the expense and logistical challenge associated with the wide application of these vaccines (*18*). Cholera and other diarrheal diseases are relatively easy to treat by intravenous and oral rehydration therapies. However, in the grip of explosive epidemics, medical facilities can be overwhelmed and significant mortality can result. For example, early in the Matlab, Bangladesh trial, the BS-WC vaccine dramatically reduced mortality by 45% in women over age 15, demonstrating that even in a community well versed in the treatment of cholera, death can still be a consequence of this disease. Cholera can have a devastating economic impact on countries that is measured not only in treatment costs but also in the deleterious effect that this disease has on food exportation and tourism. Although provision of safer water sources and sewage treatment is no doubt the best way to control cholera, estimates by the World Health Organization indicate that this goal would cost Latin America alone tens of billions of dollars.

Vibrio cholerae has often played the role of Grim Reaper (see figure), but it has also been a great educator in the public health arena and a marvelous catalyst for scientific discovery. We have now reached an historic time when the fruits of what we have learned from *V. cholerae* can be applied to effective immunization. In addition, continued studies on the properties that enable *V. cholerae* to be such a potent immunogen may help to clarify the general physiology of mucosal immunity.

References and Notes

1. B. Barua, in *Cholera*, D. Barua and W. B. Greenough III, Eds. (Plenum, New York, 1992), pp. 1–36.
2. T. Shimada *et al.*, *Lancet* **341**, 1347 (1993).
3. M. K. Waldor and J. J. Mekalanos, *ibid.* **343**, 1366 (1994).
4. M. M. Levine and N. F. Pierce, in (*1*), pp. 285–327.
5. J. D. Clemens *et al.*, *Lancet* **335**, 270 (1990).
6. M. M. Levine *et al.*, *Infect. Immun.* **43**, 515 (1984).
7. M. M. Levine *et al.*, *ibid.* **56**, 161 (1988).
8. J. J. Mekalanos *et al.*, *Nature* **306**, 551 (1983).
9. J. B. Kaper, J. Michalski, J. M. Ketley, M. M. Levine, *Infect. Immun.* **62**, 1480 (1994).
10. C. O. Tacket *et al.*, *J. Infect. Dis.* **168**, 1536 (1993).
11. D. N. Taylor, *ibid.*, in press; M. K. Waldor and J. J. Mekalanos, *ibid.* **170**, 278 (1994).
12. C. A. Wanke and R. L. Guerrant, *Infect. Immun.* **55**, 1924 (1990).
13. D. A. Herrington *et al.*, *J. Exp. Med.* **168**, 1487 (1988).
14. M. M. Levine *et al.*, *Lancet* **ii**, 467 (1988).
15. J. J. Mekalanos, *Curr. Opin. Biotech.* **5**, 312 (1994).
16. G. D. N. Pearson, A. Woods, S. L. Chiang, J. J. Mekalanos, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 3750 (1993).
17. T. Coster, A. Trofa, K. Killeen, D. Beattie, J. Kenner, J. Arthur, J. Sadoff, J. Mekalanos, D. Taylor, unpublished data.
18. J. C. Clemens, D. Spriggs, D. Sack, in Vibrio Cholerae *and Cholera. Molecular and Global Perspectives*, I. K. Wachsmuth, P. A. Blake, O. Olsvik, Eds. (American Society for Microbiology, Washington, DC, 1994), pp. 425–439.

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Rotavirus Vaccines: Success by Reassortment?

Roger I. Glass, Jon Gentsch, Jean C. Smith

Diarrhea is one of the most common diseases of children and, in developing countries, is responsible for 3.5 to 4 million deaths each year (*1*). Before 1973, infectious agents were identified in so few cases that it was difficult to envisage a strategy for prevention. In that year, R. F. Bishop and colleagues discovered 70-nm wheel-shaped (rota) virus-like particles by electron microscopy in the intestinal mucosa of infants with gastroenteritis (*2*). The identification of rotavirus has led to two decades of energetic research that established this agent as the main cause of severe diarrhea in children and may soon culminate in a vaccine recommended for routine use in children worldwide.

Early research on rotavirus was directed at the development and application of rapid diagnostic tests that allowed researchers to examine the spectrum of disease and the epidemiology of infection. The burden of disease caused by rotavirus is staggering: rotavirus is the most common cause of severe dehydrating diarrhea in children worldwide, infecting nearly every child in the first few years of life (see table) (*3*). First infections are generally associated with acute diarrhea, which in some instances can be severe, leading to dehydration and death. In developing countries, 20 to 40% of hospitalizations for childhood diarrhea and an estimated 870,000 deaths are associated with rotavirus infections each year, making it the most important single cause of diarrheal mortality among children (*4*). In the United States, although mortality is relatively low (75 to 125 deaths per year), rotavirus diarrhea incurs direct medical costs in excess of $500 million and total costs in excess of $1 billion (*5*).

Early epidemiologic studies indicated that rotavirus might best be controlled through vaccination. Longitudinal studies of children followed from birth to 2 years of age provided evidence for natural immunity: Rotavirus diarrhea infrequently occurs more than once, and repeat illnesses are less severe or asymptomatic (*6*, *7*). Furthermore, children infected as newborns are protected from disease later in life (*8*, *9*). Unfortunately, immunity is not fully protective; repeat infection can sometimes lead to disease, and adults with antibodies indicative of previous infection can develop rotavirus diarrhea while caring for sick children or traveling to developing countries where enteric infections are common (*10*).

Rotavirus vaccine development achieved a major breakthrough when human rotavirus was adapted to grow in cell culture (*11*). This advance enabled researchers to prepare vaccine seed lots, set up plaque assays to measure neutralizing antibody titers, and provide replenishable stocks of virus needed to study the molecular biology of the virus.

Rotavirus is classified in the family Reoviridae and its genome consists of 11 segments of double-stranded RNA, each coding for a viral protein. The gene-coding assignments and function of most of these proteins have been determined (*12*). Of particular interest for vaccine development are the two outer capsid proteins, VP7, a glycoprotein on the viral surface, and VP4, a protease-cleaved hemagglutinin (in some strains), which are important in virus neutralization and protection from disease. Cross-neutralization studies have identified four common serotypes of rotavirus on the basis of the VP7 glycoprotein (G_1 to G_4) and two VP4 serotypes (P_4 and P_8); all of these serotypes are found in children with diarrhea (*13*, *14*). In theory, an effective vaccine must protect against rotaviruses encoding any of these common neutralization antigens. Because the rotavirus genome is segmented, reassortment of the VP7 and VP4 genes occurs; in nature, this can lead to the development of new strains, and in the laboratory, this can be exploited for preparation of reassortant strains as vaccine candidates or for studying gene function.

In 1983, just 10 years after the discovery of rotavirus, Vesikari conducted the first rotavirus vaccine field trial, in which Finnish infants were administered a live bovine strain of rotavirus, prepared as a vaccine lot by Smith Kline–RIT (*15*). To the surprise

The authors are in the Viral Gastroenteritis Section, Centers for Disease Control and Prevention, Department of Health and Human Services, Atlanta, GA 30333, USA.

of many in the field, this vaccine, which appeared to be only marginally immunogenic in adults and children, reduced the incidence of rotavirus diarrhea by 83%. Thus, although both the VP7 (serotype G_6) and VP4 (serotype P_6) capsid proteins of the bovine vaccine strain are antigenically distinct from those in human isolates, they offered clear heterotypic protection. This strategy, in which animal strains of virus are used to prevent illness in humans, has been designated the "Jennerian" approach (*16*). The results of this trial were confirmed in a second trial in Finland and the successful development of a rotavirus vaccine seemed near at hand.

Despite this early success, the pathway toward a commercial vaccine has proved tortuous. The efficacy of the RIT strain could not be reproduced in trials conducted in three developing countries where the vaccine would be most critical to save lives. The dose of virus in the vaccine, 10^8 $TCID_{50}$ (median tissue culture infectious dose), required that the inoculum prepared in *Ceropithecus* monkey kidney cells be administered undiluted, a prospect that was commercially untenable (*17*). The RIT vaccine was withdrawn from development and replaced by two other Jennerian vaccines prepared from simian and bovine rotaviruses.

Two scientific hurdles have made the task of developing a rotavirus vaccine more costly, time-consuming, and difficult than initially thought. First, the animal models used for studying protective and heterotypic immunity to rotavirus (mice, gnotobiotic piglets, rabbits) have limited predictive value for human responses. Second, immunity to rotavirus is not well understood and no simple immune model can explain protection against disease (*3*). Mucosal immunity is believed to play a role in protection, but titer changes are difficult to measure in field specimens (stool, saliva, intestinal secretions) and elevated titers return to baseline rapidly so a single titer cannot predict protection. Cell-mediated immunity may also be important; in mice, virus-specific cytotoxic T cells appear at the intestinal mucosal surface after infection and are associated with protection against disease (*18*). Finally, humoral immunity, measured as rotavirus-specific antibodies or neutralization activity, can be easily detected, but its role in protection is also unclear. In the absence of a predictive animal model or reliable immune indicators of protection, studies to assess the efficacy of rotavirus vaccines have been confined to placebo-controlled trials in humans in which the disease experience of children is monitored intensively for 1 to 2 years after immunization.

Two live oral candidate vaccines are currently being developed and tested in field trials. The rhesus rotavirus tetravalent vaccine (RRV-TV), developed by A. Z. Kapikian and colleagues (National Institutes of Health) and by Wyeth-Ayerst Research, contains the parent strain of rhesus rotavirus (MMU18006 serotype G_3) and three single-gene reassortants prepared with VP7 genes to the three other main serotypes, G_1, G_2, and G_4 (*19*, *20*). The WI79-9 vaccine strain, developed by H F. Clark and colleagues (Wistar Institute) and by Merck, is based on a bovine parent strain that has been reassorted with the VP7 gene of human serotype G_1 (*21*). The trials conducted to date indicate that these vaccines have moderate efficacy (50 to 65%) against any rotavirus diarrhea and greater efficacy (75 to 87%) against more severe illness. Although this efficacy is less than that achieved with other childhood vaccines, it is comparable to the level of protection conferred by natural rotavirus infection and is similar to that observed for other new and recently licensed oral enteric vaccines such as cholera, where mucosal immunity may be critical. Any increase in efficacy may well require a new approach to immunization.

Several strategies are being examined to increase the efficacy of rotavirus vaccines. Low efficacy could reflect the failure of Jennerian vaccines to protect against the full range of VP7 and VP4 serotypes. Consequently, the number and diversity of these antigens have been increased by using as vaccines human rotavirus strains that have been attenuated by cold adaptation, multiple passaging, or selection of neonatal isolates that have not caused diarrhea in infants. Furthermore, current Jennerian reassortant vaccines that contain only VP7 antigens from human strains could be improved by addition of a VP4 gene from a human strain as a separate reassortant. Among the more novel approaches, microencapsulated infectious virus has been administered orally and parenterally to mice; the oral preparation penetrates and persists in the gut-associated lymphoid tissue and enhances virus-specific humoral immunity (*22*). Rotavirus-like particles devoid of nucleic acid have been produced in insect cells by expression of rotavirus genes encoding the core and capsid proteins in a baculovirus vector (*23*, *24*). These particles, when given orally or parenterally to rabbits, appear to be protective and may represent a novel approach to immunization.

The reassortant vaccines emerging from clinical trials are nearest to approval by the Food and Drug Administration for use in the United States. Once approved, they could be incorporated into the routine schedule of childhood immunizations recommended by the Advisory Committee on Immunization Practices. The impact of this new vaccine should be an immediate decline in the number of winter hospitalizations and doctor visits for childhood diarrhea. Internationally, the vaccine could play a more important role in improving child survival by decreasing the number of diarrhea-associated deaths.

Parameter	United States		World	
	Total	Risk per child	Total	Risk per child
Births	4.1 million		140 million	
Episodes of rotavirus gastroenteritis	3.5 million	1:1.2	130 million	1:1.1
Doctor visits	500,000	1:8	?	
Hospitalizations (U.S.) or cases of moderate-to-severe disease (World)	70,000 to 100,000	1:40	18 million	1:30
Deaths	75 to 125	1:40,000	873,000	1:160
Cost - Hospitalizations - Indirect	 - $ 500 million - $1,200 million		 ? ?	

Estimated annual disease burden of rotavirus gastroenteritis. U.S. data are from (*5*) and world data are from (*4*).

References

1. World Bank, *World Bank Development Report 1993: Investing in Health* (Oxford Univ. Press, New York, 1993).
2. R. F. Bishop *et al.*, *Lancet* **i**, 1281 (1973).
3. A. Z. Kapikian and R. M. Chanock, in *Virology*, B. N. Fields *et al.*, Eds. (Raven, New York, ed. 2, 1990), vol. 2, pp. 1353–1404.
4. I. de Zoysa and R. V. Feachem, *Bull. WHO* **63**, 569 (1985).
5. R. I. Glass *et al.*, in *Prospects for Public Health Ben-*

efits in Developing Countries from New Vaccines against Enteric Infections, D. A. Sack and L. Freij, Eds. (Sarec Conference Report, Goteborg, Sweden, 1990), pp. 102–107.
6. R. L. Ward *et al.*, *J. Infect. Dis.* **169**, 900 (1994).
7. D. I. Bernstein *et al.*, *ibid.* **164**, 277 (1991).
8. R. F. Bishop *et al.*, *N. Engl. J. Med.* **309**, 72 (1983).
9. M. K. Bhan *et al.*, *J. Infect. Dis.* **168**, 282 (1993).
10. D. Hardy, *Rev. Infect. Dis.* **9**, 461 (1987).
11. R. G. Wyatt *et al.*, *Science* **207**, 189 (1980).
12. N. M. Mattion, J. Cohen, M. K. Estes, in *Viral Infections of the Gastrointestinal Tract*, A. Z. Kapikian, Ed. (Dekker, New York, ed. 2, 1994), pp. 169–250.
13. M. Gorziglia *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 7155 (1990).
14. M. K. Estes and J. Cohen, *Microbiol. Rev.* **53**, 410 (1989).
15. T. Vesikari, *Vaccine* **11**, 255 (1993).
16. A. Z. Kapikian, in (*12*), pp. 409–417.
17. T. Vesikari, in (*12*), pp. 419–442.
18. P. Offit, *Adv. Virus Res.* **44**, 161 (1994).
19. D. Sack *et al.*, paper presented at the 32nd Interscience Conference on Antimicrobial Agents and Chemotherapy, Anaheim, CA, 11 to 14 October 1992.
20. P. H. Dennehy, *Pediatr. Res.* **35**, 1052 (1994).
21. J. J. Treanor *et al.*, paper to be presented at the 34th Interscience Conference on Antimicrobial Agents and Chemotherapy, Orlando, FL, 2 to 5 October 1994.
22. P. Offit *et al.*, *Virology*, in press.
23. M. J. Redmond *et al.*, *Vaccine* **11**, 273 (1993).
24. S. E. Crawford, *et al.*, *J. Virol.*, in press.

Measles Vaccine: Do We Need New Vaccines or New Programs?

Samuel L. Katz and Bruce G. Gellin

Three converging factors have rekindled interest in measles virus, measles vaccine research, and public health policies directed toward the control and eventual elimination of measles. First, after 23 years of relative obscurity, measles reemerged as a public health issue in the United States when focal outbreaks between 1989 and 1991 resulted in more than 55,000 reported cases. Second, on the international scene, the World Health Organization (WHO) formally resolved to intensify vaccination efforts in order to reduce by 95% the number of measles-related deaths and by 90% the number of measles cases before 1995. A third factor was the adverse experience encountered in several developing nations when measles vaccines containing elevated titers of attenuated virus (see below) were administered to 4- to 6-month-old infants in an attempt to overcome the maternally conferred immunity that normally prevents successful vaccination at that early age.

In the United States, measles had been a ubiquitous childhood illness so communicable that by their early teens more than 95% of each annual birth cohort (3 to 4 million) had been infected. After licensure in 1963 of live attenuated measles virus vaccine and funding of federal programs initiated in 1966, there was such a striking reduction in the number of cases that the U.S. Public Health Service targeted 1982 as the year of measles elimination (*1*). Although this goal has yet to be achieved, there were only 277 cases reported in 1993, a remarkable all-time low.

Prior to 1989, many of the small clusters of measles cases that persisted in the United States occurred among high school and college students. These cases reflected the accumulation of measles-susceptible individuals resulting from the 5% failure rate of infant immunization, as well as some cases of waning immunity. In response, both the Advisory Committee on Immunization Practices (ACIP) and the American Academy of Pediatrics (AAP) recommended administration of second doses of measles vaccine to be given at varying ages. By contrast, the measles outbreaks occurring between 1989 and 1991 showed a new and surprising shift in age prevalence—namely, there were increased numbers of cases in preschoolers and infants less than 15 months old, the usual time when primary measles immunization has been recommended. At least two factors were responsible for this shift. First, many of the cases occurred in urban-dwelling infants from poverty areas where multiple socioeconomic factors rendered these children less likely to have the recommended immunizations until school entry. Second, the widespread use since 1963 of measles vaccines in the United States had resulted in a population of young mothers who had vaccine-conferred immunity rather than immunity conferred by natural disease. The antibody levels induced by measles vaccination are generally lower than those induced by measles itself, and the measles-specific antibodies (immunoglobulin G) supplied transplacentally by these mothers diminished more rapidly in the infants. As a result, babies 6 to 12 months of age no longer had passive protection, creating a 3- to 9-month window of susceptibility before the customary administration of vaccines at 15 months of age (*2*).

In an attempt to control these outbreaks, it was recommended that vaccine

A successful vaccination campaign. Licensure of the measles vaccine in 1963 and funding of federal programs initiated in 1966 were highly effective in reducing the number of measles cases in the United States. In this 1976 photograph, schoolchildren in Highland Park, Illinois, line up for the vaccine. [Photo by Thomas S. England, Photo Researchers, Inc.]

S. L. Katz is in the Department of Pediatrics, Duke University Medical Center, Durham, NC 27710, USA. B. G. Gellin is in the Department of International Health, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD 21205, USA.