the vaccine maker

By William Wells

ILLUSTRATION BY PETER SIS

Harry Greenberg helped create a vaccine that the FDA and public health officials love. But could it be made even better?

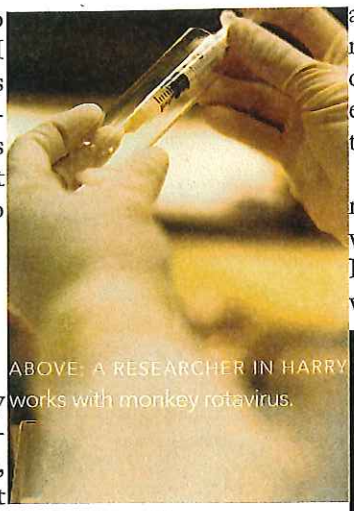
THE REGULATORY AUTHORITIES HAVE APPROVED IT, THE PUBLIC HEALTH COMMITTEES HAVE ENDORSED IT, AND NOW THE ROTAVIRUS VACCINE IS READY TO ROLL — READY TO BE ADMINISTERED TO EVERY ONE OF THE FOUR MILLION CHILDREN BORN IN THE UNITED STATES EVERY YEAR.

Without the vaccine, virtually all of those children would become infected with rotavirus within their first five years of life. As the most common cause of severe childhood diarrhea, rotavirus kills almost one million children worldwide every year. In the United States, that figure is closer to just 20 deaths — a result of simple health care measures such as oral rehydration. But there is still a monetary cost in the United States: The half million visits to physicians and the 50,000 hospitalizations cost more than a half billion dollars. Adding the indirect costs, that figure rises to one billion dollars.

That's quite a problem to tackle, says senior associate dean for research Harry Greenberg, MD, a Stanford professor of medicine who helped develop the vaccine — though he's quick to emphasize that he was only one part of a large team. "I'm a piece but in no way a keystone," he says. "Making a vaccine is a big enterprise."

Greenberg, who also serves as the associate chair of gastroenterology at the Veterans Affairs Palo Alto Health Care System, is fully aware of the enormity of a new universal vaccine. "It boggles my mind that I am part of some-

thing that will now be given to four million children," he says. "I am lucky to have been part of this story. Very few people get an opportunity to get their fingerprints on something that will help a lot of people. It's not very likely, no matter who you are."



ABOVE: A RESEARCHER IN HARRY GREENBERG'S lab works with monkey rotavirus.

The rotavirus vaccine almost died before it was born.

It takes a serious disease to justify the costly process of vaccine development. And at first glance, rotavirus appeared to be a perfect candidate for a vaccine, as it is a disease that affects everyone. "It is an egalitarian pathogen — it strikes with equal frequency at the children in the slums of Bangladesh and the hills of Palo Alto," says Greenberg. "Rotavirus spreads so efficiently that flushing toilets and hand washing don't make any difference."

But in the United States, rotavirus infection was so common, and yet so rarely fatal, that it was not a reportable disease. Therefore the data on its impact was limited.

The virus is transmitted primarily by fecal–oral contamination, although there may be some transmission via tiny droplets in the air, as with the cold virus. As few as 100 of the virus particles can start an infection, and children frequently excrete several billion particles. Each particle consists only of some strands of genetic material wrapped tightly in three protein coats. Once the virus is ingested, it enters cells lining the gut, makes copies of its genetic material, creates more of the proteins that fashion its coats, kills the intestinal cells and triggers the gut to release large amounts of fluid. The end result is a baby with diarrhea.

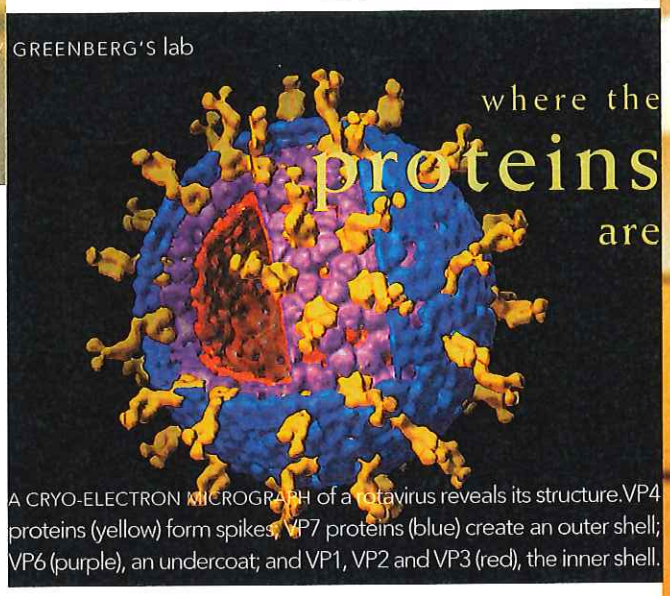
The first major study of rotavirus' impact, published in 1985 by the Institute of Medicine, reported that the diarrhea was being controlled by improved health care in the United States. "The Institute of Medicine study found that rotavirus vaccination was very important for the less developed world, but not a high priority for the developed world," says Greenberg. "That was not good, especially if you need corporate sponsorship to speed vaccine development. By and large the major pharmaceutical companies are most interested in Western diseases."

Enter Roger Glass, MD, PhD, an epidemiologist at the Centers for Disease Control (CDC) in Atlanta, Georgia. "Roger Glass, an intellectual champion, used countrywide epidemiological data to demonstrate that rotavirus was costing half a billion to one billion dollars per year in the United States," says Greenberg. "This is a substantial health care cost. Had it not been for Roger Glass there would be no rotavirus vaccine, because people said there was no need for a vaccine in the U.S."

With an economic justification for a vaccine for the West, the National Institutes of Health team working on vaccine development hoped that the same vaccine could be distributed to the developing world, where it was needed to save lives.

Albert Kapikian, MD, led the vaccine team, as part of the Laboratory of Infectious Diseases (LID) at the National Institute of Allergy and Infectious Diseases (NIAID). The team built on the ideas of Edward Jenner: Infection with an animal virus (in Jenner's case, cowpox) might be non-lethal but protective against the human virus (smallpox). There were signs that the rotavirus version of the experiment was already under way. Children who lived in rural areas where they might get frequently exposed to animal rotaviruses such as porcine or bovine rotavirus, did not appear to become ill from these strains.

The initial "Jennerian" vaccine trials using simian or bovine rotavirus in humans had mixed, but promising, results. The vaccines were definitely protective, but only for some patients. It appeared that one factor determining the early "Jennerian" vaccine's success was the serotype, or "flavor" of the infecting human virus. Human rotaviruses come in four major flavors; each has a different version of the VP7 protein on its surface. Perhaps, thought the LID team, all four versions would need to be in the vaccine to protect against any subsequent human infections. The monkey rotavirus already had a VP7 that was a close match to one of the human versions — and unlike the human rotavirus, researchers knew how to get the monkey rotavirus to grow in the laboratory. So, it seemed that the next step should be to produce monkey rotaviruses with the three other human VP7s.



A CRYO-ELECTRON MICROGRAPH of a rotavirus reveals its structure. VP4 proteins (yellow) form spikes; VP7 proteins (blue) create an outer shell; VP6 (purple), an undercoat; and VP1, VP2 and VP3 (red), the inner shell.

It was Harry Greenberg's job to create these three other viruses — each fundamentally a monkey virus, but with the monkey VP7 replaced by one of the three other human VP7s.

Greenberg already had a start on making these chimeric, or reassortment viruses. In previous work, he had been experimenting with rotavirus, playing with its natural tendency to trade one or more of its 11 pieces of genetic material with its neighbors. He discovered that when two rotaviruses reproduce in the same cell, a mixture of the genetic material from each often ends up packaged into a single virus particle.

So, to make the vaccine, Greenberg infected lab-grown monkey kidney cells with human and monkey viruses. To these cells he added specific antibodies to prevent any viruses with monkey VP7 genes from reproducing. Since the human viruses could not reproduce by themselves, and the antibodies would stymie reproduction of viruses bearing monkey VP7 genes, the only viruses that would emerge were monkey viruses with human VP7 genes. Greenberg saw that he could make the desired four-part, or quadrivalent, live vaccine by adding three of these "reassorted" viruses, each with a different human VP7, to the original monkey virus.

PHOTOGRAPHY: DAVID TSAY/JOHN PENG
MICROGRAPHY: MIKE PIQUE/MARK YEAGER

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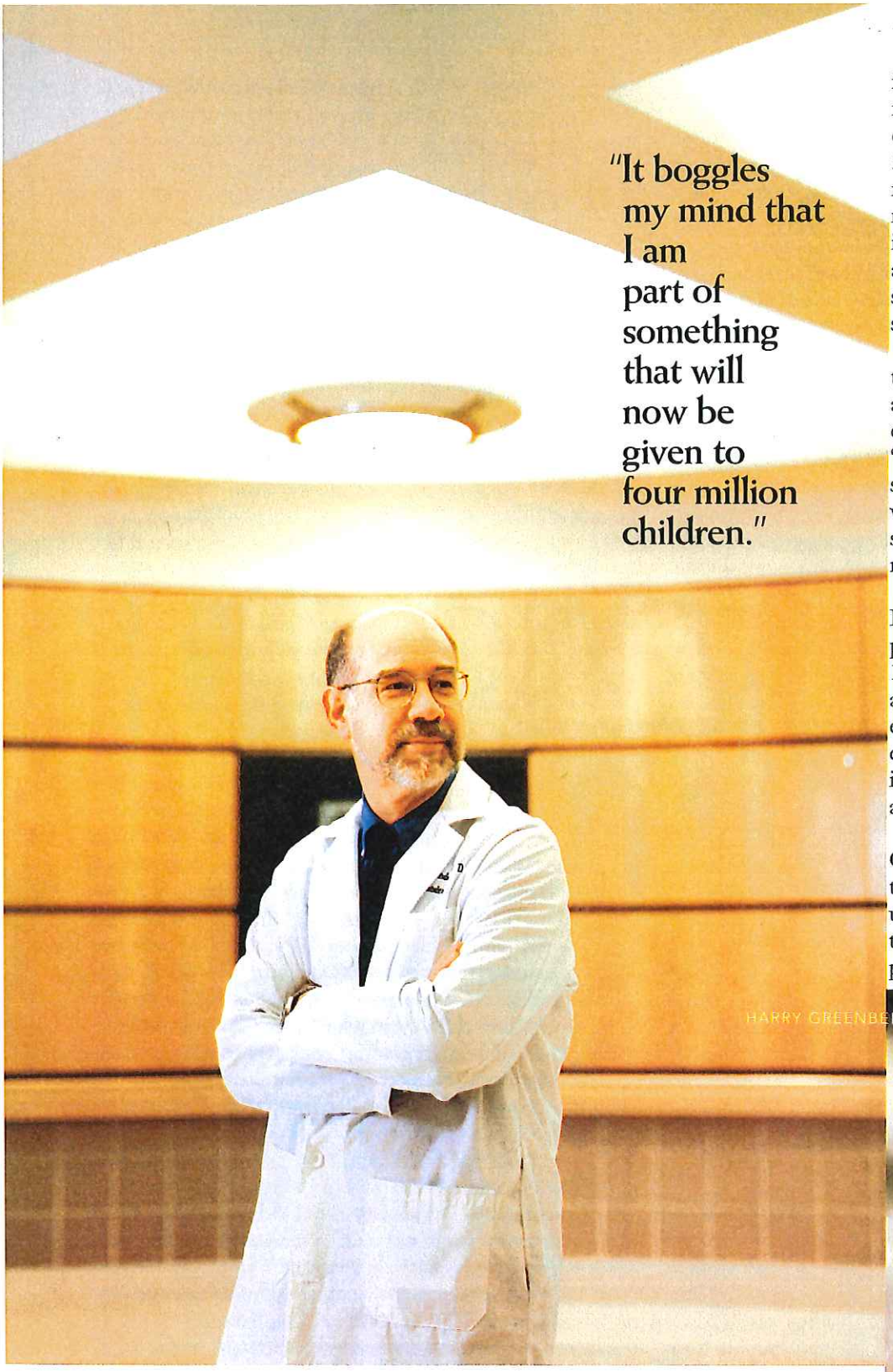
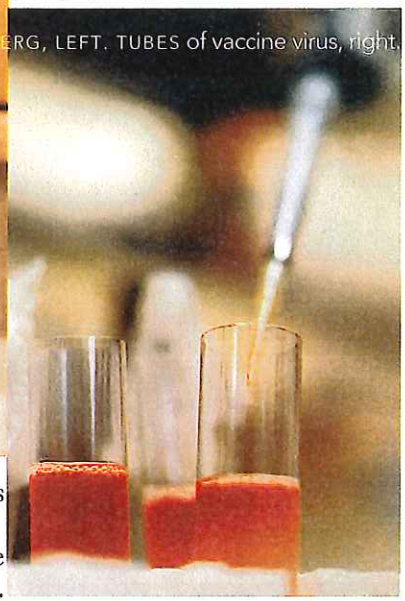


HARRY GREENBERG, LEFT. TUBES of vaccine virus, right.



Greenberg helped patent these vaccines and then left the NIAID for Stanford, and it was Kapikian and colleagues at the NIH and Wyeth who coordinated the testing of the "quadrivalent" vaccine in children. It has been a long road.

"The viruses were 'in the can' in the mid-80s, and it took over a decade to prove that the "Jennerian" concept worked in children," says Greenberg. "These strains have just been licensed in 1998, and nobody sat on their butts from 1983 to 1998."

Some of that time was spent working out what the vaccine should do. "You need to think through what you want from a medication before you design a trial," says Greenberg. "The initial aim was to stop all rotavirus infection. When you test for that, you don't get a great result: approximately 50 percent efficacy. But then people realized: Is preventing all diarrhea our most important goal, or is our most important goal keeping people alive and out of hospitals and the health care system? From a cost and morbidity standpoint, that was the big issue."

In four trials in various countries the quadrivalent vaccine was deemed a great success: It was 70–95 percent effective at preventing severe diarrhea. "Natural infection doesn't prevent all subsequent infections, but does prevent subsequent severe infection," says Greenberg. "The vaccine is almost as good."

Based on these results, the U.S. Food and Drug Administration approved the vaccine on August 31, 1998. The vaccine is called RotaShield and is manufactured by Wyeth-Ayerst Laboratories (Philadelphia). It became available in September 1998 and is given with other standard vaccines at two, four and six months of age.

Subsequently, the CDC's Advisory Committee on Immunization Practices (ACIP) recommended universal use of the vaccine. "So the utility of this vaccine should become apparent pretty quickly, since most children are likely to receive it," says Greenberg.

At $38 per dose the vaccine is not cheap. Health care economists have estimated that the cost of vaccination will exceed the cost of the hospital visits that will no longer occur. But when costs such as lost productivity of parents is taken into account, there should be substantial savings from the vaccination program.

In developing countries a price of $38 is prohibitive. "Even vaccines that cost pennies are difficult to afford for many de-

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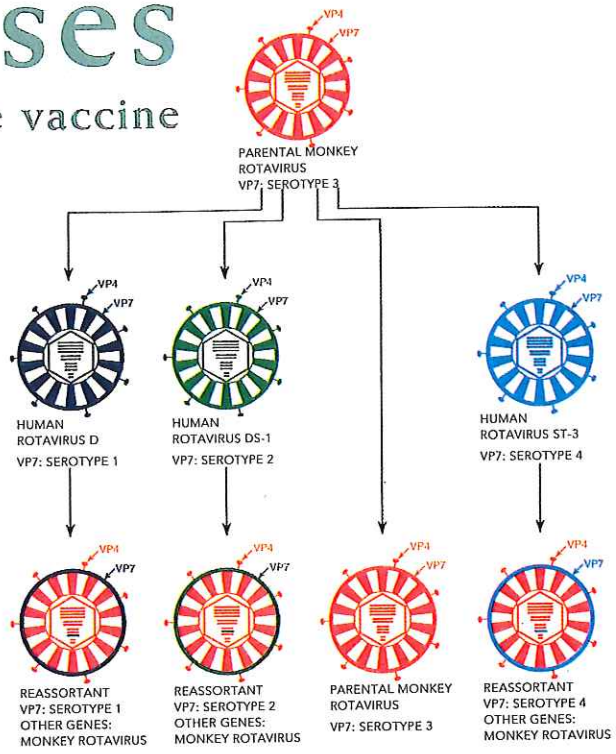
veloping countries," says Greenberg. One strategy is to charge high prices in the West to subsidize cheap or free distribution in developing countries.

But the control of this process is in the hands of the pharmaceutical company, and there is no guarantee that the vaccine will work as well in the poorer countries. One of the large trials of RotaShield was in Venezuela, at least in part to test whether the vaccine would work in a poorer country. The vaccine was highly effective in Venezuela. "Venezuela is not Park Avenue, but it is also not Zimbabwe or Bangladesh," says Greenberg. "As the socioeconomic level starts decreasing, many vaccines become less effective. Maybe the children's exposure is ten times higher, or they are malnourished. It's a higher hump for a vaccine to overcome. So it is still not clear how well RotaShield will work in many less developed countries."



THE ROTAVIRUS VACCINE consists of four viruses (diagramed in the bottom row, above) representing the four most common human rotavirus serotypes. Three of these viruses are reassortants. The variously colored knobs on the surface of each virus represent VP4 variants; the colored rings, VP7variants.

One approach for solving this problem is to get countries involved in producing their own vaccines. A potential vaccine for use in India was first isolated from an Indian nursery for newborns. Greenberg and his Indian collaborators had observed a drop in symptomatic rotavirus infection in Bangalore from 45.3% in 1988 to 1.8% during 1994, even as disease rates stayed high in nearby Delhi. This drop appeared to coincide with the appearance of the nursery strain in Bangalore. The virus in Bangalore was still infectious, but it did not appear to be causing disease; in fact it was acting like a natural vaccine, protecting children from later disease outbreaks caused by other virulent rotavirus strains.

The research teams found that the new virus was a natural reassortment virus, with eight genes apparently coming from a cow rotavirus and three genes from a human rotavirus. This genetic mixture may account for the virus' lack of virulence. This virus and a similar virus isolated by an Indian/CDC collaborative team are currently in safety tests in the United States, before being handed over to the Indian government for efficacy trials.

RotaShield is not the ultimate rotavirus vaccine for the United States. For safety reasons, dead vaccines are generally preferable to live vaccines. Various companies are looking at using just the protein coat (without genetic material the vaccine cannot reproduce and cause illness), a single protein, the DNA that codes for a single protein, or an edible vaccine, in which a plant produces a virus protein or even an entire protein coat.

The new vaccine designs are being influenced by all the basic research on rotavirus in the last 15 years, not the least of which is Greenberg's work. At NIAID Greenberg focused on VP7, because most antibodies in the blood that specifically recognized rotavirus were attaching to VP7 on rotavirus' surface. But Greenberg's more recent work has shown that antibodies in the blood may be irrelevant. Protection from rotavirus infection best correlates with the presence of special IgA antibodies. These antibodies are transported from the blood, through intestinal cells, and into the gut. There they can meet rotavirus and put it out of action before it does any damage.

Greenberg found that most of the IgA antibodies attach to VP4, the other protein that makes up the outer coat of rotavirus. So should VP4 be the key target for a vaccine? Merck and Co. Inc. (Whitehouse Station, N.J.) thinks this is possible: Whereas RotaShield has monkey VP4, the new candidate Merck vaccine has human VP4. Unfortunately the trials of this vaccine — which are in progress — may not tell us whether VP4 is the key. "Merck and Wyeth won't do a head-to-head comparison, so it will be hard to sort it out," says Greenberg.

If VP4 is the most important protein, then the number of different versions of VP7 could be irrelevant. Was the reassortment process to get four different VP7s necessary? "That was the best assumption at the time," says Greenberg. "The monovalent vaccine worked once or twice, but did not on several other occasions, so it was assumed that the quadrivalent vaccine was better. It is still not clear to me how much benefit is being gained by the quadrivalent vaccine."

"If I could do it over again I would spend more time comparing monovalent and quadrivalent vaccines," he says. "Once the train got moving with the quadrivalent, there was no going back."

THINGS GOT EVEN MORE COMPLICATED WHEN GREENBERG PUBLISHED A 1996 PAPER REVEALING A SURPRISING WEAPON USED TO FIGHT ROTAVIRUS: IgA ANTIBODIES THAT ATTACH NOT TO VP4 BUT TO VP6. Sure, there were plenty of VP4 IgA antibodies, but in the paper (published in *Science*) Greenberg showed that even if he loaded mice with these antibodies it would not prevent infection. It was VP6 antibodies that did the trick.

This was heresy. VP6 is part of the inner coat of rotavirus. Shielded by VP4 and VP7, it should be inaccessible to antibod-

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DIAGRAM: HARRY GREENBERG

THREE WORLDS COLLIDE

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works closely with other neuroradiologists, neurosurgeons, and neurologists at a hospital that is a major trauma center for the San Francisco Bay Area and one of the most respected trauma centers in the world.

Many of the patients at SFGH cannot afford medical insurance. "You see dramatic diseases that haven't been diagnosed," says Gean. "You're starting from scratch, so it's pretty challenging and you're able to make a difference in someone's life. Our team feels tremendous satisfaction."

"I wish the community knew that it's getting some of the best minds in the country," she says. "This is the county hospital, so there's little fancy wallpaper and few healthy plants in this place. But there's great medical care. I wouldn't want to work anywhere else."

The calls from lawyers take her from that work, and they are more frequent since the Boston trial. Fortunately, almost all cases settle before trial. Gean sticks to her original policy: Any opinion is based on the brain scans, without initial knowledge either of the clinical facts or of whether the requester is a prosecutor or defense attorney. That is the tactic that landed her in front of Martha Coakley in a Boston courtroom, and she does not relish the same thing happening again. "I'm still kind of licking my wounds from the first nine rounds," she says. But Gean is not about to become a lawyer-shunning hermit. "It would be a shame," she says, "to let the Martha Coakleys submerge my desire to make a difference." **SMD**

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ies. But Greenberg showed that the VP6 antibodies appeared to be latching onto rotavirus inside gut cells. Rotavirus sheds its outer coat as it enters cells, so inside the cells its VP6 was exposed to attack.

What all of this means for vaccine development is far from clear. RotaShield has monkey VP6 and yet it apparently works quite well. However, all VP6 proteins from all types of rotaviruses are very similar so that the monkey VP6 is likely to induce immunity to human strains. "The VP6 findings have caused me some trouble since they are not typical of how we think protective immunity works," says Greenberg. "They are true, and the other lines of data support these observations." Fitting them into the world of vaccines in humans is another matter.

The point is moot for RotaShield. By the time the RotaShield trial results came in, the basic science had leapfrogged ahead of the knowledge used to develop the vaccine. But it seems that the early, educated guesses somehow worked out. Perhaps the monkey VP4 or VP6 are just similar enough to the human versions to work, or maybe the mouse model of rotavirus infection doesn't exactly reflect the human situation. Further experiments should sort out those issues, but in the meantime there is an effective vaccine ready to be used. As Greenberg says, "It's a situation of 'if it's not broken don't fix it.'"

Still, Greenberg feels compelled to continue his research into rotavirus. "First, we have a chance to improve the vaccine," he says. "It works well for severe disease — it's 70 to 90 percent effective in the most severe disease. But it's less effective at protecting against mild disease."

"Second, rotavirus infection is a terrific model system to understand mucosal immunity in general," he says. Almost every animal has its own version of rotavirus, so there are plenty of variants and model systems to work with. And all those variants appear to infect through a mucosal surface, in this case, the digestive tract. Mucosal surfaces are a common site of attack for many viruses, such as HIV, and bacteria, such as cholera. If Greenberg has his way, the rotavirus vaccine could be just the beginning. Eventually the rotavirus vaccine might be used as a carrier system to deliver other vaccines to the GI tract as well. **SM**