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IAVI Report

Contents

Interfering with HIV

A fundamental biological process that was first discovered only eight years ago could revolutionize research and medicine, and may hold promise for HIV infection

By Andreas von Bubnoff

This year's Nobel Prize for the discovery of RNA interference (RNAi) to Andrew Fire and Craig Mello is the preliminary end point of a rise to prominence that can only be described as meteoric. The award came just eight years after Fire and Mello found in the nematode worm *Caenorhabditis elegans* that pieces of double-stranded (ds) RNA are much more powerful than single-stranded RNA in specifically inhibiting expression of genes with the corresponding sequence (*Nature* **391**, 806, 1998).

The dsRNA pieces Fire and Mello used were several hundred bases long, too long to specifically inhibit gene expression in mammalian cells. That's because they would induce the interferon response, a non-specific general shutdown of gene expression. But only three years later, it became clear that very short dsRNA pieces—around 21 nucleotides, so-called short interfering RNAs (siRNAs)—can specifically inhibit genes in mammalian cells as well (*Nature* **411**, 494, 2001).

It is now clear that these siRNAs inhibit gene expression through the same natural phenomenon that cells normally use to regulate their own genes. The cells do this by transcribing genes that encode micro RNAs (miRNAs), which are the functional equivalents of siRNAs. A ribonuclease protein called Dicer helps process these miRNAs into short dsRNAs that look just like siRNAs, and from there the cell treats both (siRNAs or miRNAs) the same, in that one strand is incorporated into an enzyme complex called the RNA-induced silencing complex (RISC; Figure 1). Once that strand binds a complementary target mRNA, the target MRNA is degraded or is not translated into protein. The complex acts catalytically, meaning it is recycled and can act again and again. That, combined with the exquisite specificity afforded by the nucleotide sequence matching, explains why RNAi has such potential, in research and possibly medicine too.

Gene knockout

For molecular biologists, RNAi has become a powerful and specific tool to study gene expression and function by making it much easier to knock out genes than ever before,

continued on page 2

Rotavirus vaccines rolled out

Vaccinologists battle an intestinal virus to prevent one of the leading causes of potentially deadly diarrheal disease in infants

By Kristen Jill Kresge

As a medical epidemiologist at the US Centers for Disease Control and Prevention (CDC) Umesh Parashar has spent the last 10 years of his career chasing an intestinal virus that, outside of medical circles, few people have ever heard of. Even so, rotavirus is a ubiquitous infection among infants and is the most common pathogen associated with the severe diarrheal disease known as acute gastroenteritis that is responsible for around 600,000 deaths and more than two million hospitalizations each year worldwide in children under five years of age.

The death toll in resource-poor countries and soaring medical costs in industrialized nations associated with such a pervasive infection spurred scientists into developing vaccines that could prevent the severe and all too often deadly dehydration caused by this disease, launching a 25 year quest that Parashar refers to as a "rollercoaster ride." Despite early success, the rotavirus vaccine efforts faced a serious setback when the first licensed prod-

continued on page 6

continued from page 1

says Kuan-Teh Jeang, who studies miRNAs at the US National Institutes of Health (NIH). "RNAi has now become the poor man's fast knockout tool," he says. This has made it possible to screen siRNA libraries to find host cell genes that are required for HIV replication or infection, says John Rossi of the City of Hope Comprehensive Cancer Center in Duarte, California.

Almost immediately after the discovery that siRNAs can work in mammalian cells, researchers started thinking about possible applications, including prevention and treatment of HIV. "HIV was an obvious [target]," says Bryan Cullen of Duke University Medical Center in Durham, North Carolina. Cullen's group was among the first to show that siRNA can inhibit HIV replication in cultured human T cells, one of the main cell types that HIV infects (*J. Virol.* **76**, 9225, 2002).

Initially, researchers transfected siRNAs transiently into cells, but soon they found a way to coax cells into expressing them constitutively. They infected cells with viruses engineered to insert genes for short hairpin RNAs (shRNA) into the host cell genome. The host cell processes them in a similar way to endogenous miRNAs to inhibit the expression of target genes.

These advances opened the door to a gene therapy approach by introducing cells that stably express shRNAs into HIV-infected patients. Several groups are planning to start Phase I clinical trials in the next two years.

Delivery obstacle

At the same time, Judy Lieberman's lab at Harvard Medical School is working on ways to deliver siRNAs directly to cells to treat or prevent HIV infection. One major obstacle, she says, as with all gene therapy approaches, is delivery.

"[siRNAs] don't naturally get into cells," Lieberman says. In a study three years ago she literally forced the siRNAs into the livers of mice. She used high volume injection into the blood, temporarily damaging the cell membranes of liver cells so the siRNAs got in and protected the cells from hepatitis infection. The problem was that the volume was so large that the treatment also resulted in heart failure.

In more recent experiments, Lieberman has encapsulated siRNAs into liposomes to get mucosal surfaces to take them up, for example in the genital tract of mice. She has found that this approach can silence genes for more than a week, and has shown that siRNAs targeting herpes simplex virus type 2 (HSV-2) genes can protect mice from HSV-2 infection by silencing any viral genes that enter cells in a potential transmisson event (*Nature* **439**, 89, 2006). HSV-2 is the leading cofactor for HIV transmission in the world, increasing people's susceptibility to HIV infection (see *HIV prevention in a pill?*, *IAVI Report* **9**, 4, 2005).

Prevention or treatment

Next Lieberman wants to use the approach to develop a microbicide women could use to prevent HIV transmission. "Because the silencing lasts for a while, you don't have to remember to use it immediately before you have sex," she says. It could also be cheap because it uses very small amounts of siRNA; according to Lieberman, one dose in humans could cost as little as US$8.

She is also developing a method that can direct siRNAs to HIV-infected cells inside the body. To this end she has made fusion proteins of protamine (a protein that binds and condenses the DNA in sperm) to bind the siRNA, and an antibody that recognizes proteins on target cells like the HIV Env protein. This approach can suppress HIV replication in cultured T cells, she says. In whole animals, an Env-specific antibody directed the fusion protein to tumor cells expressing Env in the flank of mice (*Nat. Biotech.* **23**, 709. 2005). These experiments show that cell-specific delivery of siRNAs is possible, but it will be a long way until clinical trials, Lieberman says. Depending on the antibody targets, the method could be used to prevent HIV infection in the first place or to treat infected patients.

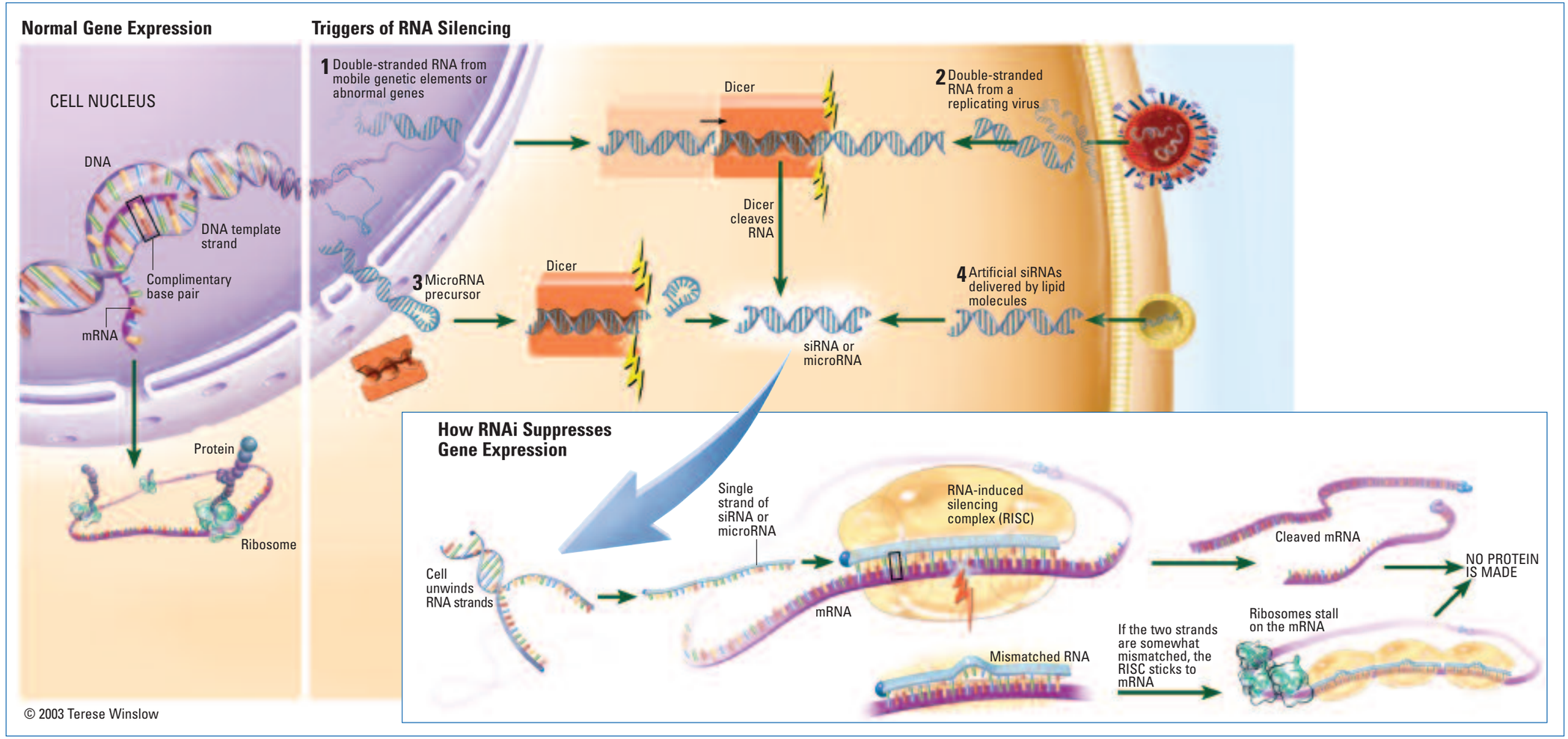

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Figure 1. Gene control by RNA interference. *Cells can control expression of their genes by interfering with the messenger RNA (mRNA) that is transcribed from any particular gene, thereby preventing that mRNA from being translated by ribosomes into active protein, as normally happens (left panel). The RNA interference (RNAi) machinery is triggered into action by small double-stranded RNA molecules with ragged ends. An enzyme called Dicer cleaves these short interfering RNAs (siRNAs) from longer double-stranded RNAs that are produced by self-copying genetic sequences (1) or viruses (2). Regulatory RNA sequences known as microRNA (miRNA) precursors (3) are also cleaved by Dicer into this short form. Researchers can also introduce artificial siRNAs into cells using liposomes (4).*

The siRNA or miRNA fragments separate into individual strands (bottom panel), which then combine with proteins to form the RNA-induced silencing complex (RISC). The RISC then captures mRNA that complements the short RNA sequence. If the match is perfect, the captured mRNA is cleaved into small fragments (top row). Less than perfect matches cause a different outcome; for instance, the RISC may remain bound to the mRNA, blocking ribosomal translation (bottom row). The end result is the same—no protein is manufactured.

> ***A constant supply of siRNAs is required to control a chronic virus infection. The best way to achieve [that] is with a gene-therapy approach in which the siRNAs are stably expressed***
>
> **Ben Berkhout**

Still, some experts say that to treat a chronic disease like HIV, gene therapy is a better approach than delivering siRNAs directly, which only has a temporary effect. "A constant supply of siRNAs is required to control a chronic virus infection," says Ben Berkhout of the University of Amsterdam. "The best way to achieve [that] is with a gene-therapy approach in which the siRNAs are stably expressed."

Berkhout and others are planning Phase I gene therapy trials. They will use an HIV-derived lentivirus to introduce HIV-suppressive shRNA genes into the genome of $CD34^+$ hematopoietic stem cells taken from HIV-infected individuals. $CD34^+$ cells give rise to T cells and macrophages, two of the major cell types infected with HIV. These $CD34^+$ cells will then be reintroduced into the patient's blood. Berkhout says that the hope is that the protected stem cells will preferentially survive and reconstitute the patient's immune system, as only the untreated, non-protected cells will be killed by HIV.

So far, animal studies suggest that the approach could be safe and efficient. In transgenic mice, shRNAs can be expressed without deleterious consequences for the host. What's more, Rossi's lab has shown that $CD34^+$ stem cells transduced with shRNA could still block HIV replication even after they differentiate into T cells and macrophages.

Safety issues

However, the safety of gene therapy in general is still a major concern. The latest major set back came when three children treated for severe combined immunodeficiency disease (SCID) in a clinical trial in France got leukemia, most recently last year, because the virus used to treat them—murine leukemia virus—had integrated into sites upstream of an oncogene, activating its expression.

But the results of a recent Phase I clinical trial suggest that an HIV-derived lentivirus did not show such dangerous insertion events after 21 months (*Proc. Nat. Acad. Sci.* **103**, 17372, 2006). In the trial, five advanced-stage AIDS patients who did not respond to at least two current antiviral regimens were treated once with autologous lentivirus-transduced $CD4^+$ T cells expressing an antisense RNA to the HIV *env* gene. "It is the first report of patients being treated with any kind of lentiviral vector," says lead researcher Carl June at the University of Pennsylvania. "We have now followed up the first two patients for three years, [and] there are no adverse events. It is safe from what we have observed." June adds that the researchers also looked at almost 200 integration sites and did not observe that the virus integrated into any regions on chromosomes known to be problematic.

What's more, the lentivirus is derived from HIV itself, which does not seem to cause cancer in HIV-infected individuals. "There has never been a known case of a viral insertion causing a cancer in any [HIV-infected] patient," Rossi says. He has also looked at about 130 lentivirus integration sites and found that it almost always integrated into introns, genomic regions that are inactive. "It's relatively benign," Rossi says of the lentivirus vector, adding that he is now ready to go into patients since experiments in mice and monkeys have indicated that it is safe.

Interference escape

Still, even if gene therapy turns out to be safe, there are additional challenges that any RNAi approach needs to overcome. One major obstacle is the high mutation rate of HIV that allows the virus to escape the RNAi inhibition. "If there is one point mutation, it doesn't work anymore," says Daniel Boden of the Aaron Diamond AIDS Research Center in New York. He found that in cultured T cells HIV can escape from shRNA targeting the HIV *tat* gene by mutating after just 25 days (*J. Virol.* **77**, 11531, 2003). Boden, for his part, is skeptical as to whether the escape problem can be solved, in part because HIV is not a clone but a quasi-species that varies greatly within an infected individual. "I don't see this as something that can be done," Boden says.

Cullen agrees that an escape of the virus is not a question of if, but when. "Every possible mutant is there 1000 times every day," he says, and the virus only has to change one nucleotide to become resistant, which is easier than changing an amino acid to become resistant to drugs. "This is very easy for the virus to escape from," Cullen says.

But Berkhout says the escape problem can be solved, for example by simultaneously targeting multiple conserved parts of the HIV genome with different shRNAs. "It just becomes a numbers game," Berkhout says. "At some point, the virus just won't escape anymore." To escape from several shRNAs, the virus would have to mutate all of the target sequences at the same time, and Berkhout thinks that's extremely unlikely. Even if it eventually escapes there could well be a fitness cost that renders the virus less virulent, he adds. So far Berkhout hasn't seen escape after two months with a combination of four different shRNAs transduced into cell lines using a lentivirus (*Mol. Ther.* **14**, 883, 2006).

Other groups are targeting host cell mRNAs to get around the escape problem, since these are less likely to mutate. One such target is CCR5, a host cell co-receptor that HIV needs to enter the cell. There is some evidence that there would be little or no side effects since some people who have a deletion in their CCR5 gene are resistant to HIV infection but seem otherwise fine.

Rossi plans to overcome escape in his gene therapy trial by using three different RNA-based mechanisms. One of them uses a ribozyme that specifically cuts the host cell's CCR5 mRNA in an enzymatic manner. The second mechanism involves shRNAs against HIV targets, and the third is a so-called decoy RNA that binds the HIV transcription factor Tat to keep it from activating the transcription of viral genes. "We think we can avoid resistance with this approach because we are going after a cellular as well as a viral target," Rossi says. In a recent study in cultured $CD34^+$ cells, he didn't see any viral replication for 72 days when using this combined approach (*Mol. Ther.* **12**, 900, 2005).

But some caution that CCR5 inhibition with RNAi may not be the best idea. Berkhout points to the recent observation that CCR5 may make symptomatic West Nile virus infection less likely. Boden says targeting CCR5 in late stage HIV-infected patients—which is what most of the trials plan to do—may not be sufficient because the virus tends to switch to a different host cell co-receptor, CXCR4, later in the course of infection. And pharmaceutical companies like Pfizer have CCR5-inhibiting drugs in clinical trials. Given the delivery problems of RNAi, these are more likely to emerge as anti-CCR5 agents than RNAi, Cullen says.

Toxic effects

Escape is not the only thing to worry about. Toxicity also needs to be addressed. Rossi says gene therapy could be less toxic then the current drug treatments for HIV patients such as RT (reverse transcriptase) and protease inhibitors. But Mark Kay's lab at Stanford University showed that mice expressing too much shRNA in all liver cells died of liver failure (*Nature* **441**, 537, 2006). "It overloaded the system," Kay says. "It gives some indication that it is very important to be in the right dosing range." The introduced shRNAs probably interfered with something in the normal processing of endogenous micro RNAs, because both use the same pathway, Kay says. However, he does not expect this to necessarily be a problem for the gene therapies planned by the labs of Rossi and Berkhout. "They are using a vector that generally gives a lower amount [of shRNA] than the vector we use," Kay says, adding that in the worst case, the treated $CD34^+$ stem cells would simply die.

Cell death is exactly what happened when Irvin Chen's lab at University of California, Los Angeles expressed an shRNA under two different promoters in T cells—the promoter that expressed more shRNA was more potent, but also killed the T cells, Chen says (*Mol. Ther.* **14**, 494, 2006). "There is a balance between potency and safety," he says. "We need the most potent shRNA combined with a promoter that expresses the minimum amount of siRNA to minimize the cytotoxic effect," says Dong Sung An, lead author of the study.

Given all these challenges, will RNAi ever be available as a treatment for HIV infection? "The reason that so many people are working on this approach is because we are optimistic that it can work," says the NIH's Jeang. "[But] we are far short of RNAi as a therapeutic agent against HIV."

It's unlikely to expect that RNAi approaches will completely replace other HIV treatments, says Stanford University's Fire. "Throwing away all the drugs and just using RNAi, I don't think that's going to happen," he says.

"I don't think RNAi has any potential against HIV in the next 5-10 years," says Cullen when asked how soon RNAi therapies will be available for patients. Jeang says he doesn't know. "If I could tell you that, I would go to Wall Street and buy a lot of stock."

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>
> **Kuan-Teh Jeang**

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continued from page 1

uct was abruptly pulled from the shelves by its manufacturer after safety concerns surfaced in post-marketing surveillance, creating higher hurdles for newer vaccines to clear and ensuring a longer road to approval and licensure.

However the continued efforts by vaccine manufacturers GlaxoSmithKline (GSK) and Merck culminated earlier this year in landmark studies showing that both company's live, attenuated rotavirus vaccines were highly effective in preventing severe gastroenteritis in infants and didn't suffer the same safety problems that sealed the fate of the previous vaccine. Now, after periods of doubt and uncertainty, Parashar is excited. "Given the challenges and the enormous resource requirements, it is just amazing that we actually have two new products," he says.

Equivalent immunity?

Parashar's enthusiasm is tempered by one thing—these vaccines are yet to be tested in efficacy trials in Africa and Asia, and scientists don't know if they will be as effective at preventing severe disease in these populations as the already completed Phase III trials indicated in infants from the US, Europe, and Latin America (*Science* **312**, 2006, 851). "That's the biggest scientific question that remains," says Parashar. The vast majority—as many as 82%—of rotavirus-related deaths occur in developing countries, but the immune responses induced by orally-administered vaccines have historically been hampered in these populations. Trials in developing countries demonstrated the need for additional doses of oral polio vaccine to stimulate equivalent immunity, and cholera vaccines and earlier versions of rotavirus vaccines performed less favorably in these settings. Before rotavirus vaccination programs can be implemented around the world, the vaccines must pass this important test.

GSK has already started two trials in Malawi and South Africa and Merck plans to initiate trials soon at sites in Africa and Asia, all of which are being conducted in cooperation with the Seattle-based public health organization Program for Appropriate Technology in Health (PATH). And although data from these studies isn't expected until 2009, organizations like the Global Alliance for Vaccines and Immunizations (GAVI; which provided PATH with a US$30 million grant), the World Health Organization (WHO), and the CDC are already actively engaged in accelerating the development and introduction of rotavirus vaccines. If implemented widely the new generation vaccines could help prevent a disease that is responsible for 5% of all childhood deaths.

Natural history

Rotavirus was first identified by Australian scientists in 1973 after being isolated from intestinal samples of children suffering from diarrhea. Although named for its wheel-like appearance when viewed by electron microscopy, rotavirus' layered structure evokes an onion. The non-enveloped virus is composed of several layers of protein that facilitate cell binding and entry but also shield the central core where the 11 segments of double-stranded RNA and the associated replicative enzymes are located (Figure 1). The middle shell is composed of the most abundant viral protein, VP6, which is common to all rotaviruses. The two proteins that comprise the outermost shell—VP7, the glycoprotein or G-protein, and VP4, the protease-cleaved or P-protein—determine the serotype of the virus and are the main targets of neutralizing antibodies, making them critical in vaccine development.

Soon after the virus was discovered diagnostic assays were developed that could detect the VP6 protein and researchers began to come to grips with the burden of rotavirus disease. They quickly found that "rotavirus was the most common cause of diarrhea in children everywhere in the world," says Parashar.

So far researchers have confirmed the identity of 14 G serotypes, 14 P serotypes, and 24 P genotypes, which have not yet been assigned to a serotype and are commonly denoted within brackets (*Expert. Rev. Vaccines* **4**, 521, 2005). Viral strains with nearly all possible combinations of these G and P serotypes have been reported in humans but, luckily for vaccine developers, there are only four combinations that predominate globally. The P[8]G1, P[8]G3, P[8]G4 and P[4]G2 account for over 80% of rotavirus-related disease, with the G1 serotype being implicated in nearly half of all rotavirus infections. The fifth most common serotype is the G9, which has been increasing in prevalence over the last decade. But Penny Heaton, head of the clinical rotavirus program at Merck, points out that the most prevalent rotavirus strain can vary widely, with several unusual strains occurring in developing countries.

Given the challenges and the enormous resource requirements, it is just amazing that we actually have two new products

Umesh Parashar

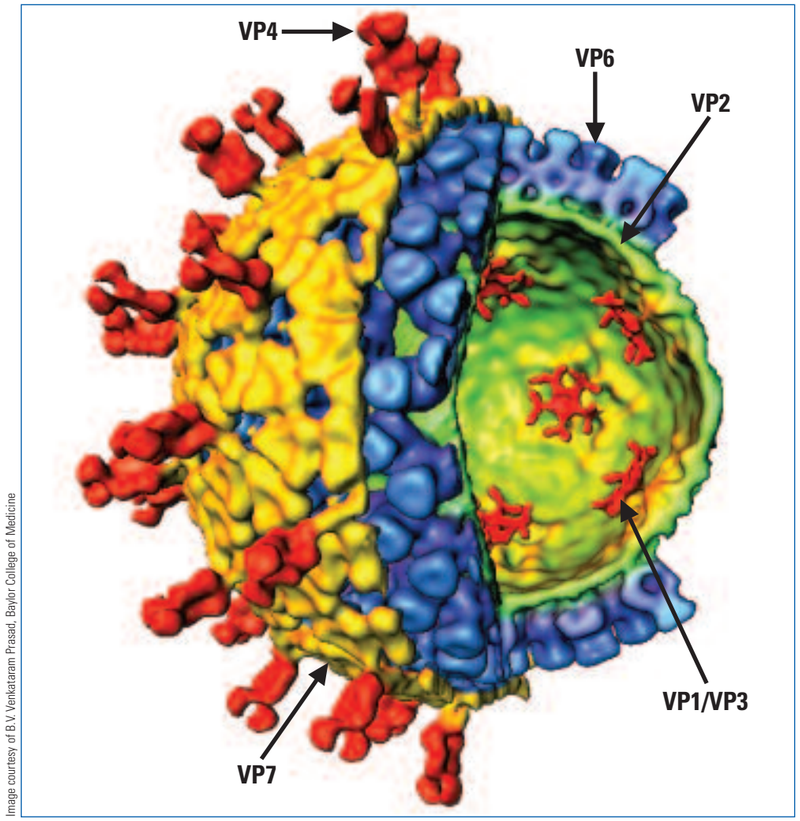

Image courtesy of B.V. Venkataram Prasad, Baylor College of Medicine

Figure 1. Reconstructed image of the rotavirus particle. *The major immune response in rotavirus infection is against the VP7 (yellow) and VP4 (red) proteins on the outer surface of rotavirus. Researchers are using the human rotavirus version of these proteins in a bovine or simian rotavirus background to construct the new vaccines, Rotateq and Rotarix.*

The virus targets the villi of the duodenal epithelium and directly infects the cells that form the lining between the inner cavity and tissues of the intestine. Rotavirus also encodes for a peptide (nonstructural protein 4) that opens chloride channels on the surface of uninfected cells, allowing it to further wreak havoc in the gut. These two mechanisms of action trigger a range of symptoms, from mild intestinal discomfort to prolonged episodes of diarrhea and vomiting, that together account for the often rapid and severe dehydration that can result from rotavirus infection.

Oral fluid replacement is the easiest way to reverse these effects but, if necessary, fluids and electrolytes can be administered intravenously. Severe cases, however, often require hospitalization and providing treatment to the 600,000 children in the US that seek medical care for rotavirus infection costs an estimated $1 billion a year. Only between 20 and 60 children die annually from rotavirus-induced dehydration in the US. In developing countries where clean water is often a rare commodity and prompt access to healthcare services is limited, failure to rehydrate rotavirus-infected infants and children results in a huge death toll, and around 1 in 200 children infected with rotavirus will die.

Even though rotavirus kills far more children in developing countries, the incidence is similar throughout the world. Alan Shaw, a researcher at Vaxinnate who worked previously on the development of Merck's rotavirus vaccine, calls rotavirus-induced gastroenteritis a "democratic disease" because it infects children regardless of socioeconomic status, water quality, or geographic location.

We don't really have a risk/benefit notion of safety in the US

Paul Offit

…children who are repeatedly infected with rotavirus develop a level of natural immunity that does not fully prevent subsequent infection but does reduce the risk of severe disease

Almost all infants have been infected at least once by the time they reach five years of age, making a vaccine the only hope for controlling the virus (*Emerg. Infect. Dis.* **9**, 565, 2003).

Clues from nature

Researchers originally set out to design vaccines that could prevent the establishment of rotavirus infection, but soon changed course when studies of natural infection showed that children who are repeatedly infected with rotavirus develop a level of natural immunity that does not fully prevent subsequent infection but does reduce the risk of severe disease. Each infection grants additional protection and after two episodes it becomes unlikely that an infant will experience severe gastroenteritis. "Efforts were then focused on developing a vaccine to mimic this effect," says Parashar.

The initial approaches to rotavirus vaccines followed the classic example of Edward Jenner's smallpox strategy. Nearly all vertebrates are infected by rotaviruses and the species barrier is substantial enough that animal viruses are safe for testing in humans. This approach, using simian and bovine rotaviruses, seemed immunogenic in initial testing but protection against rotavirus was inconsistent in larger trials and the vaccine was much less effective when tested in developing countries.

The next generation of rotavirus vaccines took advantage of rotavirus' segmented genome, which allows it to reassort during coinfection. These reassortant viruses can be selected to carry the VP4 and VP7 surface proteins of human rotavirus in a simian or bovine background, allowing the reassortant to stimulate antibody production without causing disease.

Two of these reassortant vaccine candidates based on the bovine rotavirus were developed, one at the US National Institutes of Health (NIH) and the other at Children's Hospital of Philadelphia (CHOP). A third vaccine candidate, based on a rhesus macaque rotavirus, was also developed at the NIH. This simian-based rotavirus strain, known as RRV-TV, was a tetravalent strain carrying human VP7 from three different serotypes (G1, G2, and G4) and the G3 of the parent rhesus rotavirus. This live, oral vaccine candidate looked promising in early studies and was later licensed to Wyeth for large-scale safety and efficacy studies. The vaccine, called Rotashield, eventually won approval from the US Food and Drug Administration (FDA) based on studies showing that three doses were highly efficacious in preventing severe cases of diarrhea and the resultant hospitalizations caused by rotavirus infection. Shortly after licensure in 1998 the Advisory Committee on Immunization Practices at the CDC recommended vaccination with Rotashield for all infants in the US, which secured the vaccine as part of the routine immunization schedule. At this time the product had not been tested and was not available in developing countries.

Then just nine months later physicians in the US were advised by the CDC to immediately suspend use of the vaccine after the adverse events reporting system turned up an unexpected number of cases of intussusception in infants that had received Rotashield. Intussusception is a potentially fatal bowel obstruction that happens when part of the small intestine folds over itself like a collapsing telescope. It occurs naturally in 1 of every 2000 infants and requires surgical treatment in approximately 10% of cases. If left untreated it can be fatal. There were enough hints of this rare but serious side effect in pre-licensure studies to warrant a warning in the package materials that accompanied the vaccine.

Closer analysis of vaccine recipients showed an association between receipt of the vaccine and development of intussusception, with most cases occurring within two weeks after the first vaccination. A case-control study by the CDC estimated that the intussusception risk for vaccinated infants was between 1 in 4500 and 1 in 9500 (*Vaccine* **24**, 3772, 2006). "That level of risk was not considered acceptable in the US," says Parashar. Wyeth soon withdrew Rotashield from the market and stopped manufacturing the vaccine.

Risk versus benefit

This ignited debate among scientists and bioethicists on the risk/benefit calculations for vaccines. "We don't really have a risk/benefit notion of safety in the US," says Paul Offit of CHOP. But as bioethicist Charles Weijer of Dalhousie University points out it is "imperialistic to transfer this standard of care to a country in which 1 in 200 children die of rotavirus infection," (*BMJ* **321**, 525, 2000).

Weijer calculated that even if 25% of the vaccine-induced intussusception cases proved fatal in developing countries, what he calls the worst-case scenario, it would cause 2000-3000 deaths per year, far fewer than the nearly 600,000 deaths caused worldwide each year by rotavirus-induced severe gastroenteritis. Many advocated that a vaccine that could save so many lives should still be introduced, even if it was possible that the vaccine itself would cause some deaths.

According to Parashar, all of the ethicists and most of the scientists supported testing Rotashield further in developing countries, but many representatives from these nations thought it would be politically difficult for them to promote a vaccine that was seen as unfit for infants in the US. "The risk/benefit analysis is useful and scientists understand it, but it would be difficult to explain to a layperson," adds Parashar.

Many researchers, including Parashar, now say that the biggest mistake with Rotashield—and one that resonates with AIDS vaccine researchers today—is that clinical trials in developing countries were not conducted in parallel with those in the US and Europe. "One of the challenges with this vaccine was that it hadn't already been tested in Africa and Asia," he says. Not knowing if the vaccine was even efficacious in these settings made it difficult for decision makers in developing countries to overlook the possible adverse effects.

Any discussions about testing Rotashield in developing countries soon became moot anyway because Wyeth ceased all production. The precise mechanism of Rotashield-related intussusception is still unknown, but many scientists credit it to the rapid replication of the rhesus rotavirus strain in the intestine. The peak replication for Rotashield corresponded with the occurrence of intussusception cases, says Heaton.

Further research led Lone Simonsen at the NIH to conclude that age was also a contributing factor. Infants that received the first immunization when they were older than three months, when natural cases of intussusception are more likely to occur, accounted for more than 80% of the intussusception cases reported with Rotashield. She contends that if the vaccine were only given to younger infants the relative risk of intussusception would have been greatly reduced and perhaps Rotashield would be available today (*J. Infect. Dis.* **192**, S36, 2005). But others, including the WHO, don't support the notion that age was a dominant factor because there were very few children in these studies younger than two months with which to compare intussusception rates. In a letter to the editor Simonsen warns that regulatory authorities should discourage "catch-up" immunizations—those given to infants that haven't received their first dose at the prescribed time—with the newer vaccines because they too may cause intussusception if given to older children (*N. Engl. J. Med.* **354**, 1748, 2006).

Small risk, huge trials

At the time the world received news about Rotashield, Merck was just preparing to take their lead rotavirus vaccine candidate, based on the bovine reassortant virus developed by Offit and colleagues at CHOP, into large-scale efficacy trials. Suddenly their plans changed dramatically. The Phase III trials needed to include 60,000-100,000 infants to successfully rule out the possibility of 1 in 10,000 vaccinees being at risk of intussusception. Both financially and organizationally, this would be a huge undertaking. However the company chose to move forward and began a placebo-controlled trial with their pentavalent rotavirus vaccine (Rotateq) in over 69,000 infants in 11 industrialized countries.

GSK was faced with a similar situation with their monovalent vaccine, known as Rotarix, based on an African green monkey/human reassortant strain initially developed at the Children's Hospital of Cincinnati, and they too pushed ahead with a trial involving 63,000 children in Finland and 11 countries in Latin America.

These trials were the largest, industry-sponsored vaccine trials ever conducted and both produced stellar results (*N. Engl. J. Med.* **354**, 23, 2006; *N. Engl. J. Med.* **354**, 11, 2006). Rotateq was effective at preventing 74% of any rotavirus-related gastroenteritis and 98% of severe cases, and also reduced the number of hospital visits for gastroenteritis by 86%. Immunization with Rotarix prevented 85% of severe gastroenteritis cases and associated hospitalizations and was 100% effective at reducing the most severe cases of the disease. Just as importantly, neither live-attenuated vaccine was associated with an increased risk of intussusception. "It was likely a Rotashield-specific issue," says Mark Feinberg, vice president of policy, public health, and medical affairs at Merck.

A few months after the final data were released, Merck received approval to license and market Rotateq in the US and GSK received licensure for Rotarix from the European Commission. Rotarix was already licensed in Mexico and has since also received licenses in Brazil, Philippines, and Singapore. Most recently the Advisory Committee on Immunization Practices at the CDC recommended Merck's rotavirus vaccine for all infants in the US.

These vaccines were developed without a good animal model and even after large stud-

The precise mechanism of Rotashield-related intussusception is still unknown, but many scientists credit it to the rapid replication of the rhesus rotavirus strain in the intestine

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Mark Feinberg

ies proved their efficacy, researchers have yet to identify the precise correlates of protection. This gives hope to AIDS vaccine researchers who are working under similar constraints. But Offit quickly explains that "rotavirus vaccines were much easier to make," yet it still took a quarter of a century of research and development to get two safe and effective products.

Rolling out vaccines

Before the WHO will recommend rotavirus vaccination for infants in developing countries who are at the greatest risk of contracting life-threatening gastroenteritis, the vaccines must be tested in these populations. Despite the experience with Rotashield neither manufacturer chose to run efficacy trials with the second-generation vaccines concurrently in both developed and developing countries. According to Feinberg, Merck decided that the 70,000-infant study would be run only in countries where they were confident all possible cases of intussusception could be detected and treated quickly. "Now that we know the vaccine is highly efficacious and well tolerated, we want to move forward as quickly as possible in resource-poor countries," he adds.

This is happening with the help of PATH, thanks to a large grant from GAVI, which is conducting efficacy trials in partnership with both Merck and GSK in several countries in Africa and Asia. PATH's goal is to reduce the delay between when vaccines are licensed and when they become available in developing countries. The lag time for implementing hepatitis B virus vaccine programs in some countries was around 10-15 years, but they are hopeful that for rotavirus vaccines they can reduce it to about five years.

The first step is communicating with decision makers in the 72 poorest countries of the world and explaining general information about rotavirus to prepare them for eventual introduction. "If we go to countries right now and say we want to talk about rotavirus, they say what's that?" says John Wecker, who works on the rotavirus program at PATH. These countries know they have a diarrheal disease but are unaware that rotavirus is the cause. "We want to provide a solid evidence base for developing country governments, and we have a long way to go," adds Wecker.

PATH also faces many other challenges. Wecker acknowledges that when governments are aware of rotavirus, there is some difficulty getting beyond the Rotashield data and explaining to governments that these are new vaccines. "One of the biggest things that rotavirus has taught us is that safety, or perceived safety, is paramount," says Shaw.

In the future PATH will also have to explain the intricacies that differentiate Rotateq from Rotashield so that representatives from developing countries can choose which vaccine to include in their immunization programs. Wecker says this decision will be based on the serotype coverage afforded by the vaccines as well as the dosing schedule. Rotateq includes more serotypes of rotavirus and should offer a greater breadth of protection, but requires three doses. Rotarix requires only two doses but must be dissolved in a buffer solution before it can be administered.

Researchers are torn over which vaccine may work better in developing countries. One reason used to explain the historically poorer response to live-attenuated vaccines in these settings is that infants are exposed to many more intestinal bacteria and viruses, making the gut a very busy place, says Offit. And in this crowded environment where many things are competing for the immune system's attention Offit isn't sure if the vaccine strain in Rotarix, which replicates much better than the highly-attenuated strain in Rotateq, will offer an advantage.

In the end the decision on which vaccine is adopted may come down to price. PATH is now holding consultations with the manufacturers on pricing and helping them to forecast the demand for the vaccines in developing countries (see *Cloudy with a chance of prevention: Demand forecasts and assessments*, *IAVI Report* **10**, 3, 2006). In the US, Merck's vaccine costs $180 for the three-dose course, making it one of the highest priced childhood immunizations. "The vaccine is priced commensurate with its public health value," says Feinberg, who also indicates that Merck is committed to making the vaccine available in developing countries at an affordable price.

Wecker is confident that financial subsidies offered through GAVI will also help poor countries. GAVI is also considering using advance market commitments (AMCs) that guarantee governments will buy a certain quantity of vaccine from a manufacturer at a negotiated price, but Wecker does not see this as a good model for rotavirus vaccines (see *If you build it, they will pay*, *IAVI Report* **9**, 2, 2005). "There's no reason, from our perspective, that GAVI shouldn't move forward today, and not think about rotavirus vaccines as something that is coming in the future," he argues. "They're here today."

Enrolling teens in trials

Researchers consider the special circumstances of AIDS vaccine clinical trials among adolescents

By Kristen Jill Kresge

As HIV continues to infect millions of people throughout the world, more and more of the newly infected are between the ages of 15 and 24. Young people in this age group now account for 40% of the 4.3 million new HIV infections that occurred globally in 2006. Despite these startling statistics, AIDS vaccines have so far not been tested in adolescent volunteers.

"The epidemic is becoming more youth-driven," says Linda-Gail Bekker of the Desmond Tutu HIV Centre in Cape Town, who is one of several researchers preparing for AIDS vaccine trials involving adolescents in South Africa. Studies show that despite increased efforts to reach adolescents with information about HIV prevention, young people in some communities are having sex and pursuing injection drug use at an earlier age. In the Russian Federation, where injection drug use is fueling the epidemic, 80% of the people with HIV are under 30 years of age. The greatest hope for slowing the spread of HIV is vaccinating young people before they begin engaging in activities that place them at potential risk of infection. But before a preventive AIDS vaccine can be administered to adolescents, researchers must show that it is both safe and effective in this age group, making clinical trials a necessity. This has researchers, vaccine trial sponsors, and regulatory agencies considering the obstacles for evaluating promising AIDS vaccine candidates in adolescent volunteers. "That's our big motivation," says Bekker.

Many organizations are currently working to develop guidelines and protocols that will specifically address the potentially thorny legal, ethical, and regulatory issues that are involved in the conduct of adolescent trials, including the need to protect younger volunteers from stigma and other social harms and ensuring that the concerns of parents are also adequately met. Progress in these areas will help guarantee that an effective AIDS vaccine, when available, will reach both adult and adolescent populations simultaneously and as quickly as possible, offering the greatest chance for curbing the pandemic. "I think we need to keep the pressure on," says Bekker. "As we move closer to more promising candidates, we don't want to be caught short."

An adolescent pandemic

The risk facing teenagers varies greatly from place to place. In the US, 40% of all new HIV infections are now occurring in individuals younger than 25. But the situation in sub-Saharan Africa is particularly gloomy. Two-thirds of all new HIV infections among adolescents are occurring there and young women in particular continue to be at a much greater risk of HIV infection than their male peers, due to both biological and social factors. In South Africa studies show that HIV prevalence rates approach 17% among girls age 15 to 24, four times the infection rates seen in boys of the same age. In Swaziland, a staggering 39% of young women in this age group are already HIV infected. And nearly a quarter of girls between the ages of 15 and 19 are now HIV infected in Botswana.

These trends clearly illuminate the need for introducing AIDS vaccines into these populations as quickly as possible and are helping to fuel discussions about how and when to test AIDS vaccine candidates in younger volunteers. "Everyone has been cautious about moving into adolescents with AIDS vaccines," says Michael Robertson, a lead investigator on Merck's Phase IIb AIDS vaccine trial that is being conducted through the HIV Vaccine Trials Network (HVTN). "But when you look at the epidemic in Africa, adolescents are the highest incidence group. And if you're going to make headway in dealing with the epidemic you need to involve them."

Protocol discussions

A few years ago little conversation was focused on adolescent trials but recently the discussions have gained momentum and now many groups, including the National Institute of Allergies and Infectious Diseases (NIAID) at the US National Institutes of Health (NIH), the US Food and Drug Administration (FDA), the World Health Organization (WHO), and the African AIDS Vaccine Program (AAVP) are preparing protocols and involving scientists, ethicists, and community groups in the process.

An important step in formulating these protocols is finding out what regulatory agencies will require to license a vaccine for use in adolescent populations. Most regulatory agencies, including the FDA, that oversee the approval and licensure of medicines and vaccines require that experimental products are tested in the population in which they will be used. Historically for vaccines this population has been infants, who are susceptible to many diseases that are typically contracted during early childhood and who are also at greatest risk of developing life-threatening symptoms because their immune systems haven't fully developed. Extensive childhood immunization programs have been implemented in many countries and have drastically reduced mortality rates.

But there is much less of a precedent for adolescent vaccination. A vaccine against hepatitis B virus (HBV) was the only one to target this age group until a vaccine for human papillomavirus (HPV) was recently licensed by the FDA for girls age 9 to 26 (see *Cervical cancer vaccines*, *IAVI Report* **9**, 5, 2005). Clinical trials to determine the safety and efficacy of the HBV vaccine were not conducted in adolescents until after the vaccine was approved and licensed for adults, delaying access to a critical group. It was only after the license was extended to younger people that the course of the HBV epidemic began to reverse. This offers a sobering lesson to AIDS vaccine researchers who want to ensure that a vaccine is available to adolescents and adults simultaneously.

The clinical trials, licensure, and eventual implementation of the HPV vaccine both in the US and in developing countries is also of great interest to those preparing for AIDS vaccine trials. "It's an excellent model for AIDS vaccines," says Jeffrey Safrit of the

…when you look at the epidemic in Africa, adolescents are the highest incidence group. And if you're going to make headway in dealing with the epidemic you need to involve them

Michael Robertson

Elizabeth Glaser Pediatric AIDS Foundation. The Phase III efficacy trials for Merck's HPV vaccine involved thousands of adolescent (age 12-18) and pre-adolescent girls and issues about informed consent and parental involvement, which will also be central to AIDS vaccine trials, were fully addressed for enrollment. "Many of these issues are the same ones we faced with our HPV program," says Robertson, who is using this experience to plan the company's strategy for evaluating its lead AIDS vaccine candidate in adolescent volunteers. Many researchers are also closely monitoring the acceptance of this new vaccine into immunization programs to help gauge the response to vaccines still in development that aim to prevent other sexually-transmitted infections, including HIV and herpes simplex virus type 2 (HSV-2).

Results from the HPV and HBV vaccine trials give researchers good reason to be optimistic that adolescents may respond even better to vaccination than adults. "From what we know, vaccines are generally safer in people with fewer health problems," says Jorge Flores, chief of vaccine clinical research at NIAID. There are also several physiological and immunological differences between infants, adolescents, and adults, and in clinical trials with both the HPV and HBV vaccines younger volunteers had stronger immune responses to the vaccine, which is encouraging to AIDS vaccine researchers. Fewer doses of the HBV vaccine are required to induce a similar immune response to adults and now researchers are closely studying different vaccination strategies, including response to bacterial and viral vectors, to see how age affects the induction of immune responses (*AIDS* **20**, 483, 2006).

Overall, scientists are optimistic that, if anything, a vaccine will be more effective in clinical trials involving adolescents. "The primary concern will be establishing safety data in these populations, rather than immunogenicity," says Robertson.

Key challenges

Before an AIDS vaccine trial begins enrolling adolescents many researchers, bioethicists, and international organizations are working to overcome some of the key challenges that are unique to adolescent trials. Chief among these is the need to obtain informed consent from the adolescent and their parent or guardian prior to enrollment. US and South African law both require that parental consent be provided for any trial involving minors where the vaccine isn't guaranteed to provide some benefit, and Bekker predicts that many parents may, at least initially, be reticent to allow their children to participate. A focus of any of these trials will therefore be on education and counseling for adolescents and their parents. "Once you give them the statistics, you can easily change people's perception," she says. "Parents are very aware that their children are in danger."

Trial protocols are currently being developed to protect these adolescent volunteers by tailoring the informed consent process and counseling sessions to specifically address their concerns, as well as those of their parents. But parental consent also requires striking a balance between involving parents and protecting the confidentiality and privacy of the volunteer. Adolescents may be uncomfortable disclosing their potential risk behaviors to a parent or guardian, so researchers will be given the task of making volunteers comfortable while ensuring that parents are informed about the trial. This may become even more complicated in efficacy trials where enrollment will likely hinge on the volunteer being at some risk of HIV infection either through sexual activity or drug use, says Audrey Smith Rogers, an epidemiologist at the National Institute of Child Health and Human Development, part of the NIH.

This raises legal and ethical issues about involving adolescents before they have reached the legal age for sexual consent, which varies from country to country. "The implication is that you're saying the age of consent isn't applicable," says Bekker. "I'm a bit squeamish about that, even though I've been a great protagonist." A possible solution to this dilemma is enrolling older adolescents who are over the age of sexual consent in efficacy trials and enrolling younger volunteers in smaller Phase I and II trials that deal primarily with safety and immunogenicity and don't require that participants are at high risk of infection.

Another concern in adolescent trials is the possibility that volunteers in AIDS vaccine trials may test positive on HIV antibody tests because an effective vaccine will elicit HIV-specific antibodies, which could compromise their enrollment in school, insurance coverage, or any other applications that require medical testing. Most clinical trials sites have already developed systems to handle these misunderstandings and researchers are confident that this will not be a significant barrier to adolescent enrollment.

There will be other considerations, including the social risks and stigma associated with adolescent involvement in an AIDS vaccine trial. Although these are issues central to all clinical trials, many of them are more complex or heightened in trials with younger volunteers. "Before we start a trial we need to understand as much as possible about the problems that could face adolescents who volunteer," says Flores. "Additional care must be put into the conduct of these trials."

Researchers must also be prepared to face obstacles with volunteer retention and reliability because younger people tend to be more mobile and may move away for school during the middle of a trial. Researchers remain committed to testing AIDS vaccine candidates in adolescents despite all of these confounding factors and are confident they can overcome many of the extra challenges. "I don't think these are insurmountable problems," says Rogers.

Involving expertise from outside the vaccine field is one way to facilitate these trials and many trial sponsors are already integrating organizations that are familiar with adolescent populations into the planning process. Community advisory boards will also be an important component since they can offer peer support that will help improve the experience of adolescent volunteers. "My take has always been that this can be done, but it can't be done by everyone," says Bekker. "You have to have groups that are used to working with adolescents."

Trial planning

As the planning process for adolescent AIDS vaccine trials gets underway, researchers are looking to regulatory and legal authorities for recommendations on how to proceed. In response to a bill from the US Congress requiring the FDA to advise both industry and other AIDS vaccine trial sponsors, the agency issued a guidance document in May 2006 (Development of Preventive HIV Vaccines for Use in Pediatric Populations, www.fda.gov/cber/guidelines.htm) that provided general direction on their requirements for licensure in the US.

"The big question is when is the best time to bring a vaccine into trials with adolescents," says Flores. "Some people are more conservative and say not to test a vaccine in adolescents until you know it's effective. But this doesn't take into account the urgency," he adds. The guidance document issued by the FDA suggested that strong safety and immunogenicity data for AIDS vaccine candidates should be collected in adults before adolescent trials begin. Flores suggests that when a sponsor is considering taking a vaccine candidate into efficacy trials, Phase IIb or III, they should also be preparing for adolescent trials.

The FDA document also emphasized that efficacy data collected in adults could only be extrapolated to adolescents if researchers could successfully identify the immune correlates of protection, but this can often be difficult even with highly effective vaccines. For both HPV and rotavirus vaccines (see *Rotavirus vaccines rolled out*, page 1) correlates of protection have not been identified even after very large Phase III efficacy trials.

For AIDS vaccine candidates it may therefore be necessary to run large efficacy trials in adolescents. It is unlikely that these can be done exclusively in the US since HIV incidence rates there are generally, outside of certain urban centers, too low among adolescents to support a conclusive Phase III trial, says Rogers. If efficacy trials are conducted primarily outside of the US, the FDA recommends that trial sponsors submit their plans to the agency for review and comment to ensure that this data will be applicable to adolescent approval within the US.

Other regulatory agencies are also involved, including in South Africa where researchers are leading the charge for adolescent trials due to the especially high prevalence of HIV infection in young people there. The South African AIDS Vaccine Initiative (SAAVI) is currently collaborating with the HVTN to prepare an adolescent trial protocol. The WHO and the AAVP also sponsored a meeting earlier this year in Gaborone, Botswana, to address some of the challenges related to including adolescent volunteers in AIDS vaccine trials. Flores is in the process of preparing a document on adolescent trials for the NIH which he hopes will inspire discussion among researchers on the critical issues.

And now Merck is considering testing its lead vaccine candidate in adolescents in South Africa as part of a Phase IIb trial that will start there soon in cooperation with the NIH and the HVTN. "The plans are very much in the discussion phase," says Robertson. "We've discussed expanding the planned trial and amending the age cutoff to include ado-

Some people are more conservative and say not to test a vaccine in adolescents until you know it's effective. But this doesn't take into account the urgency

Jorge Flores

The need to protect this vulnerable group from stigma and other social harms is still a substantial concern for researchers who are actively discussing the possibility of such trials in the near future

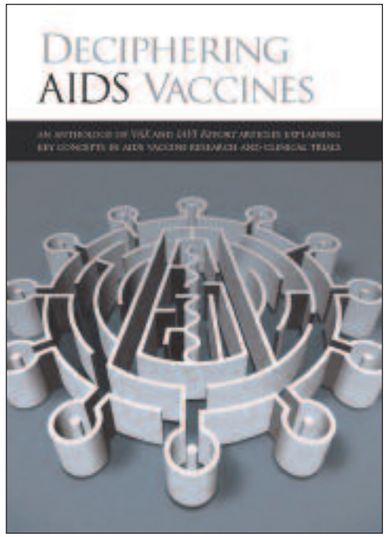
lescents, or adding another small safety and immunogenicity trial there just for adolescents."

Merck's candidate induces primarily cellular immune responses and some researchers predict this type of vaccine will not be able to prevent infection but rather slow disease progression in those who later become HIV infected. "That adds an additional level of complication," says Flores, especially for adolescents. "We're hard pressed to define what success is for this type of vaccine," and he thinks this may negatively influence how both adolescent volunteers and their parents view the trial.

Researchers are encouraged by preliminary research that indicates many adolescents are eager to participate in AIDS vaccine research. Results from a feasibility study conducted by Bekker in South Africa indicate that 53% of 256 adolescents (age 11-19) were willing to participate in a trial. However the most common reason given for participation was the perception that it would offer them protection from HIV infection. This raises the concern of behavioral disinhibition in trials, where volunteers feel a false sense of protection from a vaccine candidate that hasn't yet proven effective and as a result they may continue or increase behaviors that elevate their risk of HIV infection. Disinhibition is an important consideration in any prevention trial, but may be even more critical for adolescents. "It's a valid concern but I don't know that there's data out there to support it," says Bekker.

But the need to protect this vulnerable group from stigma and other social harms is still a substantial concern for researchers who are actively discussing the possibility of such trials in the near future. "We will have adolescents in AIDS vaccine trials within the next three years," says Flores. "And once the first trial is done, it will pave the way for all future trials."

Introducing the new VAX anthology



Deciphering AIDS Vaccines features articles originally published in *VAX* and *IAVI Report*, the only comprehensive publications on the AIDS vaccine field.

This anthology is intended to serve as a general introduction for non-scientists to AIDS vaccines, to educate and inform, to be used by trial sites, volunteers, educators, libraries and anyone else as a vaccine literacy tool.

The articles have been carefully selected to include information regarding all aspects of the AIDS vaccine field and to help the reader understand more about the science of AIDS vaccines and the clinical trials process, as well as alternate HIV prevention strategies and other vaccines that may provide lessons for the AIDS vaccine field. For more information, go to www.iavireport.org.

If you would like to receive one or more copies of the anthology, free of charge, please send your request to iavireport@iavi.org.

Vaccine Briefs

IAVI opens southern Africa regional office

IAVI recently launched a new program in Johannesburg, South Africa, to support expanding AIDS vaccine research, development, and advocacy efforts for southern Africa. The global public-private partnership already operates several regional offices worldwide in Nairobi, Kenya; New Delhi, India; Amsterdam, the Netherlands; and New York City where the global headquarters is located. The Johannesburg offices will provide an opportunity for IAVI to work closely with existing partners and programs in southern Africa, including the South African AIDS Vaccine Initiative (SAAVI), the Medical Research Council (MRC), the Desmond Tutu HIV Foundation in Cape Town, the Medical University of South Africa, the Zambia-Emory HIV Research Project, the University of Limpopo, and the Perinatal HIV Research Unit at the University of Witwatersrand.

Seth Berkley, Chief Executive Officer of IAVI, said that the new regional office will serve as a focal point for expanding AIDS vaccine programs and activities in southern Africa and will lead the organization's efforts to build capacity to conduct clinical trials in the region to the highest scientific and ethical standards. In an editorial published in South Africa's *Business Day*, Berkley also said the southern Africa program will take advantage of the region's "growing biomedical capabilities, strong regulatory systems and manufacturing base." South Africa is collaborating with India and Brazil, two other countries severely-affected by HIV/AIDS, to harness the power of their growing biotechnology sectors for the discovery of new vaccines.

South Africa is already hosting several HIV prevention studies, including a large Phase III microbicide trial and multiple AIDS vaccine trials. IAVI initiated a Phase II AIDS vaccine trial last year in South Africa with several partner organizations to evaluate the safety and immunogenicity of an adeno-associated virus vaccine candidate known as tgAAC09 that encodes clade C HIV genes, which is the primary subtype of the virus circulating in the region (see http://www.iavireport.org/trialsdb/ for more information). The Vaccine Research Center at the US National Institutes of Health, in collaboration with the HIV Vaccine Trials Network (HVTN), is also conducting a Phase II trial in South Africa with their DNA and adenovirus serotype-5 vaccine candidates. Merck and the HVTN will begin a Phase IIb AIDS vaccine trial there later this year with their lead adenovirus-based AIDS vaccine candidate.

Nasal administration of AIDS vaccine candidate

A Phase I study of an HIV protein-based vaccine was initiated in the UK in September by researchers from St. George's Vaccine Institute at the University of London in collaboration with Novartis Vaccines, Richmond Pharmacology Ltd., and the Commission of the European Union. The vaccine candidate is comprised of HIV gp140 protein with the V2 loop deleted, and is being delivered nasally along with LTK63, a heat-labile enterotoxin from *Escherichia coli* that has been shown to enhance immune responses at mucosal surfaces.

The trial will enroll 30 volunteers who will be randomized to receive either 3 nasal immunizations of the vaccine candidate and adjuvant, followed by 2 additional immunizations with the same protein vaccine administered intramuscularly, or placebo. The booster immunizations will be administered along with a liquid adjuvant known as MF59.

All volunteers will be followed for 32 weeks during which time researchers will evaluate the safety of this dosing regimen and collect preliminary information on the immunogenicity of both the vaccine candidate and route of administration. Nasal administration generally induces stronger mucosal immune responses than intramuscular injection, which are widely considered to be a necessary response for a vaccine that could prevent sexual transmission of HIV. In the study investigators will be measuring the serum IgG neutralizing antibody responses to gp140 at several intervals as well as the IgA responses in both nasal and vaginal tissues to determine the frequency and type of immune responses induced at these surfaces.

IAVI Report

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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 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 John D. Evans Foundation, The New York Community Trust, The Rockefeller Foundation, The Starr Foundation; the Governments of Canada, Denmark, Ireland, The Netherlands, Norway, 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.), Continental Airlines, Google Inc., Merck & Co., Inc. and Pfizer Inc; leading AIDS charities such as Broadway Cares/Equity Fights AIDS, Crusaid, Deutsche AIDS-Stiftung, 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

Phase I AIDS vaccine trial in infants begins in Uganda

Researchers at Makerere University in Kampala, Uganda and Johns Hopkins University in the US recently initiated the first Phase I trial of an AIDS vaccine aimed at preventing the transmission of HIV from mother-to-child during breastfeeding. According to the World Health Organization, breastfeeding remains one of the major routes of HIV transmission to infants in developing countries; it is estimated that as many as a half of all HIV-infected infants acquire the virus not during delivery but through HIV-contaminated breast milk. Alternatives to breastfeeding, such as liquid formula or powdered milk, could easily prevent these infections, but in many settings these options are either prohibitively expensive or impractical because they require access to clean water. Also, HIV-infected women who do not breastfeed their babies are subjected to stigma in many cultures where it is common practice.

Another option for preventing HIV transmission from breastfeeding is administering antiretrovirals (ARVs) to the mother. Several studies have shown that treating HIV-infected women with ARVs throughout late pregnancy, labor, and during the period they are breastfeeding is a highly effective way to prevent HIV transmission to infants (see *New strides in protecting infants from HIV*, *IAVI Report* **9**, 2, 2005). However, not all women have access to these drugs so a vaccine that could effectively protect babies during the period they are breast fed would be a major advance. To date only one vaccine trial has been conducted in infants.

This new randomized, placebo-controlled trial is being conducted through the HIV Prevention Trials Network (HPTN) and will enroll 50 infants born to HIV-infected mothers at Mulago Hospital in Kampala to evaluate the safety of a live-attenuated, recombinant canarypox virus vaccine candidate encoding HIV Env proteins from clades B and E. Forty of the infants will receive four doses of the vaccine candidate over three months and will be followed by researchers for two and a half years. The vaccine candidate, known as ALVAC-HIV vCP1521, was developed by Sanofi Pasteur and was previously evaluated in a safety trial in Uganda involving adult volunteers and in another study involving infants in the US. No serious safety issues were reported in either of these completed trials.

ALVAC vCP1521 is also now being tested in a Phase III efficacy trial in Thailand to see if it can protect adults against HIV infection. The Thai trial recently completed enrolling volunteers but final efficacy data will not be available for a few years. For more information on these and other ongoing AIDS vaccine trials, visit the *IAVI Report* vaccine trials database at www.iavireport.org/trialsdb.

New global vaccine conference to accompany annual Grand Challenges for Global Health meeting

Grant recipients through the Grand Challenges in Global Health Initiative, a US$436.6 million program funded by the Bill & Melinda Gates Foundation to increase research on diseases that primarily affect developing countries, recently convened their annual meeting in Washington, DC to highlight progress on the 48 ongoing projects. Grantees include scientists from 33 countries who are working to tackle either scientific or technological challenges that could enhance global public health (see www.gcgh.org for more information). Plans for this innovative funding mechanism were initially announced at the World Economic Forum in 2003 and the first round of grants were awarded last year in collaboration with the US National Institutes of Health (NIH).

The Gates Foundation also recently awarded the Keystone Symposia on Molecular and Cellular Biology, a US non-profit organization that hosts many high-profile scientific conferences, a three-year grant of $2.6 million to further expand their offerings of meetings that focus on global health. Keystone already sponsors several conferences concerning infectious diseases, including the annual symposia on HIV Pathogenesis and HIV Vaccines that are held in conjunction each spring (see www.keystonesymposia.org). *IAVI Report* Travel Awards will be provided to scientists from developing countries to attend this meeting in 2007.

With the new funding from the Gates Foundation, Keystone will sponsor an additional meeting on vaccines, called "Challenges of Global Vaccine Development," which will be held either immediately before or after the next Grand Challenges in Global Health Meeting. The first annual conference will take place from October 8-13, 2007 in Cape Town, South Africa and will involve 300 scientists, many of whom are investigators on one of the Grand Challenges projects. The Keystone Symposia will also use part of this grant to provide scholarships and travel awards to researchers from developing countries, and specifically to graduate students and post-doctoral fellows who are completing their studies in Africa.

Vaccine Briefs *written by Kristen Jill Kresge*