different calculus altogether," he says. "You would have to look at the risk of the vaccine versus the very real risk of acquiring infection anyway."

But for now, the field has largely given up on the idea of a live-attenuated HIV vaccine in humans. "What happened is everybody said it's not safe and they backburnered it," Haase says. Instead, the field focused more on using other viral vectors, many of which were replication deficient. But the recent failure of Merck's Ad5 vaccine candidate in the STEP trial raised questions about whether replication-defective vectors are perhaps not immunogenic enough.

Even before the conclusion of the STEP trial, several replicating viral vectors with HIV gene inserts were in various stages of preclinical development. In addition, some researchers are studying how live-attenuated strains of SIV like SIVmac239Δ*nef* protect to gain insights on how best to mimic this protection with a viral vector-based vaccine. Others are developing new, improved versions of live-attenuated SIV strains for evaluation in NHP studies (see *The mysteries of protection*, page 5). "Nothing is working, so people are going to try all sorts of things, including replicating viruses," says Louis Picker of Oregon Health & Science University.

Finding the right balance

Several researchers predict that replicating viral vectors will induce more durable and potent immune responses, but it appears that the more replication capacity the viral vector has, the less safe it becomes. "We have learned [that] with replicating vectors, there is an inverse correlation between the level of virus attenuation and vaccine efficacy," Berkhout says. "The more attenuated, the weaker the protection."

To account for these observations, Ruth Ruprecht has developed a threshold hypothesis suggesting that much of the efficacy and safety characteristics of replicating vaccines can be explained by how much they replicate (*Curr. Opin. Infect. Dis.* **17**: 17, 2004). According to this hypothesis, there must be some minimal "vaccine threshold" of replication of a live-attenuated virus to achieve protection. However, above a certain higher "disease threshold," that same live-attenuated virus can cause disease. The goal is to find a vaccine virus that replicates within a "window of opportunity," Ruprecht says, exceeding the vaccine threshold but staying below the threshold at which the virus can cause disease. James Hoxie of the University of Pennsylvania agrees. "If it replicates too much, you are going to get disease," he says, "if too little, you will have a wimpy virus that can't generate any protection."

This appears to be the case with live-attenuated versions of SIV. In general, the more genes that are removed from SIV, the more replication is compromised, and the safer it is. But it also becomes less efficacious, Picker says. He is currently conducting the first systematic comparison of the immune responses to different versions of live-attenuated SIVs in a study involving 120 rhesus macaques. Until now NHP studies have been much more limited in size. Picker's study will compare T-cell, innate, and antibody responses induced by different live-attenuated SIV strains.

Chris Parks of IAVI's AIDS Vaccine Development Laboratory says the inverse correlation between safety and efficacy generally holds true for viral vaccine approaches (see Figure 1). Some of the most effective vaccines to date are typically based on live-attenuated viruses, such as those against measles, mumps, and rubella, Parks says, while DNA-based or protein subunit vaccines are typically safe, but not as efficacious. There are notable exceptions to this, such as the Hepatitis B vaccine, which is a protein subunit and is highly efficacious. But the gp120 Env protein subunit AIDS vaccine

that was developed by VaxGen and tested in a Phase III efficacy trial was not effective.

Replicating vector systems

Several different replicating viral vectors are currently under investigation that can carry HIV or SIV genes, but do not integrate into the host genome. Researchers hope that replicating vectors will be more efficient since replication is thought to stimulate the immune system over a longer period of time. It is also possible that replicating vectors will induce mucosal immune responses, which are considered important for protection from HIV infection (see *The great barrier*, *IAVI Report*, March-April, 2008). In the case of polio and measles, studies showed that the live-attenuated version was better at protecting from infection than an inactivated version, says Frédéric Tangy of the Institut Pasteur in Paris.

Replicating vectors have other advantages as well. They induce better innate immune responses, according to Tangy. Also, less virus is required for vaccination because more virus gets made through replication in the host cell. For example, Tangy is using measles vaccine as a replicating vector, and only 1,000-10,000 copies of the virus are necessary to achieve an immune response, he says, much less than the one billion copies of the replication-deficient Ad5 vector used in the MRKAd5 vaccine candidate. The high dose of replication-deficient Ad5 used in the STEP trial might have generated additional target cells for HIV by recalling a memory response to preexisting Ad5 immunity. "This is one possibility of increasing infection due to vaccination," Tangy says.

Still, there is little evidence that replicating viral vectors will be superior for AIDS vaccine candidates and experts say that the assumption that they will work better is still hypothetical at this point. Berkhout says it's not really known how the immune responses to replication-competent vectors are different from those induced by replication-deficient vectors. And not everyone believes that replicating vectors will be able to protect as well as live-attenuated HIV or SIV. "I would have my doubts that these other replicating systems will mimic an HIV-1 infection, because it's happening in different cell types," Berkhout says. "But the tests should be done. I would be happy if I am wrong."

IAVI's Vector Design Consortium has several investigators working on novel replication-competent viral vectors. IAVI also has a vaccine development program in partnership with DNAVEC, a biotechnology company in Tsukuba, Japan, to develop a replication-competent Sendai virus vector. This vector is expected to be in Phase I clinical trials by 2010. It is considered safe for humans because mice are the natural host and its ability to replicate in humans is greatly restricted, preventing it from causing illness, Parks says. In addition, Sendai infects the upper respiratory tract, raising hopes that it might induce mucosal immunity. IAVI is also planning to do intranasal administration of the [Sendai] vaccine with a device similar to the one used to administer FluMist to generate mucosal immune responses. The safety of intranasal administration will be studied in mice, rabbits, and monkeys.

Tetsuro Matano's group at the University of Tokyo showed a few years ago that a DNA prime followed by a boost with a Sendai vector expressing HIV *gag* could protect five of eight rhesus macaques from intravenous SIVmac239 challenge, in that they had undetectable viral loads five weeks after challenge (*J. Exp. Med.* **199**, 1709, 2004). In the study, both replication-competent and replication-deficient Sendai vector vaccines showed some protection: The replication-competent version protected two out of four monkeys, and the deficient version protected three out of four. There was no observed difference in the induced immune response between the replication-competent and incompetent Sendai vector in this study, Matano says. "We have not shown evidence that replication can result in longer duration of the immune response," Matano adds.

IAVI is also collaborating with Picker's lab to develop Cytomegalovirus (CMV) for use as an AIDS vaccine vector. Picker is currently studying the efficacy of simian CMV vectors carrying SIV genes in rhesus macaques. One advantage of using CMV is that it results in persistent infection and induces a high level immune response for life. There are concerns that preexisting immunity could be an issue because many people have been exposed to the human CMV, but so far, preexisting immunity doesn't seem to make a difference in immune response in studies with NHPs. "Preexisting immunity doesn't affect CMV, unlike virtually all other vectors today," says Picker. His group is currently developing attenuated strains of CMVs by deleting immune evasion genes to handicap the virus. These strains remain immunogenic and efficacious, but are less likely to cause disease in immunocompromised people, Picker says.

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>
> ***Ben Berkhout***

The mysteries of protection

While live-attenuated AIDS vaccines may be off limits in humans, understanding how they protect may provide clues about how to develop other, safer vaccines with similar levels of protection. Even though SIVΔ*nef* was described over 15 years ago, it's still unclear just how it protects in rhesus macaques. "It's just been curious [that] nobody has been able to figure out exactly why," says Louis Picker of Oregon Health & Science University. The protection could be due to cellular, humoral, innate immunity, or some combination of these. "None of this has yet been ruled out," says Ruth Ruprecht of Dana Farber Cancer Institute. Viral interference might also be at play, she adds, meaning that once one type of a virus is in the host, it could interfere with a second virus being able to "set up shop."

Researchers are now conducting studies to try to elucidate the mechanism of protection. "If we had some insight into how [it protects], we would have an idea what an efficacious vaccine might actually look like," says Ashley Haase of the University of Minnesota. The goal is to understand and then mimic the mechanism with another approach that's safer, for example with another persistent virus such as CMV, Picker says.

In one project, in conjunction with IAVI's Live Attenuated Consortium (LAC), Haase will combine tetramer staining with *in situ* hybridization to observe where the immune responses to Δ*nef* occur. The technique will make it possible to directly count the ratio between effector and HIV target cells. "We can actually see the battle going on," Haase says. "We can directly see where the tetramer positive cells are spatially in relationship to the infected cells."

He already knows from natural history studies that the higher that ratio, the better the control of infection. Haase has injected Δ*nef* into 18 monkeys and plans to challenge them soon. The hypothesis is that in vaccinated animals, many effector cytotoxic T lymphocytes (CTLs) eliminate HIV-infected cells at the portal of entry before they even have a chance to expand and disseminate the infection systemically. One theory is that Δ*nef* continues to replicate at a low level, thereby continuing to stimulate the immune response. "That's why we think these attenuated viruses may actually prove to be superior to [non-replicating vectors]," Haase says. "That's what we are trying to prove."

But maintaining a constant stimulation of the immune system is not the only reason why live-attenuated viruses protect. They also express most viral genes, whereas many non-replicating vectors only express a few. "There might be a more narrow immune response," says Matt Reynolds, a member of David Watkins' laboratory at the University of Wisconsin-Madison. Live-attenuated viruses also direct the immune system toward the same cells that will be infected by the pathogenic strains, Reynolds adds.

Researchers are also developing better live-attenuated vaccines, in part because the ones that are available so far don't protect well enough. For example, a more recent study showed that SIVΔ3 protection from homologous challenge disappeared several years after vaccination, Ruprecht says (*J. Virol.* **79**, 8131, 2005). And neither Δ*nef* nor Δ3 completely protect against heterologous challenge with SIV E660. E660 is derived from SIV-infected rhesus macaques and is believed to more closely resemble naturally occurring SIV. It is not a clone of a single SIV strain, but made up of several SIV subspecies. In one study, two out of four Δ3 vaccinated animals got sick by 96 weeks after the E660 challenge (*J. Virol.* **73**, 8356, 1999).

Recently, David Watkins' group vaccinated 10 macaques with SIVmac239Δ*nef* and challenged them with an intravenous injection of E660. Eleven months after the challenge, only four of the 10 could suppress viral load.

Others are working on improved live-attenuated SIVs beyond Δ*nef* and Δ3. James Hoxie of the University of Pennsylvania has been developing a live-attenuated SIV that, so far, appears to be safe and protect against heterologous SIV challenge in pigtail macaques. The strain, called SIVmac239ΔGY (*J. Virol.* **75**, 278, 2001), has a deletion of two amino acids in the cytoplasmic tail of the SIV Env protein. This motif is highly conserved in all SIVs and HIVs and mediates endocytosis of Env by binding to clathrin adaptor complexes, Hoxie says. Without GY, Env is incorporated less into virions and perhaps more Env that's not incorporated into virions remains on infected cells, making them more susceptible to immune responses by the host.

After vaccination, ΔGY initially replicates at levels similar to that of wild-type SIV, but then an undetectable level of virus is established in the context of an emerging immune response. "This is a virus that's not a wimp," Hoxie says, "it replicates quite well." In fact, he adds, it replicates to higher levels than what has been reported for Δ*nef*.

The immune responses elicited by ΔGY seem to be, at least in part, mediated by $CD8^+$ T cells, since depleting these cells 600 days after ΔGY vaccination leads to a brief burst of ΔGY replication. And so far, ΔGY appears to protect from heterologous challenge: Two pigtail macaques vaccinated with ΔGY seven years ago remain healthy three years after E660 challenge, Hoxie says. In another experiment, two out of three animals vaccinated four years ago remain healthy two years after the E660 challenge. The third animal died after its $CD8^+$ T cells were experimentally depleted because it couldn't control its E660 levels anymore.

Still, Hoxie cautions that it remains to be seen if ΔGY is safer than Δ*nef*, because it took years for some of the Δ*nef* vaccinated animals to get sick. "I wouldn't try to argue that this is a safe virus," Hoxie says. One animal did develop a high viral load and AIDS within the first month of ΔGY infection after the virus developed an apparent compensatory mutation. However, such mutations only seem to occur soon after vaccination, Hoxie says.

In a different approach, Ben Berkhout's group at the University of Amsterdam has engineered a live-attenuated SIV that depends on the antibiotic doxycycline to replicate (*J. Virol.* **81**, 11159, 2007). After two to three weeks, when administration of the antibiotic is stopped, replication also ceases. That, he hopes, will limit replication and thereby the ability of the virus to mutate and become more virulent. He has tested the system in cell culture and in SCID mice that have a human immune system. He will soon test it in macaques in collaboration with Neil Almond at the UK-based National Institute for Biological Standards and Control, as part of IAVI's LAC.

"That's where this [live-attenuated approach] is taken to the next level," says Paul Gorry of the Macfarlane Burnet Institute. However, Gorry also says he doubts a regulatory agency will allow testing an approach like Berkhout's in humans. "Even though you can turn it on and off," adds Chris Parks of IAVI's AIDS Vaccine Development Laboratory, "you are going to end up with nucleic acid integrating into the host. I think that's the drawback of the system."

Berkhout does not necessarily believe his approach will make it into human clinical trials anytime soon, but he says it will be useful for elucidating the mechanism of protection by live-attenuated vaccines. *–AVB*

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Vesicular stomatitis virus (VSV), a virus that naturally infects cattle, is also being explored as a replicating vector. Data from John Rose's lab at Yale University indicated that VSV expressing HIV genes could protect rhesus macaques from SHIV challenge (*Cell* **106**, 539, 2001). "The data from Rose's lab was the first to demonstrate that VSV was a promising HIV vaccine vector candidate," Parks says. Earlier studies showed that VSV can spread to the central nervous system of young mice exposed to the virus, although monkeys vaccinated intranasally or through intramuscular injection did not experience any CNS complications, Parks says. Wyeth later developed highly-attenuated VSV vectors that retained high levels of immunogenicity in preparation for clinical trials (see *Renewed promise*, *IAVI Report,* Sept.-Oct., 2005).

Measles is another possible replicating vector for AIDS vaccines. It is considered safe since measles vaccine has been used in millions of people, and inexpensive because manufacturers are already making it, Tangy says. Measles, similarly to HIV, targets T cells, macrophages, and dendritic cells. Tangy is currently collaborating with GSK to develop a measles vector-based AIDS vaccine candidate expressing HIV *gag*, *pol*, and *nef* for a Phase I clinical trial.

Preexisting immunity to measles is an obvious concern but animal experiments suggest it might not be an issue (*J. Virol.* **78**, 146, 2004). In the experiments, researchers first primed animals with standard measles vaccine and then, one year later, gave them two injections with the measles vaccine encoding HIV proteins. This regimen was able to induce HIV-specific immune responses. Tangy's group is also working on a chimeric virus where the surface glycoproteins of measles are replaced with a modified HIV surface gp160 Env protein. The hope is that this chimeric virus will circumvent preexisting immunity from measles vaccination and will enter HIV-specific target cells with CD4 and CCR5 or CXCR4 receptors.

Marjorie Robert-Guroff of the US National Cancer Institute is also planning a Phase I safety trial of an orally-administered replicating adenovirus serotype-4 vaccine. She says this was shown to be safe and effective in protecting American soldiers against acute respiratory disease. A group at St. Jude Children's Research Hospital has initiated a Phase I safety trial with a replicating vaccinia virus vector based on the smallpox vaccine (*Eur. J. Clin. Microbiol. Infec. Dis.* **23**, 106, 2004). The vector is given in combination with DNA and a protein in an approach called D-V-P (DNA-Vaccinia-Protein).

Regulatory concerns

A variety of other replicating vectors are also currently in development and many groups are now considering the possibility of additional regulatory hurdles to advancing them into clinical trials. There are some safety concerns for replicating vectors and regulatory agencies, like the US Food and Drug Administration (FDA), might set more stringent requirements for preclinical studies of candidate vaccines that use replicating vectors.

One concern is that replication could go uncontrolled in immunocompromised people. Shiu-lok Hu of the University of Washington in Seattle recalls a case in the early 1990s when individuals in a therapeutic AIDS vaccine trial died after receiving a vaccine preparation containing replication-competent Vaccinia virus that was insufficiently inactivated. To address this issue, Heinz Feldmann of the National Microbiology Laboratory of the Public Health Agency in Canada is currently checking whether immunocompromised macaques infected with SIV get sick from vaccines that use a VSV vector to carry the Marburg or Ebola virus glycoprotein gene. So far, he says, it seems that there are no problems.

Another concern might be that using animal viruses as vaccine vectors exposes humans to viruses they wouldn't otherwise come in contact with.

Possible shedding of a replicating virus in urine or stool is also being explored. Tangy says the clinical trial of a measles vector-based vaccine will need to measure where in the body the vector replicates, whether it is shed by the body of vaccinated people, and if the released virus is infectious. "We have to document the shedding and biodistribution," Tangy says.

Despite these concerns, some say it is a necessary avenue to pursue. "My view is let's find something that works and then we can go and make it safe," Picker says, referring to preclinical studies. "There is no point in making a vector safe if it doesn't work." But at the same time, researchers insist other strategies should not be neglected. "I don't know what it takes to have a safe and effective vaccine," Ruprecht says. "As long as we don't know what it takes, we need [to] test various approaches."

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>
> ***Ruth Ruprecht***

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have declined significantly since the advent of highly active antiretroviral therapy (HAART) in 1996—plummeting by more than 70% between 1995 and 2004. Once the leading cause of death among Americans ages 24-44 in 1995, HIV is now usually a chronic condition if managed effectively with some combination of the 32 different antiretroviral drugs that act on the virus or its target cells in different ways to limit viral replication (*Mon. Vital Stat. Rep.*, **45**, 11, 1997).

But what disconcerts public health researchers is that the latest surveillance data reflect a static epidemic in which the HIV incidence in the US has not changed much since 1994. Despite continued efforts to improve education and promote effective and available interventions like condoms, public health agencies have had little success in controlling the number of new infections.

This worrisome situation will be highlighted in a much-anticipated surveillance report from the CDC that incorporates more comprehensive data from state registries and a more accurate method of identifying recently HIV-infected individuals using the serologic testing algorithm for recent HIV seroconversion (STARHS) technology. The STARHS method employs a combination of assays to draw its conclusion. A standard enzyme immunoassay (EIA), which is used to diagnose HIV infection by the detection of HIV-specific antibodies, is coupled with a less sensitive or "detuned" antibody test called BED HIV-1 Capture EIA. Antibody responses are generally weak soon after an HIV infection occurs, so if HIV-specific antibodies are detectable by the less sensitive assay, researchers conclude that the individual was *not* recently infected. If antibodies are detectable by the normal EIA assay, but not the less sensitive one, researchers using the STARHS methodology conclude that this individual was recently HIV infected. This model has been increasingly used around the world to estimate incidence in the absence of studies that directly track HIV incidence by following cohorts of uninfected individuals over time.

The new HIV incidence figures calculated using this method were submitted for publication in an academic journal by the CDC last year to make sure the methodology, emerging data, and conclusions were scientifically rigorous. The agency says the data is still undergoing review. The new incidence estimates are widely expected to be announced sometime this year and are expected to show that the number of new HIV infections for 2006 are significantly higher—perhaps by as much as 20,000 infections—than the annual estimate of 40,000 HIV infections per year often cited by public health departments to describe the steady pace of the epidemic in the US since 1994. While there is already plenty of speculation surrounding what the revised HIV estimates actually mean—discussions fueled mainly by the agency's slow pace in releasing the data—those familiar with the new methodology say the more accurate and timely epidemiological data probably won't be portrayed by the CDC as a major resurgence in overall incidence.

"Most likely it is just an upward adjustment and a more accurate estimate of what has been occurring in the last decade," says Walt Senterfitt, a California epidemiologist involved with Community HIV/AIDS Mobilization Project (CHAMP), a national alliance of prevention activists.

> ***[A vaccine] is needed universally and we have reached a point, particularly in the US, where we can't get beyond the 40,000 new infections a year***
>
> **Anthony Fauci**

What the new incidence estimates will dramatize is how little progress the US has made in preventing the spread of new HIV infections among adults, particularly within high-risk populations. The updated incidence data should provide a much clearer picture of where the epidemic is heading in the US, helping to identify populations and individuals at highest risk of HIV infection. This could eventually offer researchers conducting clinical trials for vaccines, microbicides, and other biomedical interventions more reliable incidence estimates to use when designing efficacy trials.

Vaccinations in the Phase IIb STEP trial, which had enrolled 3,000 men and women in North and South America, the Caribbean, and Australia, were stopped in September after Merck's adenovirus serotype 5 (Ad5)-based candidate, MRKAd5, showed no protection against infection (see *A STEP back?*, *IAVI Report*, Sept.-Dec., 2007). The majority of volunteers in this trial were men who have sex with men (MSM), but 1,100 were also women at high risk of HIV infection, 473 of them at US sites. During the trial only one female volunteer in either the vaccine or placebo group became HIV infected. Investigators involved in the STEP trial initially speculated that perhaps the women enrolled were not at particularly high risk of HIV infection, but Susan Buchbinder, a principal investigator for the STEP trial, said recently at the Keystone HIV Vaccines Symposium that there were high pregnancy rates among the female volunteers, corroborating the fact that they were having unprotected sex. She said that the low HIV incidence in women during the trial was more likely due to the lower HIV prevalence among heterosexual men in the US as compared to MSM.

More accurate incidence data will also help eliminate other problems with clinical trial design. After analyzing several non-vaccine HIV prevention trials in preparation for an Institute of Medicine (IOM) Report on the methodological challenges of conducting such trials, Harvard University biostatistics professor Stephen Lagakos and IOM Senior Program Officer Alicia Gable concluded that clearing up design deficiencies, including basing trials on unreliable incidence data, is key to overcoming the kinds of problems that led to

premature termination of some recent late-stage non-vaccine HIV prevention studies (*N. Engl. J. Med.* **358**, 1543, 2008).

Readjusted incidence numbers

Harold Jaffe, who headed up the CDC's National Center for HIV, Sexually Transmitted Disease (STD), and Tuberculosis Prevention when the Atlanta agency began developing its new HIV monitoring system, considers the system a more accurate way of calculating HIV incidence than the previous methods of back-calculation and data synthesis. Back-calculation looked at the number of individuals currently diagnosed with AIDS, then moved backward in time to estimate when and how many HIV infections would have had to occur per year to add up to current AIDS caseloads.

"Until HAART became available in the late 1990s, we believed it was possible to make reasonably accurate incidence estimates using back-calculation," says Jaffe. "Once treatment became available widely, that method became unreliable. We decided that we needed a new system," adds Jaffe. Once effective therapies altered the incubation of HIV, the CDC moved on to data synthesis, which generates HIV incidence estimates using the size of the populations at risk and the relationship between HIV prevalence and incidence. Data synthesis was used by the CDC in 1994 to determine that there were about 40,000 new HIV infections occurring in the US each year. This incidence estimate has essentially been used ever since.

The CDC expanded its existing case surveillance system several years ago to include STARHS to try and tease out recent HIV infections from longstanding ones in the population. "Fundamentally, it's a much better approach than what we had before and it's an advance in estimating incidence," says Jaffe. But the STARHS methodology also has its critics. Three years ago, the Joint United Nations Programme on HIV/AIDS (UNAIDS) recommended that countries not use the BED or detuned assay because it appeared to have overestimated HIV incidence in some African countries and Thailand. But in early studies validating its use on subtype B infections from US cohorts with known rates of seroconversion, the BED assay performed well, says Jaffe.

In either case, Jaffe predicts the new incidence estimates will stir controversy among those who feel AIDS prevention dollars are being squandered, as well as those who believe efforts are underfunded. About 4% of the US$23.3 billion allocated by the government in fiscal year 2008 to fight HIV/AIDS globally was spent on domestic prevention efforts, according to a Kaiser Family Foundation analysis.

Anthony Fauci, director of the National Institute of Allergy and Infectious Diseases, says there is no single reason for why the US has hit a "brick wall" in reducing the number of new HIV infections. He says AIDS has lost the terrifying persona that once served as a powerful incentive for careful behavior, while poverty, substance abuse, homophobia, and poor healthcare continue to put a disproportionate percentage of African Americans at risk for HIV.

"This makes it even more compelling for us to find a vaccine for HIV," says Fauci. "It is needed universally and we have reached a point, particularly in the US, where we can't get beyond the 40,000 new infections a year."

Because most HIV infections in the US today are transmitted sexually or by the sharing of used needles among injection-drug users, prevention efforts in the US have tended to center around condom promotion and distribution, needle exchange, HIV counseling and testing within high-risk communities, and sex education, including abstinence-only campaigns. Some of these interventions, notably syringe exchange, appear to have helped to reduce transmission of HIV, the latest data shows. The share of new AIDS diagnoses attributable to injection drug use dropped from a high of 31% in 1993 to 18% in 2006, and although the methodologies used in evaluating syringe-exchange programs are subject to a number of limitations, these studies generally have found that needle exchange has had a major hand in reducing HIV transmission within this high-risk group, CDC surveillance data from 2006 shows. There are currently 185 known syringe-exchange programs operating in 36 states, according to the CDC.

But many of the behavioral interventions launched by state and local health agencies, grass-roots organizations, and faith-based groups over the years have not been well-studied, and epidemiologists and social scientists tracking the epidemic tend to think the approaches have had minimal, if any, effect in reducing infections within communities shouldering the biggest burden of HIV/AIDS—men who have sex with men (MSM) and African Americans.

"I think the reality is that HIV prevention through behavioral change, which is what we have available for adults, isn't that effective," says Jaffe, now at Oxford University. "Fundamentally, it's hard to change human behavior. When you look at published studies on prevention techniques, they have been done on a small scale. It is difficult to say how well they would work in the general population. I think we need to be asking harder questions."

From 2003-2006, the most recent period for which data is available, the estimated number of HIV/AIDS cases in the US increased among MSM but remained stable among adults and adolescents who contracted HIV through high-risk heterosexual contact, according to the 2006 HIV/AIDS Surveillance Report. MSM and persons exposed through high-risk heterosexual contact accounted for 82% of all HIV/AIDS cases diagnosed in 2006, the CDC report says, basing its estimates on data collected from 33 states and five US-dependent areas that have had confidential name-based HIV reporting since at least 2003 (see Figure 2). Confidential name-based reports include data on patient demographics, HIV risk behaviors, laboratory and clinical events, and virologic and immunologic status. State and local health departments collect the data and forward it to the CDC, minus the patient's name and other personal identifiers.

Although the estimated number of newly-diagnosed HIV/AIDS cases remained stable among blacks and Latinos while increasing among whites, blacks still accounted for 49% of all HIV/AIDS cases in 2006, the CDC surveillance report found.

Women represented 26% of HIV/AIDS cases diagnosed in 2006, compared to just 8% in 1985, and the CDC estimates there are about 300,000 women living with HIV/AIDS in the US today. Black women accounted for two thirds of new AIDS cases among all women in 2006, according to the CDC surveillance report.

Though perinatal transmission has declined dramatically in the US since the start of the epidemic, mostly because of the delivery of prompt antiretroviral therapy to pregnant women and their babies, there were still 609 infants who contracted HIV before or during birth from their mothers during the years 2002-2006, the CDC reports. The CDC recommends HIV screening during prenatal visits and five states even mandate it, but hundreds of infants still slip

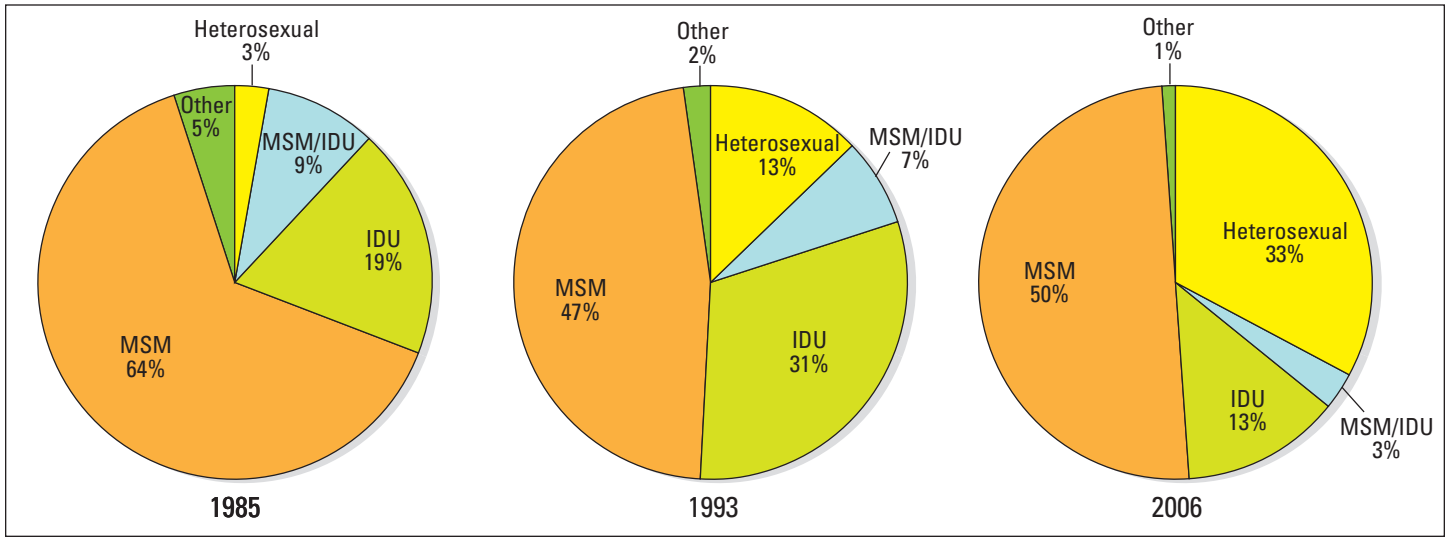

Figure 2. Shifts in HIV transmission patterns in the US based on estimates from the Centers for Disease Control and Prevention. *Although men who have sex with men remains the major transmission category, high-risk heterosexual transmission now accounts for one-third of new HIV/AIDS cases. The use of syringe-exchange programs has been a key factor in recent declines in HIV infections attributable to injection-drug use. Data for 2006 reflect the most recent statistics available.*

through the cracks because so many women are becoming newly infected with HIV every year.

"Mother-to-child transmission remains a continued problem because of the several hundred thousand women infected with HIV," says Dr. James Curran, of Emory University, an epidemiologist who investigated the very first cluster of AIDS cases in 1981 for the CDC. "We have testing during pregnancy and rapid use of antiretrovirals and other mechanisms. And it still is not eliminated."

Infected and undetected

The difficulties in reducing HIV in the US have been underscored by another statistic that public health agencies believe is partly to blame for the static rates of transmission. Using back-calculation methods, the CDC estimates about 25% of the 1.2 million individuals living with HIV/AIDS are unaware that they are infected. Because some of the people with unrecognized HIV infection may transmit the virus unknowingly, perhaps for years because of the virus' long latency period, the CDC expanded its routine testing recommendations two years ago to include all adolescents and adults ages 13-64, rather than just those in high-risk groups.

It is unclear as of yet whether this recommendation will help identify infected individuals, providing them with earlier access to treatment and care services and possibly lowering the chances that they will transmit the virus to others. State and local health departments support the new testing guidelines for physicians, clinics, and hospitals even as they struggle for ways to implement the new guidelines and the burden of caring for the additional cases that will surely surface.

"It's just like blood pressure or diabetes, if you don't know you have a problem you can't get help," says Tom Liberti of the Florida Department of Public Health, which tested 300,000 individuals last year, more than any other state.

Whatever long-term impact the testing guidelines will have in altering the status of the US epidemic, tracking it continues to be a complex epidemiological exercise that, paradoxically, seems to grow more difficult as public health agencies become more skilled at collecting and analyzing data. Compounding the confusion has been the mosaic of surveillance systems adopted by states since the start of the epidemic. Some have used confidential name-based systems, others have favored code-based reporting that replace the patient's name with their initials, date of birth, or other secret identifier and still others have used a hybrid system.

It took 21 years, for instance, for all states and dependent areas to implement HIV case reporting—the last ones just started in 2004. And it wasn't until 2005 that the CDC recommended all states and dependent areas adopt confidential name-based HIV infection reporting to "better monitor the scope of the epidemic." States are finally on board, but it will be at least three years before the CDC is able to establish trends, particularly at the state level.

Curran says HIV incidence is also hard to determine in the US because compared to AIDS-ravaged areas like sub-Saharan Africa, the incidence in the US is fairly low and the epidemic is not equally distributed across the geographic population. "When you have unequal distribution, it becomes even more difficult to find out what the incidence is," says Curran. "For example, a household survey would under-represent many of the people at high risk for HIV."

While measuring HIV incidence is very hard, getting it lower remains the goal, Curran says. "Each year, new people become at risk and the availability of good therapies has shifted attention away from the horrible death rates," says Curran. "So we now have a whole generation of young people who have grown up without having AIDS be so visible. Those people have a different feeling of risk."

Social scientists and epidemiologists who have studied the epidemic over the years aren't entirely sure what's driving the racial disparities in HIV infection among Americans, particularly black and white MSM. A meta-analysis published last June of 53 different studies found behavioral risk factors did not appear to solely explain the elevated HIV rates among black MSMs (*AIDS* **21,** 2083, 2007).

Greg Millett, the CDC behavioral scientist who led the study, says lower rates of antiretroviral use and higher rates of undiagnosed HIV infection among black men might explain the disparity, or the fact that the occurrence of STDs was higher among black MSM. "But we just don't know," says Millett. "The fact that the epidemics might be different in sub-populations of MSM has not really been looked at."

Ron Stall, a behavioral scientist at the University of Pittsburgh, evaluated US incidence data from 1995 to 2005 and estimated that slightly more than 2% of MSM in the United States were becoming infected every year. Stall, who presented his results in February at the 15th Conference on Retroviruses and Opportunistic Infections, said HIV prevention needs to incorporate multiple mechanisms—from better access to healthcare and treatment for co-morbid conditions and policies that promote gay men's health, to individual interventions such as condom distribution—to change the course of the epidemic in the MSM community.

"The risk levels among gay men are driven partly by the behavioral risks," says Stall. But he also notes that high incidence within some communities is also driven just by the sheer fact that HIV prevalence is so high. "Just by being a human being in these [areas with] very high prevalence rates, over the long haul the chances of being infected are pretty good."

Adaora Adimora, a professor of medicine at the University of North Carolina who has studied HIV rates in the African American community, particularly African American women, says there are many issues that need to be addressed for HIV prevention to work. These include the high incarceration rates among black men and the lack of services offered to them upon release from prison, high rates of STDs among African Americans, inadequate healthcare, and other factors that have put an extraordinarily high number of black women at risk for HIV. Studies have also found that fewer HIV-infected black MSM were likely to be on HAART, despite markedly higher HIV prevalence (*AIDS* **21,** 2083, 2007).

"You really have to regard this as the truly urgent situation that it is," says Adimora. "HIV prevention is going to require a fresh look." She says behavioral interventions are a good idea but fall short. "To the extent that factors outside the individual are playing a significant role in driving HIV incidence, condom use alone won't be enough," Adimora says.

AIDS advocates frustrated by the failures in controlling the US epidemic want a national AIDS strategy that incorporates more money for prevention, more rigorous studies of existing prevention methods, and better access to healthcare. "There is a mindset out there now that we will never have behavioral or social strategies that work," says Julie Davids, executive director of CHAMP. "No, we are not going to have a magic bullet, but we need to have a combination of approaches that could be rooted in a biomedical intervention." Biomedical interventions could include a preventive vaccine, microbicide, or pre-exposure prophylaxis for HIV.

With the cost of treating AIDS growing yearly in the US, advocates are also increasingly worried about how state and local governments, which shoulder most of the cost, will be able to afford programs over the long haul. In the US, HIV/AIDS spending through Medicaid—a government health insurance program that is one of the primary funders of treatment for HIV-infected individuals—increased from $1.3 billion in 1994 to $6.3 billion in 2006, according to a report by the Kaiser Family Foundation. This has reinforced the need for effective HIV prevention strategies.

> ***We are not going to have a magic bullet, but we need to have a combination of approaches that could be rooted in a biomedical intervention***
>
> ***Julie Davids***

But those on the frontlines of the US epidemic say a big challenge is keeping AIDS awareness and prevention on the radar screen. Advocacy for HIV prevention has never reached the same level as treatment advocacy in the US. Scores of activists terrified by the specter of AIDS in the 1980s and 1990s fought to save the sick and dying, and had little time to take care of the healthy, says long-time AIDS activist and playwright Larry Kramer.

Kramer, who helped establish the landmark HIV advocacy and activist groups Gay Men's Health Crisis in 1982 and AIDS Coalition to Unleash Power (ACT UP) in 1987, says many activists also found AIDS vaccine science confusing and incomprehensible. He says the government has done a poor job communicating the scope of the research, while the failure of some vaccine candidates hasn't helped. "It never caught on, even until this day," says Kramer. "It has always been a mysterious bunch of hocus-pocus."

Fauci, whose long career in AIDS research and public service put him in the center of the discovery of HIV/AIDS, the fight for treatment and its profound impact on millions of lives, and now the continuing quest to develop an AIDS vaccine, says in some ways the US has become a victim of its success. "Since we have good therapies and people are living normal lives, the perception of what a risk behavior might ultimately mean is different than what it was," says Fauci. But in an editorial he authored recently in *Science* magazine, marking the discovery of HIV as the causative agent of AIDS 25 years ago, he said, "New infections far outstrip our ability to treat everyone infected with the virus: around three people are newly infected for every person put on therapy—and current HIV therapy is a life-long commitment." He called the discovery of a safe and effective HIV vaccine "our best hope for ultimately ending the pandemic."

Small loans: Big hopes

Can economic empowerment programs give women the skills and power to reduce their risk of HIV?

by Catherine Zandonella

The shanty areas of Nairobi, Kenya are home to thousands of adolescent girls and young women, many who have migrated from rural areas. More than half of the girls aged 15 to 17 in these slums are living without parents and the vast majority are not attending school. Many are too poor to afford school fees, while others are forced to drop out of school to take care of extended family members affected by HIV/AIDS. The poorer and more isolated the girls, the higher their risk of HIV.

Here and in many other regions of the world, women have little standing to negotiate HIV prevention in their personal relationships. Intimate-partner violence, as well as poverty, is intricately linked to a higher risk of HIV throughout sub-Saharan Africa.

Establishing gender equality in these communities is a priority, and this goes hand in hand with economic empowerment. "If young people have financial capacity, you would expect a stronger ability to negotiate sexual relationships," says Evelyn Stark, a microfinance specialist at the Consultative Group to Assist the Poor (CGAP). Empowering women economically could help them work their way out of poverty, gain independence, refuse unwanted sexual advances, and successfully negotiate condom use, contributing, some researchers hope, to an eventual reduction in HIV transmission.

One way to supply women with financial capacity and independence is through microfinance initiatives. The idea is to provide women with small loans, typically just a few hundred US dollars, which could provide the foothold they need to start small businesses. Perhaps even more importantly, microfinance programs can provide a forum for women to give and receive moral support, which may help them challenge the acceptability of intimate-partner violence, learn to expect better treatment from their partners, and begin to mobilize public awareness about gender-based violence and HIV infection.

> ***If young people have financial capacity, you would expect a stronger ability to negotiate sexual relationships***
>
> ***Evelyn Stark***

Microfinance programs have already provided economic opportunity for millions of women worldwide. Now a handful of researchers are testing the hypothesis that these programs can also foster an environment that empowers women in their sexual relationships, allowing them to create societal changes that could help stabilize the AIDS epidemic in sub-Saharan Africa, where 75% of all new HIV infections occur in females between the ages of 15 and 24.

Microfinance and HIV

Microfinance programs typically provide small loans, savings, or other financial products, including credit and insurance, to individuals who could not historically access loans because they lacked the types of collateral—land or personal savings—that banks and lending institutions require. In the 1970s, microlending emerged as a viable way to stimulate economic development among the poor. Since then it has been applied successfully throughout the world. The pioneer of this concept is Muhammad Yunus, founder of Bangaladesh's Grameen Bank and recipient of the 2006 Nobel Peace Prize.

In microfinance programs, loans can be provided directly to single individuals or to small groups of collective borrowers. Although there are many ways to run a microlending program, one of the most popular is based on the concept of group lending, in which borrowers pool their savings as collateral for a loan. Although the loans are made to individuals, it is the group that is held responsible for repayment. In some models, the group's savings is used to make the payments in case of default, while in other models a village leader determines how to handle repayment. In either case, the success of these programs—repayment rates are typically well above 90%—depends in large part on the group pressure to repay the loans.

During the 1970s and 1980s when microfinance programs were first initiated, microfinance institutions (MFIs) focused mainly on loans and less on training or education. However, throughout the 1990s, concerns that microfinance programs were failing to reach the very poor led to the practice of teaming credit services with training on business development, literacy, and community building skills. "Microenterprise development [which includes microfinance, market development, and business readiness] is one of the leading economic tools being applied globally," says Mary McVay, a program director at the Small Enterprise Education and Promotion (SEEP) Network.

On the surface microfinance programs and HIV/AIDS programs seem to have little in common. But advocates of microfinance initiatives, especially in sub-Saharan African countries where AIDS is so shockingly prevalent, cannot ignore the disease. Loan programs suffer when participants or employees become ill or must leave their business to care for family members with HIV/AIDS. As a result, the US Agency for International Development (USAID) and other organizations are now supporting the integration of microfinance programs and HIV/AIDS education. One such program, Defining Options, was created by the non-governmental development agency, Development Alternatives, Inc. (DAI), with support

IMAGE participants in South Africa organized their village's first march for the international campaign "16 Days of Activism to End Domestic Violence," and made headlines in their local newspaper. Photo courtesy of The Steelburger Newspaper.

from USAID. The program advises MFIs that are partnering with AIDS service organizations. "One thing we try to encourage the MFIs to think about is their policies with regard to affected clients," says DAI's Lauren Mitten. This includes offering credit insurance, which will pay off the loan in case of death, or health insurance to clients affected by HIV/AIDS.

The popularity of microfinance programs, it turns out, also makes them excellent venues for reaching people with messages about HIV prevention, as well as stigma reduction. In Mozambique, 32 MFIs regularly reach an estimated 100,000 clients, who tend to be mature African women who are leaders in their communities. "This is an incredible platform for a whole range of public health and HIV/AIDS interventions," says Guy Winship, who initiated the link between HIV/AIDS education and microfinance during his tenure as managing director of FINCA Uganda, one of the country's largest microfinance organizations.

Women's Empowerment

Educating women about HIV/AIDS is an important step, but many public health researchers hope that microfinance programs can go even further, helping women gain the self-esteem and negotiating power they sorely lack in their personal relationships.

A study in South Africa's Eastern Cape found that roughly 30% of young men reported perpetrating physical or sexual violence against their main sexual partner during the past year. These same men also engaged in significantly higher levels of HIV risk behavior than their non-violent peers. Many women fear bringing up HIV prevention with their partners because it might arouse suspicions of infidelity, and result in physical violence.

Promoting female empowerment is the goal of an ongoing program in South Africa called Intervention with Microfinance for AIDS & Gender Equity (IMAGE). Female empowerment involves acquiring knowledge and understanding of gender relations, developing a sense of self-worth and the right to control one's life, gaining the ability to exercise bargaining power, and developing the ability to create a fair social and economic order. The IMAGE study combines gender-based health education conducted by Rural HIV and Development Action Research (RADAR), a collaborative program between the University of the Witwatersrand and the London School of Hygiene and Tropical Medicine, with microcredit provided by the Small Enterprise Foundation (SEF), a microfinance institution with over 40,000 clients in South Africa. "We wanted to pair microfinance with specific training on gender and HIV," says Julia Kim, a senior researcher with RADAR. "Bringing women together to meet their basic needs and the resulting social capital and engagement with communities would be kind of a springboard for HIV prevention and talking about gender-based violence that you don't get with most health interventions."

In the IMAGE study, women participated in a microfinance program in which they received loans to start small businesses and routinely engaged in educational sessions that covered topics such as healthcare, gender relations, domestic violence, and HIV prevention.

The project was designed as a randomized trial and researchers followed several thousand households over a two- to three-year period in Limpopo Province, a rural region of South Africa. Villages either received the microfinance/education intervention immediately or were used as a control group, which was offered the intervention at the end of the study period. After two years of

follow up, researchers used questionnaires to evaluate the direct effect of the combined intervention program on participants' economic well-being, their levels of empowerment, and the rates of intimate partner violence. HIV risk was also assessed among female participants who were considered at highest risk, in this case, those younger than 35.

The results were encouraging. Researchers found that households that received the microfinance and training intervention had improved their economic status. They also observed improvement in the level of women's empowerment using all the markers they originally set: self-confidence, financial confidence, willingness to challenge gender norms, autonomy in decision making, perceived contribution to and communication within the household, status of their relationships, and membership in social groups. Futhermore, levels of intimate partner violence were reduced by 55% among women in households that received the loans and training as compared to other households, during the last year of the study (*Lancet* **368**, 1973, 2006 and *Am. J. Public Health* **97**, 1794, 2007).

Also, among women younger than 35, there was a significant increase in uptake of voluntary counseling and testing services for HIV, higher levels of condom use with nonspousal partners, and improved communication about HIV/AIDS within their households, Kim says.

Now, new research from the IMAGE study is comparing the combined microfinance and training package with microfinance services alone. Preliminary findings suggest that although microfinance provides clear economic gains, the combined intervention offers broader benefits, particularly in relation to women's empowerment and reducing intimate-partner violence. "The findings suggest there is added value in terms of the gender training," says Kim.

A panacea?

Despite positive results, there is a concern that microfinance will be oversold as an intervention that can empower women, says Stephanie Urdang, currently with Rwanda Gift for Life. "If a woman can figure out with some support how to generate income," says Urdang, "then clearly she is in a much stronger position to resist violence, to be able to be independent, to make her own choices. But sometimes people have seen this as a panacea—that all women need is a leg up and once they've got an income, then they can move ahead and take control of their lives."

Indeed, economic empowerment alone does not automatically enable women to control their own sexual and reproductive health. In some cases, it may make it more difficult.

This was apparent in one program, known as Shaping Health of Adolescents in Zimbabwe (SHAZ), that sought to directly empower young women with microfinance initiatives. It started in 2001 with the aim of providing small loans to adolescent females and young women who were interested in starting a business. But rather than empowering the vulnerable girls who participated in the study, in many cases having income drew the attention of men in the community and made the girls in the SHAZ program subject to more sexual advances because it drew attention from men in the community. As journalist Helen Epstein wrote last year in her book, *The Invisible Cure*, "The researchers had not anticipated that their program to 'empower' these poor women was actually placing them right in the path of HIV."

Researchers found that the social networks established by the girls were what provided the most benefit and many participants reported greater knowledge of safe-sex practices at the conclusion of the SHAZ study. The few girls that did succeed economically received strong support from friends, guardians, or family members, especially ones who already possessed business skills. "It is not the money that empowers them," says Epstein. "It is the collective solidarity and support that they get from each other. That comes from them coming together either through a program that is organized, or spontaneously through a kind of social movement for women's rights."

A similar conclusion was reached by researchers involved in another microfinance program centered on reaching young girls who live in the slums of Nairobi. This program, called Tap and Reposition Youth (TRY), provided business education, mentoring, and small loans through a multiphase initiative undertaken by The Population Council and implemented by the Kenyan microfinance institution, K-Rep Development Agency (KDA).

Through churches and youth groups, the TRY program recruited 25 women between the ages of 16 and 22 to join five-person

> ***It is not the money that empowers them. It is the collective solidarity and support that they get from each other***
>
> ***Helen Epstein***

lending groups. Only 12% of participants lived with both parents, while others lived in single-parent households, were themselves head of the household, or lived with a boyfriend or husband. One quarter of the girls who participated in the TRY program reported having traded sex for money, rent, or gifts.

With increasing poverty comes an increased likelihood that their first sexual experience was non-consensual, occurred at an earlier age, and didn't involve a condom. "You have girls who have been involved in [HIV education] programs for a long time say 'I had to have sex with my boyfriend without a condom because I needed to pay the rent,'" says Judith Bruce of The Population Council. "They have complete information, they are just economically vulnerable."

The key is to have the girls select the level of risk, rather than us impose it

Judith Bruce

All participants received six days of training on business planning, life skills, and gender roles before they started contributing small amounts of money each week to a group savings account, which constituted collateral for a loan. After the loan was secured, each participant was allowed to take a portion of the money, ranging from US$40 to $200, on a rotating schedule to establish a small enterprise such as a food stand, a business buying and selling used clothes, or hairdressing.

The program got off to a strong start, but eventually repayment rates started to slip and girls dropped out of the program to protect their savings. At one point, loan officers required an adult guarantor to pledge to repay the loan in case a girl defaulted. This had the unintended consequence of increasing the girls' vulnerability, rather than reducing it.

As with SHAZ, the traditional microfinance structure worked for only a small subset of girls in the TRY program—those with the most advanced knowledge of business and with strong family or entrepreneurial support. Researchers noted that for many of the girls, the weekly meetings and social connection was far more important than starting a business. In response the TRY researchers beefed up the social support and counseling aspects of the program, adding seminars addressing gender-based violence, women's rights, communication in relationships, family planning, and HIV/AIDS education. Despite the popularity of these programs, girls continued to drop out, largely over concerns about losing their savings. The project officers have now instead focused on a savings club based on the same group model as the initial microlending program.

Even though the TRY program had limited success as a microfinance initiative, researchers did find it had promising results. "Given limitations, [the] findings are not conclusive," says Annabel Erulkar, of the Population Council, who worked on the TRY project. "However, there are indications that, among girls for whom microfinance is appropriate, it may result in greater negotiating ability within their relationships, including negotiating for safer and consensual sex."

Targeted approach

While microfinance may not be the magic bullet for reducing HIV transmission, study results indicate that targeted approaches offer several benefits. Bruce and her colleagues who worked on the TRY project have suggested a tiered approach to microfinance, in which younger, more vulnerable girls begin with basic financial education and life skills training and then move on to financial, vocational, and business training. Once the fundamental elements are in place, they can then take advantage of economic options, such as microcredit. "The key is to have the girls select the level of risk, rather than us impose it," says Bruce, referring to the progression from learning about finance and saving to eventually taking out a loan.

Another approach is to implement even more targeted and shorter term initiatives. One example of this is a microfinance program along the shores of Lake Victoria in Kenya, where the HIV prevalence hovers around 35%. The Kenya Business Development Services Program, with support from USAID, offers one-day loans to very poor women who participate in a practice known as "sex for fish." Instead of trading sex for fish, which the women could then sell at market for a profit, they receive small loans to buy fish. At the end of the day, after they have sold the fish, they can repay the loan and keep the profit.

This type of program, which is targeted at certain populations, and others that are more broadly aimed at changing societal norms, could help alter the vulnerability of girls and young women and are one more way researchers are attempting to impede the spread of HIV in sub-Saharan Africa.

Study shows role of Th17 cells

There is a growing body of evidence showing that bacterial translocation through the damaged mucosal tissues of the gut plays an important role in the chronic immune activation observed in HIV-infected individuals. Studies in recent years have suggested that depletion of the T helper type 17 (Th17) subset of $CD4^+$ T cells might be part of the reason this bacterial translocation occurs, in that it leads to impaired mucosal immune responses against incoming microbes.

Now a team led by Daniel Douek of the Vaccine Research Center at the National Institute of Allergy and Infectious Diseases (NIAID) has found further evidence to support this explanation. In people with Job's syndrome, a genetic immune disorder characterized by recurrent and severe bacterial and fungal infections, Douek and colleagues observed a lack of Th17 cells (*Nature* **452**, 773, 2008). This, Douek says, for the most part explains their increased vulnerability to attacks by bacteria and fungi. "Probably the lack of the Th17 cells is enough to account for the infections that they get," he says.

The study found that T cells producing interleukin 17 (IL-17), the cytokine made by Th17 cells, were barely detectable in peripheral blood mononuclear cells (PBMCs) from Job's syndrome patients. In addition, naïve T-cells from those patients were unable to differentiate into Th17 cells. "[The Th17] cells are not there nor can they be elicited," Douek says. This is the first description of a human genetic disease where Th17 differentiation is absent. "Job's syndrome is kind of like the Th17 knockout human," he adds.

Douek also has unpublished observations that Job's syndrome patients have bacterial lipopolysaccharides in their plasma, similar to what his group has observed before in HIV-infected individuals (*Nature Medicine* **12**, 1365, 2006). This suggests they might also suffer from bacterial translocation through their guts. "In HIV infection we have bacterial translocation and the absence of Th17 cells," Douek says. "So I think Job's syndrome adds a nice confirmation for a hypothesis that Th17 cells may be important in HIV disease pathogenesis."

A number of studies suggest that depletion of Th17 cells and an insult to the gut play an important role in explaining the chronic immune activation in AIDS patients (see *Scanning the scientific horizon*, *IAVI Report*, Jan.-Feb., 2008). For example, Satya Dandekar's group at the University of California in Davis found that injecting *Salmonella typhimurium* into the guts of SIV-infected rhesus macaques resulted in the spread of the bacteria to other organs, whereas in uninfected macaques, the bacteria remained in the gut. She also found that the IL-17 response to the injected microbes was blunted in SIV-infected macaques. IL-17 is thought to induce antimicrobial immune responses in the gut epithelium, Dandekar says. Others have shown that Th17 cells in the gut of sooty mangabeys, natural SIV hosts that don't develop AIDS, remain stable.

Donald Sodora of the Seattle Biomedical Research Institute says the latest data from Douek and colleagues are an additional piece of evidence for a key role of Th17 cells in preventing translocation by commensural gut bacteria, as has been postulated to occur during HIV and SIV infection. "It's a good paper especially for identifying what Th17 cells are doing," says Sodora, who studies immune activation during HIV and SIV infection.

Douek says he got the idea to look for Th17 cells in Job's syndrome patients when others showed last year that these patients have a mutation in the *stat3* gene (*Nature* **448**, 1058, 2007; *N. Engl. J. Med.* **357**, 1608, 2007). Because it was already known at the time that STAT3 is involved in the differentiation of Th17 cells (see, for example, *J. Biol. Chem.* **282**, 9358, 2007), it seemed plausible that Job's patients had a defect in Th17 cells. "We sort of put two and two together," Douek says. —*Andreas von Bubnoff*

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The International AIDS Vaccine Initiative (IAVI) is a global not-for-profit organization whose mission is to ensure the development of safe, effective, accessible, preventive HIV vaccines for use throughout the world. Founded in 1996 and operational in 24 countries, IAVI and its network of collaborators research and develop vaccine candidates. IAVI's financial and in-kind supporters include the Alfred P. Sloan Foundation, the Bill & Melinda Gates Foundation, the Foundation for the National Institutes of Health, The John D. Evans Foundation, The New York Community Trust, the James B. Pendleton Charitable Trust, The Rockefeller Foundation, The William and Flora Hewlett Foundation; the Governments of Canada, Denmark, Ireland, The Netherlands, Norway, Spain, Sweden, the United Kingdom, and the United States, the Basque Autonomous Government as well as the European Union; multilateral organizations such as The World Bank; corporate donors including BD (Becton, Dickinson & Co.), Bristol-Myers Squibb, Continental Airlines, Google Inc., Henry Schein, Inc., Merck & Co., Inc. and Pfizer Inc; leading AIDS charities such as Broadway Cares/Equity Fights AIDS and Until There's A Cure Foundation; other private donors such as The Haas Trusts; and many generous individuals from around the world. For more information, see www.iavi.org.

Vaccine Briefs

World AIDS Vaccine Day and HIV discovery commemorated

May 18 marked the 11th annual commemoration of World AIDS Vaccine Day, which is observed to honor the thousands of people working around the world to develop an AIDS vaccine. The significance of this day stems from a Morgan State University commencement address delivered in 1997 by then-US President Bill Clinton in which he called for renewed commitment toward the development of an AIDS vaccine. In the wake of some recent setbacks in the AIDS vaccine field, several organizations consider 2008 to be a particularly important year to raise awareness and support for continued efforts to develop an effective vaccine.

This year, organizations around the world coordinated educational campaigns and awareness activities to commemorate the day, including voluntary counseling and testing services for HIV. Several organizations in South Africa sought to heighten awareness about the importance of continuing AIDS vaccine research in light of last year's failure of Merck's leading AIDS vaccine candidate, which was also tested in South Africa in a Phase IIb test-of-concept trial, known as Phambili.

On May 20, leading HIV/AIDS researchers marked another important day—the 25th anniversary of the study published in the journal *Science* by Luc Montagnier and colleagues at the Institut Pasteur and La Pitié-Salpêtrière Hospital that described HIV as the causative agent of AIDS. A meeting was held at the Pasteur Institute in Paris in observance of this day at which leading scientists discussed ongoing HIV/AIDS research efforts, including sessions focused on the current strategies for AIDS vaccine development. Since the discovery of HIV, over 60 million individuals have been infected with the virus and more than 25 million have died. Alan Bernstein, the executive director of the Global HIV Vaccine Enterprise, wrote in an editorial published in the May 9 issue of *Science*, "The only end for a journey that began 25 years ago should be the development of a safe and effective HIV vaccine."

In another commentary piece in *Nature* magazine, Anthony Fauci, director of the National Institute of Allergy and Infectious Diseases (NIAID), reflected on the advancements made over the last quarter of a century in understanding and combating HIV/AIDS. He also called for a renewed commitment to overcoming the challenges in AIDS vaccine development and said, "It is now clear that we were naïve to think there would be a straight path from the discovery and characterization of HIV to the development of a vaccine." On May 20, NIAID announced a new US$15.6 million, five-year program that will provide grants to 10 research teams to study the critical role of B cells in HIV infection with the aim of developing antibody-based AIDS vaccine approaches. Past NIAID grants focused primarily on T-cell based AIDS vaccine candidates, but following results from recent trials and the HIV Vaccine Summit held by Fauci in March, NIAID decided to place renewed emphasis on vaccine approaches that elicit neutralizing antibodies against HIV (see *Balancing AIDS vaccine research*, *IAVI Report*, March-April, 2008). *—Alix Morris, contributing writer*

Super sequencing

Since the start of the epidemic, HIV has managed to outrun and outmaneuver scientists. It has done this in part by developing high levels of genetic diversity that have enabled the virus to rapidly spawn mutations capable of withstanding immune pressure. But the chase is on. Ultra-deep pyrosequencing technology, a fairly new high-throughput statistical method that produces DNA sequences in much greater numbers than previous techniques, is allowing scientists to inch closer to the most diverse region of the virus, the third hypervariable or V3 loop.

During April's 15th Annual Workshop on HIV Dynamics and Evolution in Santa Fe, New Mexico, two separate papers—one now in publication—showed how ultra-deep sequencing can be used to produce huge amounts of genomic data about the viral population, including detailed identification of thousands of low-frequency viral variants. The ultra-deep sequencing helps identify, quantify, and track these minority virus populations as they evolve.

These hard-to-detect variants moving through the viral population are of great interest to AIDS vaccine researchers working to design effective immunogens that will induce the broadest immune responses against the greatest possible number of circulating HIV variants. Getting a clearer view of how HIV's genetic material evolves over time could be the retroviral version of stealing the opponent's playbook. And because the V3 amino acids are so diverse—as much as 35% can differ between strains within the same clade of HIV—this section of HIV's envelope is integral in helping the virus evade the immune system.

Pyrosequencing allows researchers to sequence a single strand of DNA by assembling the complimentary strand alongside it, one base pair at a time. This permits researchers to detect which base was actually added at each step. It's faster, cheaper, and less labor-intensive than the traditional Sanger method. The chief limitations with pyrosequencing are shorter reads—only about 250 nucleotides compared to 1,000 with the Sanger method—and data that is more error-prone.

The annual HIV Dynamics and Evolution Workshop, which attracts about 100 HIV scientists, began meeting to encourage dialogue among specialists who use a range of mathematical models and other computational tools to study viral and cellular dynamics, as well as viral evolution and population genetics. Other highlights from this year's workshop included the description by University of Arizona researcher Mark Worobey of the oldest putative case of HIV disease identified to date from a lymph node sampled in early 1960 from a female patient in the former Central African country of Zaire, now the Democratic Republic of the Congo. The country has long been considered a hub for the dispersal and diversification of AIDS pandemic viruses, and the clade A-like strain detected in the woman showed the existence of distinct subtypes, evidence that its HIV group M ancestor dated back to at least the 1930s. The sample is also the only sequence-confirmed symptomatic group M HIV infection prior to the late 1970s, researchers said. *—Regina McEnery*