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"New Hope for Defeating Rotavirus". Scientific American, Roger I. Glass, MD, PhD, April 2006.

NEW HOPE FOR DEFEATING ROTAVIRUS

Although its name is unfamiliar to many, rotavirus is the leading cause of severe childhood diarrhea worldwide and a frequent killer of young children in developing nations. Now—after 30 years of investigation—vaccines that may well conquer it are ready for market

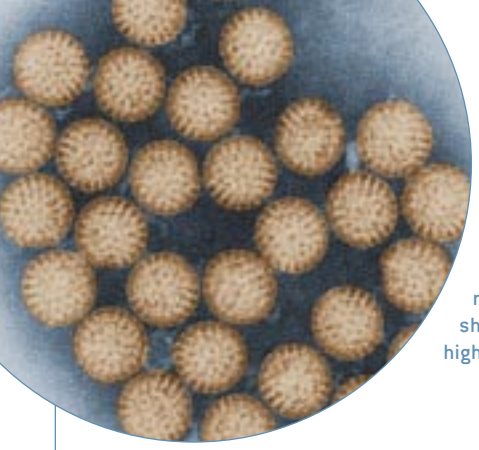
By Roger I. Glass

The thought of a murderous virus often conjures images of patients suffering from Ebola virus in Africa, SARS in Asia or hantavirus in the U.S. Yet those evildoers have taken far fewer lives than rotavirus, whose name is virtually unknown. This virus infects nearly all children in their first few years of life. It causes vomiting followed by diarrhea. The diarrhea is often so severe that, if left untreated, it can lead to shock from dehydration and then death. Worldwide, rotavirus kills an estimated 610,000 children every year, accounting for about 5 percent of all deaths among those younger than five years. In the U.S., few children perish from the virus, but as many as 70,000 require hospitalization for it annually, and several million suffer quietly at home.

Scientists, though, are now about to break the grip of this devastating disease. In January—some three decades after investigators first identified the pathogen—researchers reported that two rotavirus vaccines had proved successful in massive clinical trials. The process of developing rotavirus vaccines has been more difficult and complicated than anyone imagined, full of setbacks and surprises. But today both the World Health Organization and the Global Alliance for Vaccines and Immunization consider rotavirus vaccine a top priority, and the final battle to get immunizations to the young children who so desperately need them has begun.

INFANT ILL with severe diarrhea caused by rotavirus will be saved by rehydration therapy. But too many children in impoverished countries, where access to health care is limited, go untreated and die from the virus.

GEOFF WARD



ROTAVIRUS PARTICLES look wheellike (hence the Latin name *rota* for "wheel") through an electron microscope. The particles shown here are colorized and highly magnified.

Identifying the Contagion

ROTAVIRUS was first identified as a cause of human disease in 1973 by Ruth Bishop, a young microbiologist working on gastrointestinal diseases at the Royal Children's Hospital in Melbourne, Australia. At the time, investigators were perplexed by diarrhea in children. Although the disorder was common and frequently severe, the causative agent was rarely identified. Searching for clues, Bishop's group looked through an electron microscope at biopsied tissue from the duodenum, or small intestine, of acutely sick children. What they saw astounded them: an infestation of wheel-shaped viruses in the epithelial cells that form the intestinal lining.

My own involvement with rotavirus began in 1979, when my wife and I moved to Bangladesh to work at the International Center for Diarrheal Disease Research. Young and idealistic, we were drawn by the prospect of helping children in a country where severe diarrhea was a leading cause of death. The center's hospital in Dhaka admitted so many patients with unspecified "intestinal" flu annually that some had to be cared for in hallways and in tents outside. Believing the cause of their diarrhea to be bacterial, we were surprised to find many of the children were suffering not from cholera, salmonella, shigella or *Escherichia coli* but from rotavirus, about which we knew little. With the help of a simple test, we determined that rotavirus was responsible for the admittance of between 25 and 40 percent of all children younger than five to our hospital for diarrhea.

Overview/*Rotavirus Victory*

- Almost every child in the world contracts a rotavirus infection at least once, yet the disease has poor name recognition. Often it is dismissed as stomach or intestinal flu, even by health care workers.
- The disease exacts a devastating toll on young children, every year hospitalizing tens of thousands of them in the U.S. and killing more than 600,000 in poorer countries.
- Since the virus's discovery some 30 years ago, researchers have unraveled many of its secrets, in the process realizing that only a vaccine is likely to curb it.
- Today, after many snafus and false starts, the race to find a vaccine is almost won: several rotavirus vaccines have now proved safe and effective.

Studies from around the globe yielded similar results. What is more, they revealed that rotavirus was not only widespread but a major cause of death in the poorest nations. By 1985 such data compelled the Institute of Medicine to put rotavirus infection atop a list of diseases for which vaccines were urgently needed in the developing world.

At the same time, surprisingly little was known about the incidence and distribution of rotavirus in the U.S. In 1986, when I returned to the U.S. Centers for Disease Control, the disease was rarely diagnosed and, in fact, was not even listed in the *International Classification of Diseases*. Having seen the impact of the disease overseas, my co-workers and I were intent on finding out whether it was affecting many people in the states.

But how does one assess the burden of a disease that is rarely diagnosed, is never listed as the cause of hospitalization in discharge records, and goes unrecognized by a majority of pediatricians who commonly treat it? My colleague, Mei-Shang Ho, began by looking at U.S. data on childhood hospitalizations. She found that diarrhea was a common cause of hospital stays, accounting for 12 percent of hospitalizations in children younger than five, and that most cases were coded as being of unknown etiology. Further studies revealed that a lion's share of the undiagnosed cases were attributable to rotavirus. Three other interesting facts about rotavirus in the U.S. emerged as well. First, infection follows a distinctly seasonal pattern, peaking from December to March; second, the vast majority of children hospitalized for this virus are younger than five years; and third, regardless of season, rotavirus causes most cases of severe diarrhea in young children.

Epidemiologists now know that rotavirus is far and away the leading cause of childhood diarrhea worldwide, infecting virtually all children between the ages of three months and five years. Unlike bacteria that spread via contaminated food and water and thus disproportionately affect people in poor regions, rotavirus shows no regard for geographic borders. Indeed, the very ubiquity of the pathogen—with Americans facing the same risk of infection as Bangladeshis—suggests the virus is highly contagious, spreading as easily as, say, a cold virus. And, as is true of cold viruses, sanitation and clean drinking water have little power to block transmission.

Molecular and clinical studies bear witness to its virulence. Just 10 virus particles can start trouble in a young child. A virus-laden droplet landing on a baby's thumb or toy is all it takes. Popped into the mouth, the virus makes its way to the epithelial cells lining the small intestine, where it replicates at astonishing speed: within 24 hours, 10 viruses become millions, filling and killing the cells with their proteins, toxins and newly made particles. Soon the gut epithelium sloughs, and a flood of fluids and electrolytes exits the body in diarrheal bursts. Without rehydration therapy, a child can lose as much as 10 percent of his or her body weight and go into shock in just one or two days.

Fortunately, children who survive their first infection suffer no long-term consequences, and few ever experience an-

CHARLES D. HUMPHREY *U.S. Centers for Disease Control and Prevention*

other bout of rotavirus diarrhea. They have natural immunity—that is, their immune system has become primed to quickly recognize and prevent replication of rotavirus when it next invades. But because so many children become severely ill with the first infection, scientists consider a vaccine that could mimic this natural immunity to be the best hope for saving lives.

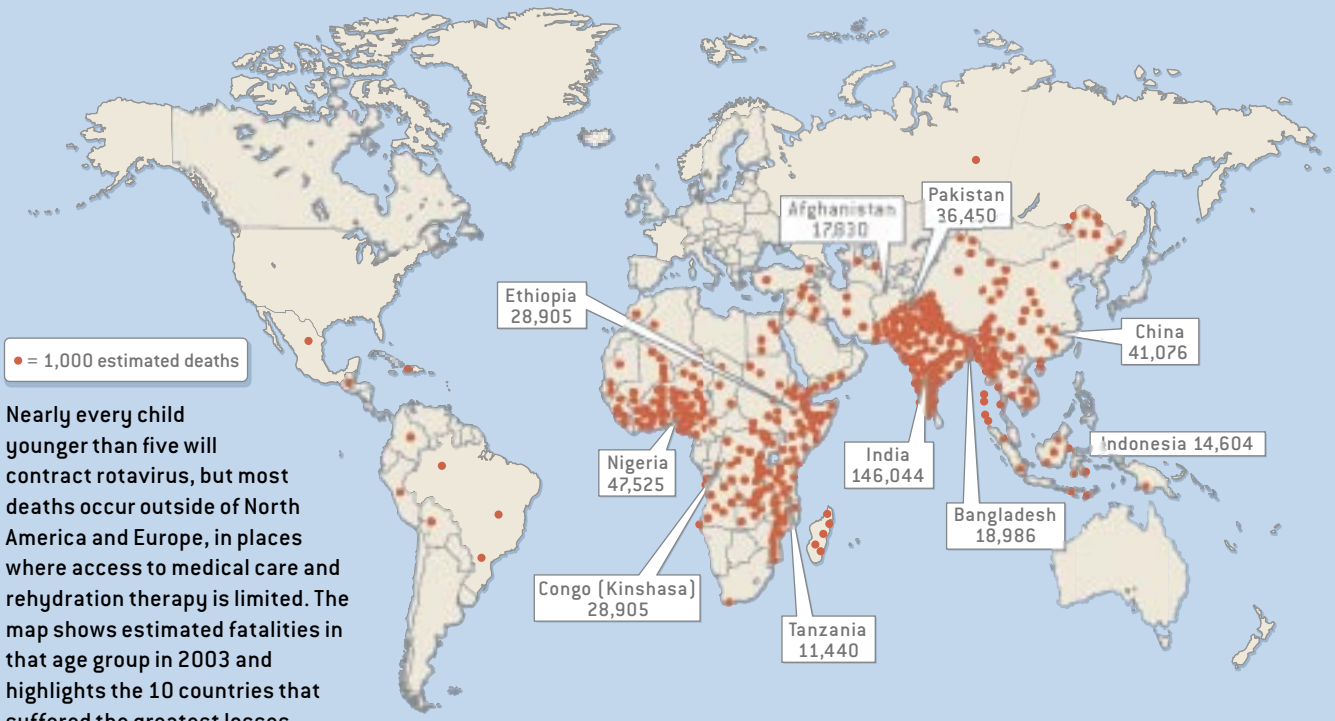
Quest for a Vaccine Begins

VACCINES are powerful weapons in the human arsenal against infectious disease and among the most effective interventions in public health. Made from either live or killed microorganisms or from their key proteins, vaccines trick a recipient's immune system into believing it is under attack. In response, the immune system produces antibodies against the vaccine (which poses no biological threat), just as it would against the virus itself. And as in natural immunity, should the disease-causing agent ever invade, the immune system is fully primed, ready to pump out antibodies to immobilize it.

Twenty years ago several pharmaceutical companies became interested in developing a vaccine against rotavirus. With a potential market both large in size and global in scope, the high costs of vaccine development appeared reasonable. In addition, distribution would be easy even in remote places: rotavirus vaccine could be added to the Universal Program for Childhood Immunization, which under the auspices of the WHO and UNICEF already delivers routine vaccines to about 80 percent of the world's children.

Although different approaches to vaccines have been considered—human versus animal strains, live versus killed viruses, whole virus or protein subunits—rotavirus researchers followed the lead of Albert Sabin, creator of the oral poliomyelitis vaccine. Sabin believed that live vaccines, which can replicate somewhat but are too weak to trigger disease, best mimic the protection acquired through natural infection. Also, in the case of rotavirus, oral vaccines would prompt an immune response where it is most desirable—in the gastrointestinal tract. Vaccine developers quickly focused on live but weakened, or attenuated, strains of rota-

Global Distribution of Deaths from Rotavirus



Nearly every child younger than five will contract rotavirus, but most deaths occur outside of North America and Europe, in places where access to medical care and rehydration therapy is limited. The map shows estimated fatalities in that age group in 2003 and highlights the 10 countries that suffered the greatest losses.

Bangladesh falls near the bottom of that group but has the highest per capita death rate from the disease. Rural inhabitants there often have to travel far, by slow means (*left photograph*), to get help. Babies with profuse diarrhea who reach a hospital in Dhaka are placed on cots that drain directly into buckets meant to catch the watery excrement. In the right photograph, a mother at the hospital is feeding a rehydration solution to her infant.

LUCY READING-IKKANDA; SOURCE: UMESH D. PARASHAR *CDC (map)*; ASEM ANSARI *International Center for Diarrheal Diseases Research, DHAKA, BANGLADESH (left photograph)*; JEAN C. SACK *ICDDR (right photograph)*

Rotavirus Up Close

Structural studies reveal that rotavirus, shown below in two cutaway views, consists of three protein layers that encase the genome. Its structural proteins—those present in particles that spread from person to person—are called VPs and are denoted by numbers.

VP7 forms the outer surface and is studded with VP4 spikes. These two proteins elicit a host's disease-fighting immune response and thus play a central role in vaccines. VP4 also facilitates viral entry into cells, as do VP5 and VP8 (*not shown*), which result from cleavage of VP4 in a host's body. VP6 composes the middle layer and is required for gene transcription, a process essential to the synthesis of viral proteins in infected cells. VP2 makes up the inner shell, and VP1 and VP3 are enzymes involved in copying viral genes.

The genome comprises 11 segments of double-stranded RNA tightly coiled and packed together. These segments code for the VPs as well as for nonstructural proteins (NSPs), including a toxin called NSP4 that is made after the virus enters cells.

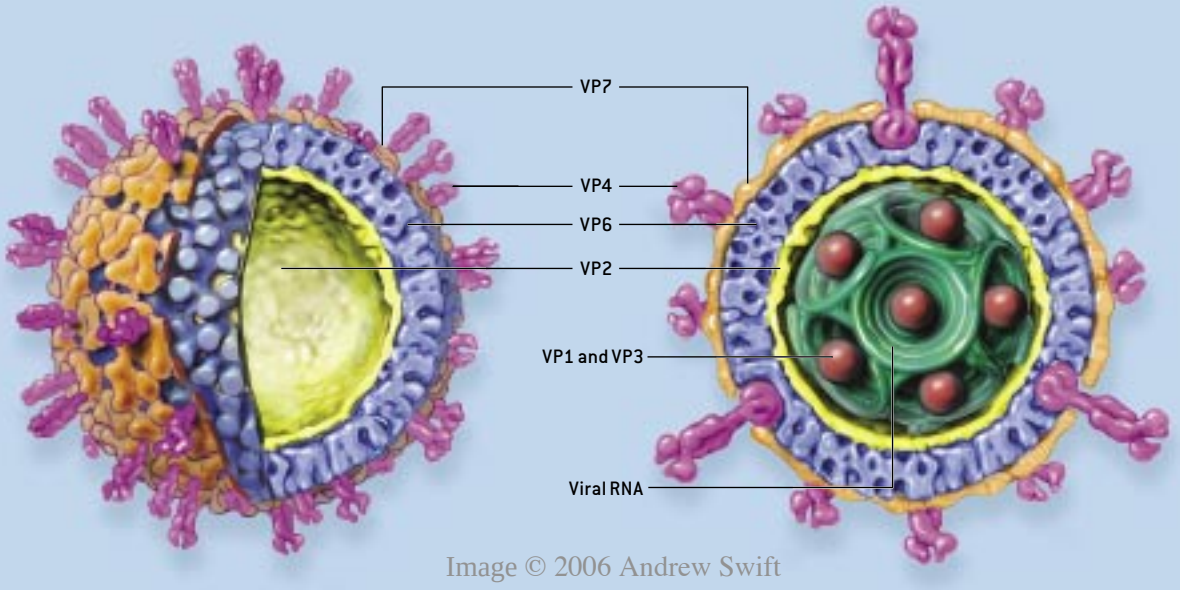


Image © 2006 Andrew Swift

ANDREW SWIFT; SOURCE: "EMERGING THEMES IN ROTAVIRUS CELL ENTRY, GENOME ORGANIZATION, TRANSCRIPTION AND REPLICATION," BY HARIHAN JAYARAM, M. K. ESTES AND B. V. VENKATARM PRASAD IN *VIRUS RESEARCH*, VOL. 101; 2004

virus that could be administered by mouth, without needles.

In 1983 the first rotavirus vaccine was ready for testing. Francis Andre of Smith Kline-RIT (now GlaxoSmithKline Biologicals) in Rixensart, Belgium, and Timo Vesikari, a pediatrician at the University of Tampere in Finland, prepared and tested a vaccine derived from a rotavirus strain found in cows. They chose a bovine rotavirus because it grew well in culture and was thought to be naturally attenuated in humans.

From all vantages, the first trial, conducted in Finland, was a landmark success: the vaccine reduced the chances that a vaccinated child would get severe rotavirus by 88 percent, demonstrating that immunity could be induced with a live oral vaccine. Moreover, the vaccine had no troubling side effects.

THE AUTHOR

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Encouraged, Smith Kline-RIT launched trials in other countries, and by the late 1980s the end of rotavirus-related deaths seemed at hand. But then results from trials in Africa and Peru proved inconsistent and disappointing. Lacking certainty about the reasons for the troubles—although poor health, untreated infections, malnutrition and parasites are known to affect a child's immune response to vaccines—the company put its rotavirus program on hold.

Back to the Drawing Board

RESEARCHERS at the National Institutes of Health and the Wistar Institute in Philadelphia sought to explain the failure of the RIT vaccine. Possibly, the bovine strain was overattenuated—that is, it was too weak to replicate and elicit a good immune response under challenging conditions. They began looking for new formulations. Albert Kapikian of the NIH, for example, identified a rhesus strain of virus, and Fred Clark and Stanley Plotkin of Wistar identified another bovine strain that might replicate more vigorously. The strains were prepared for clinical trials, but these, too, showed both success and failure. Several more years were needed to rethink the science.

Meanwhile other researchers were unraveling the virus's molecular structure. Though wheellike in cross section, rotavirus is actually a three-layered sphere containing 11 seg-

ments of double-stranded RNA, each of which consists of a single gene encoding a protein. The proteins fall into two basic types: ones that are structural (composing the virus) and ones that are nonstructural (made within infected cells). The structural viral proteins, or VPs, are numerically named: VP1, VP2 and so on, as are the nonstructural proteins, or NSPs, which participate in viral replication and in deranging intestinal function.

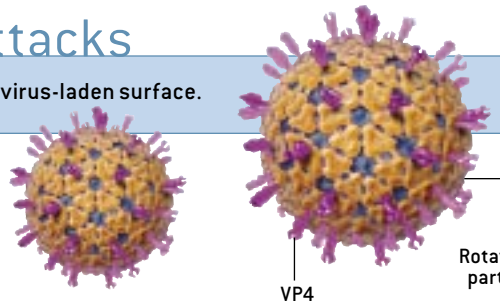
The outermost shell, important in eliciting the host's immune response, has been a focus of attention in vaccine development. VP7 fashions its lumpy surface, and the VP4 protein forms the spikes on the outside of the "wheel." VP6, the most abundant protein in the virus, sits underneath VP7 and participates in producing viral proteins in infected cells. A nonstructural molecule called NSP4 is a toxin that may play a role in triggering profuse diarrhea.

The proteins come in several varieties, and separate strains sport different mixes of proteins. When two viral strains infect the same cell, their gene segments can reassort just like figures on a slot machine, forming new combinations and thus novel versions of the virus. New reassortant viruses arise constantly, but as is true of most mutations, few offer survival advantages to the virus. Consequently, of the 42 unique rotavirus strains identified to date based on their combinations of VP7 and VP4 varieties, only four or five account for more than 90 percent of rotavirus disease worldwide.

Exploiting the natural ability of rotavirus to reassort its genes, Kapikian and his NIH colleague Harry Greenberg developed a laboratory method to create reassortants that had features useful for vaccines but would not cause disease in humans. They began by making a reassortant virus that combined 10 genes from a monkey rotavirus—giving it the property of attenuation—with one gene encoding a surface protein, VP7, from a human strain. They made three such reassortants, each displaying a different human version of VP7, and one purely rhesus virus, displaying a fourth VP7 found in both monkey and human rotaviruses. They mixed all four into a cocktail called a tetravalent vaccine intended to offer

Wreaking Havoc: How Rotavirus Attacks

Highly infectious, rotavirus can be picked up from the air or by touching a virus-laden surface.



1 The virus enters the body through the mouth, often via a contaminated thumb. The viral particles then pass through the stomach and into the small intestine

Epithelial cell

Viral subparticle

2 VP4 proteins attach the virus to epithelial cells lining the gut

3 Then the VP4 spikes and the outer shell are shed, and the rest of the particle (the subparticle) enters the cytoplasm

Toxin

4 There the viral genes direct production of thousands of new viral particles and of toxin molecules that can poison even uninfected cells and instigate fluid release from intestinal tissue

5 Waves of new virus stream out of infected cells to invade healthy ones, and the cycle repeats itself

6 Dead epithelial cells and fluids from the stomach and tissues fill the gut and exit the body as profuse diarrhea

Dying cell

Fluid and other matter leaving the body

For clarity, viral particles are depicted much larger than scale

Image © 2006 Andrew Swift

ANDREW SWIFT; SOURCE: PHILIP R. DORMITZER *Harvard Medical School*

protection against the four most prevalent human strains of rotavirus.

In 1991 the Food and Drug Administration granted the pharmaceutical company Wyeth Ayerst (later Wyeth Pharmaceuticals) permission to make and test this vaccine, which they named RotaShield. Over the next five years it launched large-scale clinical trials in the U.S., Finland and Venezuela, verifying RotaShield's safety, ability to induce a protective immune response, and lasting efficacy. In 1998 RotaShield was licensed by the FDA and recommended by the CDC's Advisory Committee on Immunization Practices and the American Academy of Pediatrics for routine immunization of all American children. Over the next nine months more than 600,000 children received an estimated 1.2 million doses of RotaShield.

These were heady times. The vaccine still had to be tested on undernourished children in developing nations, where live oral vaccines for other diseases—including polio and cholera—were known to be less effective than elsewhere. Also, the price per dose was still high for most developing nations. But for the first time, the world had a tool with which to combat rotavirus, and many of us were jubilant.

Then disaster struck. In 1999 several infants suffered a serious complication within two weeks of receiving the vaccine: a segment of the intestine folded into a nearby region (like a part of a telescope collapses into another), creating a blockage called intussusception. The condition can be excruciatingly painful and must be quickly reversed with either an air or fluid enema or fixed surgically. In rare cases, the intestine perforates and the infant dies. The CDC, which was monitoring experience with RotaShield, called for an immediate halt to the immunization program, thereby sinking a vaccine that had taken 15 years and several hundred million dollars to launch.

The agency initially estimated the risk to be one intussusception in 2,500 vaccine recipients, which was considered unacceptable. Later studies pegged the probability at only one in 11,000. Then Lone Simonsen of the NIH correlated risk with age: infants younger than three months were in less danger than older ones. If the vaccine were given only to young babies, the likelihood of intussusception could drop 10-fold, to perhaps one in 30,000.

The new data raised new questions. Was this risk acceptable in the U.S., where children are often hospitalized but rarely die of rotavirus? Were the odds more palatable in the developing world, where one child in 200 dies of rotavirus? If 150 lives could be saved for each complication from intussusception, might the risk be justified? Given these statistics, was it unethical, in fact, to withhold a vaccine that might save half a million lives a year? Or no matter what the risk-benefit analysis showed, was it unethical to market a vaccine in the developing world that had been withdrawn from use in the U.S.?

The CDC and the WHO called a meeting of policymakers from developing countries. After heated discussion, science bowed to politics. As a high-ranking Indian official said, "I know this vaccine would save 100,000 children in my country. But when the first case of intestinal blockage occurred, I would not be forgiven for allowing a vaccine that had been withdrawn in the United States to be used in my country."

Back on Track

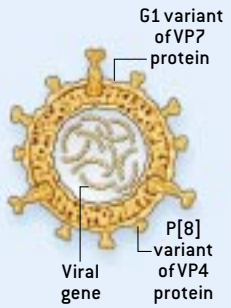
RESEARCHERS continued to study the link between vaccination and intussusception. Children who contracted rotavirus naturally had no greater incidence of blockage than other children, so why should vaccination per se raise their risk? Some began to suspect the problem was specific to the rhesus strains, not an effect common to all live oral rotavirus vaccines.

Making a Rotavirus Vaccine

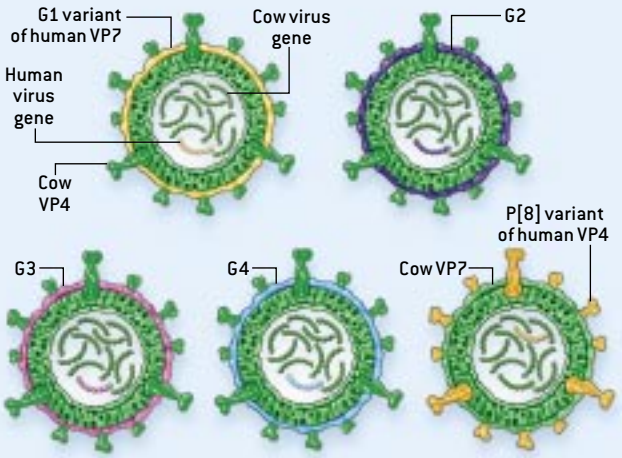
Two rotavirus vaccines that recently proved highly effective in large clinical trials are depicted schematically below.

ROTARIX

Made by GlaxoSmithKline, Rotarix consists of a single strain of a human-infecting rotavirus that provides protection against many strains. The vaccine features common variants of VP7 and VP4—G1 and P[8], respectively. Because such a human virus could potentially cause disease if it were fully functional, the manufacturer weakened it through a standard cell culture method that prevents it from causing symptoms but enables it to replicate enough to trigger an immune response.



ROTATEQ



Made by Merck, RotaTeq contains five genetically distinct viruses called reassortants. These reassortants are produced by combining 10 cow (*green*) rotavirus genes with one of five human (*other colors*) rotavirus genes, thereby generating mainly cow viruses that display a protein from the human virus on the surface. Four of the reassortants have a gene that codes for a variant of human VP7 (either G1, G2, G3 or G4), and one reassortant carries a gene for the P[8] form of the human VP4 spike. The end result is a pentavalent vaccine, which specifically protects against the four most prevalent human strains of rotavirus yet has too many cow genes to cause disease in people.

Image © 2006 Andrew Swift

ANDREW SWIFT

Betting the intussusception problem could be overcome, two vaccine makers renewed their interest in rotavirus. GlaxoSmithKline dusted off its program and pressed forward with a new monovalent vaccine derived entirely from a single attenuated human strain. Because natural rotavirus infection was not associated with intussusception, they reasoned their vaccine would similarly not increase the risk of this complication. In addition, the company would select for study only infants who were six weeks to 13 weeks old, a stage when natural intussusception is rare. At the same time, Merck developed a pentavalent vaccine derived from five human-bovine reassortant strains that together would target the major strains of rotavirus. Merck scientists knew bovine strains did not grow or replicate as well as the rhesus strain and also did not cause the low-grade fever many children developed after being immunized with the rhesus vaccine. Also, the company would limit eligibility in its clinical trials exclusively to infants six to 12 weeks old.

Both companies conferred with the FDA on their plans to conduct clinical trials. The FDA, wanting to ensure that the next generation of rotavirus vaccines would be safer than RotaShield, insisted that the trials be large enough to detect any risks, however small, that might be associated with the vaccine. An initial target of 60,000 participants per trial was set, making these the largest and most expensive safety trials of any vaccine ever tested before licensing. Not only would the trials be costly, but the undertaking itself was risky—each one would instantly collapse if the rate of intussusception among vaccinated babies exceeded that of nonvaccinated ones. The developers pressed on with some trepidation.

Now, six years after the intussusception debacle, the rotavirus gamble is paying off. GlaxoSmithKline and Merck have completed their clinical trials, and the results for both vaccines are encouraging. They offer from 85 to 98 percent protection against severe rotavirus diarrhea. Moreover, the vaccinated children showed no more cases of intussusception than did nonvaccinated children.

The GlaxoSmithKline vaccine, Rotarix, was tested primarily in Latin America. Since 2004, it has won approval from more than 20 countries and, most recently, from the European Union; it is under review in the U.S. Merck, in contrast, targeted the U.S. market first, wanting to prove that its vaccine, RotaTeq, is safe here before introducing it elsewhere. The company has gained approval in Mexico and the U.S. and expects to have it for Europe this year; such approvals are a prelude to introducing the vaccine to many countries.

Vaccine manufacturers in the developing world are also interested in rotavirus. Unlike those that require sophisticated bioengineering techniques, a rotavirus vaccine, like that for polio, can be made using traditional tissue culture methods and so is within the reach of smaller companies. Today more than 10 makers in India, China, Indonesia and Brazil are preparing live oral rotavirus vaccines; a Chinese firm has already gained approval to sell its product.

BABY ANDREW was a subject in the large-scale study that evaluated the safety and effectiveness of the RotaTeq vaccine.

MERCK & CO., INC.

Future Challenges

THE PROSPECT of new vaccines fuels hope that rotavirus's grip may soon be broken. Still, hurdles remain. Because many policymakers in developing countries have not heard of rotavirus, they fail to understand its dire consequences. Surveillance efforts in more than 40 countries—being conducted by Joseph Bresee and Umesh D. Parashar of the CDC, with the WHO and the Program for Appropriate Technology in Health—are just beginning to provide data that decision makers will need before welcoming the vaccines into their nations. In addition, confirmation that live oral vaccines are safe and effective in the poorest areas is still lacking. Moreover, the vaccines, which cost several hundred million dollars each to develop, must be affordable to those responsible for the 135 million children born worldwide every year.

Yet momentum is building, and many of us hope that within a decade, this major cause of diarrhea and principal killer of children in the developing world will be eliminated by the most cost-effective public health measure we have today: immunization. With help from a committed global community, rotavirus will soon join such microorganisms as polio, smallpox and diphtheria, which have been vanquished by vaccines and are now sidelined and obscure. Epidemiologists hope the anonymity that has historically characterized this disease will define it once again, its regained obscurity a true testament to the power of vaccination. SA

MORE TO EXPLORE

Global Illness and Deaths Caused by Rotavirus Disease in Children. U. D. Parashar et al. in *Emerging Infectious Diseases,* Vol. 9, No. 5, pages 565–572; May 2003.

The Future of Rotavirus Vaccines: A Major Setback Leads to New Opportunities. Viewpoint. R. Glass et al. in *Lancet,* Vol. 363, Issue 9420, pages 1547–1550; May 2004.

Safety and Efficacy of an Attenuated Vaccine against Severe Rotavirus Gastroenteritis. G. Ruiz-Palacios et al. in *New England Journal of Medicine,* Vol. 354, pages 11–22; January 5, 2006.

Safety and Efficacy of a Pentavalent Human-Bovine (WC3) Reassortant Rotavirus Vaccine. T. Vesikari et al. in *New England Journal of Medicine,* Vol. 354, pages 23–32; January 5, 2006.