ROTAVIRUS INFECTIONS

R. KATYAL*, S.V. RANA, K. SINGH

Department of Gastroenterology, Postgraduate Institute of Medical Education and Research, Chandigarh 160 012, India

Received February 17, 2000; accepted September 11, 2000

In 1976, John Rohde, highlighting the importance of diarrhea as prime killer of children in the developing world, beckoned the scientific community to "take science where the diarrhea is". The World Health Organization estimates that one billion diarrheal episodes occur in infants annually resulting in 3.3 million deaths, making diarrheal disease a major contributor to infant mortality in developing world (Bern *et al.*, 1992). The need for simple, effective and inexpensive intervention to treat diarrhea and to prevent its occurrence is urgent and abundantly clear. Among the etiological agents of acute infectious diarrhea rotaviruses account for nearly 25% of hospital admissions in India with vomitting and diarrhea followed by severe dehydration in very young children below 2 years of age (Broor *et al.*, 1985). In developing countries, it has been estimated that more than 870,000 children die from rotavirus infection every year (Perez-Schael, 1996). The discovery of rotavirus by Bishop and colleagues in 1973 initiated a line of research that has progressed rapidly towards the goal of prevention of rotavirus diarrhea (Bishop *et al.*, 1973).

Characteristics of rotaviruses

Rotaviruses belong to the *Reoviridae* family with characteristic double shelled 70 nm wheel-like particles with 11 double-stranded RNA (dsRNA) segments as genome. The RNA segments of the genome of rotavirus perform different functions. The genome codes for 6 structural proteins (VP1, VP2, VP3, VP4, VP6, and VP7) and 5 non-structural proteins (NSP1-NSP5). Cleavage of VP4 (85 K) by protease such as trypsin into VP5 and VP8 is necessary for efficient virus infection of cells (Arias *et al.*, 1996). Rotaviruses have been classified into 6 serogroups (A to F) and subgroups depending on their reactivity with monoclonal and polyclonal antibodies. Besides groups and subgroups rotaviruses can be differentiated into serotypes. The serotype defined by VP7 (glycoprotein) is referred to as G and that defined by VP4 (protease-sensitive protein) is referred to as P. Currently, 14 G and 8 P serotypes have been described on the basis of their deduced sequences (Estes, 1996). Of the non-structural proteins, NSP4 deserves special mention and is currently defined as the first viral enterotoxin (Ball *et al.*, 1996) and the cellular basis for the induced mobilization of intracellular calcium (Dong *et al.*, 1997). Mutations in the NSP4 glycoprotein associated with altered virus virulence have also been reported (Zhang *et al.*, 1998). Evidence accumulated to date has demonstrated that VP3, VP4, VP7, NSP4 and perhaps additional viral proteins and host factors affect the virulence of these viral pathogens (Desselberger, 1997).

*E-mail: pgi.chd.nic.in!medinst; fax: 0172-744401.

Abbreviations: CTL = cytotoxic T lymphocyte; dsRNA = double-stranded RNA; EIA = enzyme immunoassay; ELISA = enzyme-linked immunosorbent assay; EM = electron microscopy; HRV = human rotavirus; PAGE = polyacrylamide gel electrophoresis; PCR = polymerase chain reaction; p.i. = post infection; RRV = Rhesus rotavirus; T_h = T helper; VLP = virus-like particle

Characteristics of rotavirus infection

There is a wealth of information describing the pathological changes found in the gut of infected animals, but very little comparable information from human population is available. Generally, rotaviruses infect the mature absorptive epithelial cells lining the upper two-thirds

of the villi of the small intestine, resulting in severe acute diarrhea in the young. The infection is restricted to the mucosal surface and the cells containing rotaviral particles occur in the lamina propria and regional lymph nodes. The infection usually spreads from proximal small intestine to the ileum over a period of 1–2 days (Bass and Greenberg, 1991). Although rotavirus infection is mainly restricted to small bowel, recent investigations of mice with combined immunodeficiency as well as of immuno-compromised children indicate that rotavirus can also infect the liver (Kanwar *et al.*, 1993; Gilger *et al.*, 1992). Numerous reports associating rotavirus with pneumonia, pharyngitis, otitis media and respiratory symptoms exist, though precise relationship of such respiratory manifestations to the virus in nuclear. Central nervous system manifestation of rotavirus infection has been reported infrequently (Hongou *et al.*, 1998; Lundgren *et al.*, 2000).

Rotavirus infection is frequently asymptomatic in neonates and adults and this led to efforts to develop vaccines containing neonatal strains (Bishop *et al.*, 1983). The first neonatal vaccine strain M37, isolated in Venezuela, showed conflicting results. Three additional neonatal strains are currently under development: RV3a strain isolated from newborns at the Children's Hospital in Melbourne (Bishop *et al.*, 1976) and the 116E and 1321 bovine-human reassortant strains isolated from newborns in India (Bhan *et al.*, 1993; Das *et al.*, 1993). Additionally, in some developing countries, nosocomially acquired neonatal rotavirus infections are not uncommon (Kilgore *et al.*, 1996) occurring in up to 40–50% of infants hospitalized for 3 days or longer who usually had no symptoms. In symptomatic infection, the incubation period is 1–3 days and the illness usually lasts 5–7 days. Rotaviral gastroenteritis is generally characterized by frequent vomiting, which starts early in the illness and is commonly associated with mild to moderate dehydration with normal serum sodium concentrations and mild acidosis. Vomiting and diarrhea may both be present initially, but in at least half of the cases vomiting precedes diarrhea. In a study by Rodriguez *et al.* (1977), vomiting persisted for an average of 2.6 days and diarrhea persisted for an average of 5 days with a range of 1–9 days. Fever is common, but signs or symptoms of dysentery, such as bloody diarrhea and tenesmus, indicate concurrent bacterial infection.

How does the host respond to rotavirus infection? A likely scenario is that rotaviruses replicate in mature villus cells, probably cross the basement membrane, enter the lamina propria or attach to membranous epithelial cells (M cells) and enter Peyer's patches. In either of these two sites, rotaviruses are most likely processed by antigen-presenting cells and presented to helper T (T_h) lymphocytes, cytotoxic T lymphocytes (CTLs) and B lymphocytes within Peyer's patches and lamina propria. After virus-specific T_h lymphocytes within the intestine release B lymphocyte growth and differentiation factors, rotavirus-specific IgM and IgA appear at the intestinal mucosal surface. Coincident with the appearance of rotavirus-specific plasma cells in the circulation, B and T_h lymphocytes, rotavirus-specific IgM and IgA also appear in the serum 4–6 days post infection (p.i.). Within one month of infection, virus-specific B and T lymphocyte precursors are probably distributed throughout intestinal and non-intestinal lymphocyte populations (Colomina *et al.*, 1998; McNeal *et al.*, 1998).

Rotavirus excretion has been identified by several techniques that enable detection of 10^5–10^6 particles per ml, such as enzyme immunoassay (EIA), electron microscopy (EM) of negatively stained fecal extracts; polyacrylamide gel electrophoresis (PAGE) of dsRNA extracted from rotavirus-positive stools has reduced the threshold of rotavirus detection to as low as 1000 virus particles per ml (Wilde *et al.*, 1991). Besides these methods of direct detection of rotavirus excretion it has been suggested that fluctuations in rotavirus copro-IgA indirectly indicate replication of rotavirus in the small bowel (Coulson *et al.*, 1990). Also, duration of a rotavirus infection must be established by most sensitive technqiues as human infection can be caused by just ingestion of 1 PFU of the virus (Ward *et al.*, 1986). Historically, EM was used for confirmation of the diagnosis because it was able to demonstrate the characteristic morphology of rotavirus particles. Rapid virus detection assays in stools have replaced older techniques. The most economical and appropriate assay used presently is the enzyme-linked immunosorbent assay (ELISA) or the agglutination test (Kapikian and Chanock, 1996). Dot-blot hybridization and polymerase chain reaction (PCR) assays for rotaviruses are more cumbersome and expensive but may provide much greater sensitivity (Flores *et al.*, 1983; Wilde *et al.*, 1991). Cell cultures for rotaviruses are not available. The optimum time to collect stool for virus assays is between the 1st and the 4th day of illness, although shedding can occur for weeks, depending on clinical manifestations. Several assays now exist for measuring the serological response to the rotavirus infection (Kapikian and Chanock, 1996). As with other pathogens, the detection of rotaviruses or serological tests often are not helpful during acute illness.

Natural history of rotavirus infections and the goal of rotavirus vaccine

Rotavirus infections occur repeatedly in humans from birth to old age, and the majority of infections are asymptomatic or associated with mild gastrointestinal symptoms (Bishop, 1994). This means that even natural infection does not provide complete protection against a subsequent infection or mild diseases associated with it. A

realistic goal of rotavirus vaccine is to prevent a severe rotaviral gastroenteritis during the first two years of life, the period when the rotavirus disease is most serious. Based on the accumulated experience, the priority was given to the production of a live attenuated, orally administered heterologous rotavirus vaccine which was dubbed "the Jennerian approach" (Kapikian *et al.*, 1986). Its modified version, i.e. the modified Jennerian approach was largely supported by the results of field trials (Christy *et al.*, 1993; Bernstein *et al.*, 1995) with variable effectiveness in different settings (Vesikari, 1993; Conner *et al.*, 1994). An approximately 70% effectiveness against severe diarrhea is provided as observed in recent trial of an effective rotavirus vaccine (RRV-TV), which is a combination of 4 different common serotypes of rotavirus (Joensuu *et al.*, 1998; Marwick *et al.*, 1998; Ward *et al.*, 1998).

Vaccines nearing licensure could provide an important new weapon to decrease diarrheal morbidity and mortality in both developed and developing countries. Many new approaches to immunization being pursued are the use of virus-like particles (VLPs) (O'Neal *et al.*, 1998), DNA vaccines (Herrmann *et al.*, 1996), microencapsulated viruses (Moser *et al.*, 1998), and other live strains for oral delivery (Burns *et al.*, 1996). Besides, inhibitors (Katyal *et al.*, 1999) and antioxidants (Sodhi *et al.*, 1996) may also help in preventing rotavirus diarrhea.

Studies in animals have also tried to pinpoint the best antigen candidate. The importance of serum rotavirus IgA antibody has been shown using backpack tumor carrying B cell-deficient adult knock-out mice (Nakagomi *et al.*, 1996). In this model, it was observed that an IgA antibody to inner core protein VP6, rather than to surface proteins VP4 or VP7 provides the best protection against rotavirus (Burns *et al.*, 1996). After vaccination, the strongest immune response is directed towards this antigen (Hashino *et al.*, 1994). The importance of this finding may be in the fact that VP6 is a common group antigen of all human and animal group A rotaviruses, providing a common antigen for protection against heterologous strains of the virus. Each approach will benefit from more detailed studies of the mechanisms of pathogenesis and immunity to the disease.

Problems surmounted for vaccination

Longitudinal and inpatient studies provide evidence that rotavirus is the commonest agent consistently identified in severe diarrhea. Although great progress has been made to reduce morbidity and mortality of infectious diarrhea by imperative improvement of hygiene standards and substitute re-hydration therapies, the progress in preventive measures such as vaccine development has been extremely complex and slow.

The major aim of developing a rotavirus vaccine is to prevent severe diarrhea in young children. It is important to keep in mind that the vaccine is not meant to prevent the disease but to prevent the occurrence of severe diarrhea, because it is unrealistic to assume that a vaccine would provide a better immune response than natural infection. In the latter, total protection usually occurs in 50% of infants while protection from severe disease may occur in more than 90% of naturally infected children. The formation of an effective vaccine strategy is extremely difficult due to the antigenic diversity of human rotaviruses, the complexity of immune response and the diversity of epidemiological settings. The principal problems encountered in development of a rotavirus vaccine have been the serotype specificity of the immune response necessitating the inclusion of multiple serotypes and difficulty in setting the appropriate balance between attenuation and the ability to replicate effectively in the intestine.

An immunization with the RIT 4237 vaccine gave encouraging results in Finland. However, it could not be confirmed elsewhere in the world as the vaccine failed to protect against other serotypes (Ramchandaran *et al.*, 1998) (Table 1).

The protection did not always correlate with the serological response to vaccination and subsequently the vaccine was withdrawn. The Rhesus rotavirus (RRV) reassortant vaccine failed to provide protection due to the presence of pre-existing antibodies and unknown interfering substances present in the hemopoetic system of the infants. Another vaccine, prepared from the naturally occurring attenuated nursery strain M37 (G1 serotype), adapted to monkey kidney cells and replicated very poorly in the gut mucosa (Smith *et al.*, 1995).

In the modified Jennerian approach, the vaccine contains genes of RRV and some of the important genes from human rotavirus (HRV). The major problem with this vaccine (RRV-TV) turned out to be a poor response in developing countries with low socio-economic population. Only 20% and 35% efficacy was seen in Peru and Brazil, respectively (Vesikari, 1999; Vesikari *et al.*, 1991).

Besides, there appear to be a number of factors as far as developing countries and development of rotavirus vaccines are concerned. All vaccines appear to be less effective in tropics when compared to developed countries due to several reasons. Children in these regions may harbor other enteric viruses which may interfere with the immune response. Breast feeding may be another cause of interference. Maternal antibodies may also decrease the effectiveness of the vaccine.

As rotavirus infects only the small intestine, the protection against the disease is believed to be associated with the antibody at the mucosal surface which is extremely difficult to measure in children . In a study of immunity to rotavirus with mouse as a model, it was observed that most protective immunity was directed against VP6 and involved an IgA which is not associated with neutralization *in vitro*. This antibody was not

Table 1. Results of efficacy trials with rotavirus vaccines in developed and developing countries

Vaccine/country	Age of vaccines	No. of enrolled vaccinated/unvaccinated	No. of doses	Vaccine efficacy (%)	
				Diarrhea	Severe disease
(A) RIT 4237					
Developed countries					
Finland (1985)	6-12 months	168/160	2	58	82
Finland (1990)	<1 week,7 months	124/128	2	40	89
Finland (1991)	< 1 week	123/122	1	0	22-100
Developing countries					
Rwanda (1986)	2-5 months	106/107	1	0	0
Gambia (1987)	10 weeks	170/83	3	33	
Peru (1989)	3-8 months	122/123	1	0	0
USA[a] (1992)	2-18 months	99/100[b]	1,2,3[b]	24	0-47
(B) WC3					
Developed countries					
USA (1988)	3-12 months	49/55	1	76	100
USA (1990)	2-12 months	103/103	1	0	
Developing countries					
Central African Republic (1991)	3 months	237/235	2	0	0
Shanghai (1996)	1-2 months	2	50		
(C) Rhesus rotavirus vaccine					
Developed countries					
USA (1986)	5-20 weeks	14/10	1	100	
USA (1988)	4-12 months	53/51	1	48	80
Sweden (1989)	2-4 months	85/88	1	0	0
USA (1990)	2-11 months	63/49	1	29	29
Finland (1990)	2-5 months	100/100	1	38	67
USA (1992)	2-4 months	76/73	1	66	
Developing countries					
Venezuela (1990)	2-5 months	108/107	1	0	0
USA[a] (1991)	1-10 months	151/151	1	64	85-90
(D) Tetravalent vaccines: RRV-TV and WC3-QV					
Developed countries					
RRV-TV/USA (1995)[c]	4-26 weeks	332/330	3	57	82
RRV-TV/USA (1995)[d]	3-5 months	1190/1208	3	68	61-100
RRV-TV/USA (1996)[d]	5-25 weeks	403/400	3	49	80-100
Wc/qv Finland (1997)	2-6 months	206/199	3	67	69
Developing countries					
RRV-TV/Peru (1996)[c]	2-4 months	219/209	3	24	0-40
RRV-TV/Brazil (1996)[d]	8-18 weeks	1112/1095	3	48	88
RRV-TV/Venezuela (1997)[c]	1-5 months	233/233	3	35	46
RRV-TV/USA (1997)[a,d]	2-6 months	347/348	3	50	64

Data from Midthun and Kapikian (1991).

[a]Conducted among a low-socioeconomic, rural population of native Americans.

[b]Only data for the 3-dose regimen are given. Among children receiving 1 or 2 vaccine doses, the vaccine efficacy against all disease was 0; for severe disease, the efficacy was 0–63% and 15–59%, for 1- and 2-dose regimens, respectively,

[c]Vaccine dose of 4 x 10^4 PFU.

[d]Vaccine dose of 4 x 10^5 PFU.

active in the gut lumen but was alive when it reached the gut epithelium from the basolateral side, where it presumably inactivated the virus during its transcytosis into the lumen (Burns *et al.*, 1996). There is an urgent need for definitive studies on the mechanism of mucosal immunity and the antigens responsible for protection against rotavirus infection.

A number of candidates are being evaluated recently as rotavirus vaccines, namely DNA and VLPs. Oral DNA vaccines have been tested in animals; they induced a good response and protected the animals against challenge (Vesikari, 1991). A heterotypic protection from rotavirus infection in mice vaccinated with VLPs has been shown to

have implications for the development of parental vaccines against the rotavirus disease. Immunization by both the mucosal and parental routes provided partial protection and the combined effect of the two immunizations appeared to be additive (Chen *et al.*, 1998).

All oral rotavirus vaccines, regardless of origin, are likely to have many similar properties including disadvantages (e.g. a suppression of uptake of oral poliovirus which may compromise immunogenicity in developing countries).

The first and foremost problem encountered is the protective efficacy of the vaccines in developing countries. There may be several reasons for this: (a) children in these regions may harbor other enteric viruses which may interfere with the immune response; (b) breast feeding may be another cause of interference; (c) maternal antibodies may also decrease the effectiveness of the vaccine; (d) the serotypes responsible for infection in these countries may differ from those present in the vaccines. This is exemplified by a recent study from New Delhi, India, where it has been observed that the prevalent serotype was G9 which is not present in the RRV-TV vaccine (Jiang *et al.*, 1999). To be effective, a vaccine would have to contain this serotype as heterotypic immunity is usually poor.

Recently, Centers for Disease Control and Prevention (McNeal *et al.*, 1999) have analyzed the cost effectiveness of minimization and estimated that, given a vaccine efficacy rate of 50% and a vaccine cost of $ 30 per dose, a rotavirus immunization program would prevent above one million cases of rotavirus diarrhea, 58,000 hospitalizations and 82 deaths per year. A single vaccine program would cost $ 243 million per year but would yield a net saving of $ 79 million from the perspective of the health-care system and $ 466 million from the perspective of society. The incremental cost-effectiveness is a saving of $ 459 per case prevented from the perspective of society and $ 78 per case prevented from the perspective of the health-care system.

References

Arias CF, Romera P, Alvarez V, Lopez S (1996): Trypsin activation pathway of rotavirus infectivity. *J. Virol.* **70**, 5832-5839.

Ball JM, Tian P, Zeng CQY, Morris AP, Estes MK (1996): Age dependent diarrhoea induced by a rotavirus non- structural glycoprotein. *Science* **272**, 101-104.

Bass DM, Greenberg HB (1991): Pathogenesis of viral gastroenteritis. In Field M (Ed.): *Diarrhoeal Diseases*. Elsevier, New York, pp. 139-157.

Belhom T (1999): Rotavirus diarrhea. *Curr. Probl. Pediatr.* **29**, 198-207.

Bern C, Martines J, de Zoya I, Glass RI (1992): The magnitude of global problem of diarrhoeal disease: a ten year update *Bull. WHO* **70**, 705-714.

Bernstein DI, Glass RI, Rodgers G, Davidson BL, Sack DA (1995): Evaluation of rhesus rotavirus monovalent and tetravalent reassortant vaccines in US children *J. Am. Med. Assoc.* **273**, 1191-1196.

Bhan MK, Lew JF, Sazawal S, Das BK, Gentsch JR, Glass RI (1993): Protection conferred by neonatal rotavirus infection against subsequent diarrhea. *J. Infect. Dis.* **168**, 282-287.

Bishop RF, Barnes GL, Cipricin E, Lund JS (1983): Clinical immunity after neonatal rotavirus infection: a prospective longitudinal study in young children. *N. Engl. J. Med.* **309**, 72-76.

Bishop RF, Davidson GP, Holmes IH, Ruck BJ (1973): Virus particles in epithelial cells of duodenal mucosa from children with viral gastroenteritis. *Lancet* **1**, 1281-1283.

Bishop RF (1994): Natural history of human rotavirus infections. In Kapikian AZ (Ed.): *Viral Infections of the Gastrointestinal Tract.* 2nd ed., Marcel Dekker, Inc., New York–Hong Kong, pp. 131-167.

Bishop RF, Hewstone AS, Davidson GP (1976): An epidemic of diarrhea in human neonates involving a reoviruslike agent and enteropathogenic serotypes of Escherichia coli. *J. Clin. Pathol.* **29**, 46.

Broor S, Singh V, Venkateshwarlu Gautam S, Mehta S, Mehta SK (1985): Rotavirus diarrhoea in children in Chandigarh, India. *J. Diarrhoeal Dis. Res.* **3**, 158-161.

Burns J, Sidat-Pajouh, M, Krishnaney AA, Greenberg HB (1996): Protective effects of rotavirus VP_6-specific IgA monoclonal antibodies that lack neutralizing activity. *Science* **272**, 104-107.

Chen SC, Jones DH, Fyan EF *et al.* (1998): Protective immunity induced by oral immunization with rotavirus DNA vaccine encapsulated in microparticles. *J. Virol* **72**, 5757-5761.

Chiristy C, Offit P, Clark HF, Treanor J (1993): Evaluation of a bovine human rotavirus reassortant vaccine in infants. *J. Infect. Dis.* **168**, 1598-1599.

Colomina J, Raga J, Gil MT, Buesa J (1998): Virus-specific serum and fecal antibodies response in children with acute rotavirus gastroenteritis. *Enferm. Infec. Microbiol. Clin.*. **16**, 55-60 (in Spanish?).

Conner ME, Matson DO, Estes MK (1994): Rotavirus vaccines and vaccination potential. *Curr. Top. Microbiol. Immunol.* **185**, 286-337.

Coulson BS Greenwood K, Masendyez PJ *et al.* (1990): Comparison of rotavirus immunoglobulin. A coprocon-version with other indices of rotavirus infection in a logitudinal study in childhood. *J. Clin. Microbiol.* **28**, 1367-1374.

Das M, Durm SJ, Woode GN, Greeenberg AB, Rao CD (1993): Both surface proteins (VP4 and VP7) of an asymptomatic neonatal rotavirus strain (1321) have high levels of sequence identity with the homologous proteins of a serotype 10 bovine rotavirus. *Virology* **193**, 374-379.

Desselberger U (1997): Viral factors determining rotavirus pathogenicity. *Arch. Virol.* (Suppl.) **13**, 131-139.

Dong Y, Zeng CQ, Ball JM, Estes MK, Morris AP (1997): The rotavirus enterotoxin NSP4 mobilizes intracellular calcium in human intestinal cells by stimulating phospholipase C-mediated inositol 1,4,5 triphosphate production. *Proc. Natl. Acad. Sci. USA* **94**, 3960-3965.

Estes MK (1996): Rotaviruses and their replication. In Fields, BN, Knipe DM, Howley PM (Eds): *Fields Virology*. Lippincott-Raven Publishers, Philadelphia, pp. 1625-1655.

Flores J, Purcell RA, Perez I, Wyatt RG, Boeggeman E, Sereno M *et al.* (1983): A dot hybridization assay for detection of rotavirus. *Lancet* **1**, 555-559.

Gilger MA, Matson DO, Connen ME, Rosenblatt HM, Finegold MJ, Estes MK (1992): Extra intestinal rotavirus infections in children with immunodeficiency. *J. Pediatr.* **120**, 912-917.

Hashino Y, Kapikian AZ (1994): Rotavirus vaccine development for the prevention of severe diarrhoea in infants and young children. *Trends Microbiol.* **2**, 242-249.

Herrmann JE, Chen SC, Fynan EF, Santoro JC, Greenberg HB, Robinson HL (1996): DNA vaccines against rotavirus infections. *Arch. Virol.* **12**, 207-215.

Hongou K, Konishi, T, Yagi S, Arachi K, Mejwaski T (1998): Rotavirus encephalitis mimicking afebrile seizure and lesions in infants. *Pediatr. Neurol.* **18**, 354-357.

Jiang B, Estes MK, Barone C, Barnick V, O'Neal CH, Ottaiano A, Madore HP, Conner ME (1999): Heterotypic protection from rotavirus infection in mice vaccinated with virus-like particles. *Vaccine* **17**, 1005-1013.

Joensuu J, Koskenniemi E, Vesikari T (1998): Symptoms associated with rhesus human reassortant rotavirus vaccine in infants. *Pediatr. Infect. Dis. J.* **17**, 334-440.

Kanwar SS, Singh V, Malik AK, Vinayak VK, Komal HS, Pal SR, Mehta SK, Mehta S (1993): Hepatic involvement in rotavirus gastroenteritis in a homologous murine model. *Immunol. Infect. Dis.* **3**, 77-81.

Kapikian AZ, Chanock RM (1996): Rotaviruses. In Field BN, Knife DM, Howley PM (Eds): *Fields Virology.* 3rd ed., Lippon-scott-Raven Publishers, Philadelphia, pp. 1657-1708.

Kapikian AZ, Flores J, Hoshino Y, Glass RI, Midthun K, Gorzilgia M, Chanock RM (1986): Rotaviruses: the major etiologic agent of severe infantile diarrhoea may be controllable by a "Jenneriah approach to vaccination *J. Infect. Dis.* **153**, 815-822.

Katyal R, Ojha S, Rana SV, Vaiphie K, Singh K, Singh V (1999): Protective efficacy of trypsin inhibitor on the gut following rotavirus ingestion in malnourished infant mice. *Ann. Nutr. Metab.* **43(5)**, 319-328.

Kilgore PE, Unicomb LE, Gentsch JR Allert MJ, McElroy CA, Glass RI (1996): Neonatal rotavirus infection in Bangladesh: strain characterization and risk factors for nosocomial infection. *Pediatr. Infect. Dis. J.* **15**, 672-677.

Lanata CF, Midthun K, Black RE, Butron B, Huapaya A, Penny ME *et al.* (1996): Safety, immunogenicity and protective efficacy of one and three doses of the tetravalent rhesus rotavirus vaccine in infants in Sima. Peru. *J. Infect. Dis.* **174**, 268-275.

Linhares AC, Gabbay YB, Mascarenhas JD, De Freitas RB, Oliveria CS, Bellesi N *et al.* (1996): Immunogencity, safety and efficacy of rhesus-human reassortant rotavirus vaccine in Belem, Brazil. *Bull. WHO* **74**, 491-500.

Lundgreen O, Peregrin AT, Persson K, Kordasti S, Whoo I, Svensson L (2000): Role of enteric nervous system in the fluid and electrolyte secretion of rotavirus diarrhea. *Science* **287**, 491-494.

Marwick AJ, Rennels MB, Zito ET, Wade MS, Mack ME (1998): Oral tetravalent rotavirus vaccine can be successfully co-administered with oral poliovirus vaccine and a combined diphtheria, tetnus, pertussis and Haemophilus influenzae type b vaccine. *Pediatr. Infect. Dis. J.* **17**, 913-918.

McNeal MM, Rae MN, Ward RL (1999): Antibody responses and protection stimulated by sequential oral parenteral immunization of mice with rotavirus. *Vaccine* **17**, 639-645.

McNeal MM, Rae MN, Conner ME, Ward RL (1998): Stimulation of local immunity and protection in mice by intramuscular immunization with triple-or double layered particles and QS-21. *Virology* **243**, 158-166.

Midthem K, Kapikian AZ (1996): Rotavirus vaccines: an overview. *Clin. Microbiol. Rev.* **9**, 423-434.

Moser CA, Speaker TJ, Offit PA (1998): Effect of water based microencapsulation on protection against EDIM rotavirus challenge in mice. *J. Virol.* **72**, 3859-3862.

Nakagomi O, Nakagomi T (1996): Rotavirus vaccines: A perspective. *Microbiol. Immunol.* **40**, 1052-1062.

O'Neal CM, Clements JD, Estes MK, Conner ME (1998): Rotavirus 2/6 virus particles administered intranasally with cholera toxin, Esherichia coli heat-labile toxin (LT) and LT-R 192G induce protection from rotavirus challenge. *J. Virol.* **72**, 3390-3393.

Perez-Schael I (1996): The impact of rotavirus disease in Venezuela. *J. Infect. Dis.* **174** (Suppl. 1), 519-521.

Ramchandaran M, Vij A, Kumar R *et al.* (1998): Lack of maternal antibody response to P serotypes may predispose neonates to infections with unusual rotavirus strains. *Clin. Diagn. Lab. Immunol.* **5**, 527-530.

Rodriguez WJ, Kim HW, Arrobio JO, Brandt CD, Chanock RM, Kapikian AZ *et al.* (1977): Clinical features of acute gastroenteritis associated with human reovirus-like agent in infants and young children. *J. Pediatr.* **91**, 188-193.

Smith JC, Haddix AC, Tentsch SM, Glass RI (1995): Cost effectiveness analysis of a rotavirus immunization program for the United States. *Pediatrics* **96**, 609-615.

Sodhi CP, Katyal R, Rana SV, Attri S, Singh V (1996): Study of oxidative stress in rotavirus infected mice. *Indian J. Med. Res.* **104**, 245-249.

Vesikari T, Ruuska T, Koivu H-P, Green KY, Flores J, Kapikian AZ (1991): Evaluation of the M 37 human rotavirus vaccine in 2- to 6 month old infants. *Pediatr. Infect. Dis. J.* **10**, 912-917.

Vesikari T (1993): Clinical trials of live oral rotavirus vaccines: the Finnish experience. *Vaccine* **11**, 255-261.

Vesikari T (1999): Rotavirus vaccines: development and use for the prevention of diarrheal disease. *Ann. Med.* **31**, 79-35.

Ward RL, Bernstein DI, Young EC, Sherwood JR, Knowlton DR, Schiff GM (1986): Human rotavirus studies in volunteers: determination of infectious dose and serological response to infection. *J. Infect. Dis.* **154**, 871-880.

Ward RL, Dinsmore AM, Goldberg G, Sander DS, Rappaport RS, Zito ET (1998): Shedding of rotavirus after administration of the tetravalent rhesus rotavirus vaccine. *Pediatr. Infect. Dis. J.* **17**, 386-390.

Wilde J, Yolken R, Willoughly R, Eiden J (1991): Improved detection of rotavirus shedding by polymerase chain reaction. *Lancet* **337**, 323-326.

Zhang M, Zeng CO, Dong Y, Ball JM, Saif LJ, Morris AP *et al.* (1998): Mutations in rotavirus non structural glycoprotein NSP4 are associated with altered virus virulence. *J. Virol.* **72**, 3666-3672.