Clin. Immunother. 3 (6): 476-484, 1995
1172-7039/95/0006-0476/$04.50/0

Developments in the Treatment of Rotaviral Gastroenteritis

Oral Therapy with Immunoglobulins and Prospects for a Vaccine

Alfredo Guarino, Roberto Berni Canani and *Stefania Russo*

Department of Pediatrics, University 'Federico II' of Naples, Naples, Italy

Contents

Summary

Rotavirus is the most frequent agent of gastroenteritis in both industrialised and developing countries. It is responsible for a high number of deaths, hospitalisations and nosocomial infections. Besides rehydration, no specific therapy effective against the virus has so far been developed. However, immunotherapy involving oral administration of human serum immunoglobulin meets the criteria for ideal drug therapy for the following reasons: (a) commercially available immunoglobulin preparations have high neutralising antibody titres directed toward rotavirus; (b) the antibodies survive the gastric barrier and remain active throughout the intestine; (c) no adverse effects are associated with oral administration of immunoglobulin.

Preliminary studies have shown the efficacy of a single oral administration of immunoglobulin to children with severe and protracted rotaviral diarrhoea. Subsequently, a double-blind case- and placebo-controlled trial showed that immunotherapy was effective in decreasing the severity of symptoms and the duration of diarrhoea, viral excretion and hospital stay in children admitted with acute rotaviral gastroenteritis. The cost effectiveness of immunotherapy was also

clearly proved. This therapy should therefore be considered for children admitted with rotaviral diarrhoea.

Because rotavirus is a large scale worldwide problem, attempts at making an effective vaccine are being pursued. Initial approaches were based on the use of high-passage attenuated rotavirus strains obtained from animals. Genetically engineered strains were also used. However, clinical trials have been only partially successful. The use of new tools available from molecular biology may help to overcome the problem of efficacy and duration of vaccine-induced immunity.

1. Role of Rotavirus as an Enteric Pathogen

Infantile diarrhoea remains a major cause of morbidity and mortality worldwide. Rotavirus is the leading cause of acute gastroenteritis in developing as well as in industrialised countries.[1] The major epidemiological features of rotaviral diarrhoea are summarised in table I.

Rotaviral infection is among the most contagious of diseases. It may spread not only through the faecal-oral route, but also through hands, fomites and even through the air.[2] The impact of rotaviral diarrhoea varies depending on socioeconomic conditions.

In industrialised countries, rotavirus is a major cause of morbidity in children below 3 years of age. Diarrhoea due to rotavirus is usually mild and self-limiting. However, rotavirus is responsible for 2 to 8 hospital admissions per annum per 1000 children, which is over 100 000 hospital admissions and approximately 580 000 days of hospitalisation in the US per year.[3] The case fatality rate for rotavirus infection is not negligible, since approximately 150 deaths are reported each year in the US.[4] The estimated cost of hospital care is approximately $US30 million per year.[3]

Table I. Major epidemiological features of rotavirus-induced diarrhoea

Single most common agent of diarrhoea in infants
Peaks in winter season in temperate climates
Sometimes epidemic, usually sporadic
Major cause of nosocomial infections
Protracted excretion in immunodeficient patients
Spread through oral-faecal route, hands, airborne droplets
Long survival in environment or water
Asymptomatic carriers
High prevalence of antibodies in children

In developing countries, rotavirus is responsible for over 125 million cases of gastroenteritis annually, of which 18 million cases are considered severe. The case fatality rate is dramatically high and rotavirus is responsible for 800 000 to 900 000 deaths per year.[1]

Rotavirus gastroenteritis shows a typical seasonal pattern with a winter peak in temperate climates, whereas it is present all year round in tropical countries.[5,6]

2. Rationale for the Use of Immunotherapy

2.1 Existing Treatment

As in all forms of viral gastroenteritis, the management of fluid and electrolyte balance in patients is the essential treatment. Bismuth salicylate has been shown to be beneficial in improving clinical manifestations as an adjunct to oral rehydration solution.[7] It has been suggested that Bifidobacteria, which predominate in the intestinal flora of breast-fed infants, may be active against rotavirus.[8,9] *In vivo* and *in vitro* evidence has been reported suggesting that oral administration of a particular lactobacillus, strain GG, is able to counteract rotaviral infection.[10-12] The mechanism of this effect is unknown, but preliminary data suggest that it may be immunologically mediated.[13]

Specific antiviral drugs for rotavirus illness in humans are not currently available. Several substances, such as protease inhibitors and nucleoside and nucleoside triphosphate analogues, have been suggested to have some activity against human

rotavirus through an inhibition of viral replication and transcription, but the practicality of the administration of those drugs to humans has not yet been demonstrated.[14]

An ideal drug therapy of diarrhoeal infectious disease should decrease stool output, preventing dehydration. Furthermore, an ideal drug should have a clear microbicidal effect, leading to earlier clearance of the responsible agent and also preventing its spreading. It is also desirable that the drug can be administered orally, preferably as a single dose, that it does not interfere with normal gut function, and is free from major adverse effects. Finally, an ideal drug should be widely available at low cost and proven to be cost effective.[15]

At present, the antidiarrhoeal drugs available for infantile diarrhoea include antisecretory and/or proabsorptive agents, drugs active on intestinal motility, drugs that alter stool consistency and, finally, antimicrobials.[16] However, immunotherapy for viral diarrhoea is a novel approach that may meet the criteria of ideal drug treatment for this condition.

2.2 Immunology of Rotaviral Infection

The mechanisms of the immune response of the host and the duration of effective immunity against rotavirus infection are not fully understood.[17-19] Rotavirus infection has an age-related pattern (fig. 1). Most children requiring hospitalisation are younger than 24 months, and are usually in the age range 3 to 15 months.[20] This is confirmed by the finding that most children have developed antirotavirus antibodies by 2 years of age,[21] and virtually every child in the US has been exposed to rotavirus by 4 years of age.[22]

Adult subjects can easily be infected with rotavirus, but they are usually symptomless. It has been suggested that this is related to a protective effect of specific serum antibodies acquired earlier in life.[23] However, there is evidence that suggests the importance of local antibody in prevention and treatment of this diarrhoeal disease. It is already known that the presence of antibody in serum does not prevent rotavirus infection.[24-26] In the neonate, rotavirus infection is usually symptomless or causes a very mild diarrhoea. There is no significant difference in rotavirus-inhibitory activity in cord blood sera of infected and noninfected infants, suggesting that cord blood antibodies do not provide protection against neonatal rotavirus infections. On the contrary, antirotavirus antibodies can be detected in breast milk and breast-fed infants are protected against viral diarrhoea.[27] To provide protection it is therefore necessary for the antibody to reach the small intestine, where rotavirus is localised.

2.3 Use of Human Serum Immunoglobulin

Having established that local humoral immunity has a protective role against rotavirus, 3 problems remain to be solved:

- a source of specific antibody
- whether the antibody can survive the gastric barrier to reach the small intestine, the target site of rotavirus infection
- whether the specific antibody exerts an effective antiviral activity at the intestinal level.

Data have been obtained in the last few years that answer all 3 questions. First, as already mentioned,[1] rotavirus is an ubiquitous agent and most adults have significant titres of antirotaviral antibody.[23] Therefore it is predictable that human serum immunoglobulin preparations will contain significant titres of antirotaviral antibodies. Indeed, high antibody titres against the 4 rotaviral serotypes of medical importance have been detected in commercially available human serum immunoglobulin preparations.[28,29]

Secondly, orally administered heterologous antibodies can resist proteolytic degradation during gastrointestinal passage.[29-31] Recently, it has been shown that some reduction of rotavirus neutralising activity does occur after gastric-phase digestion, and that this is due to the acidic pH and not to proteolytic degradation.[32] However, specific antirotaviral activity has been found in the stools after oral administration of immunoglobulins.[33]

Thirdly, *in vitro* as well as *in vivo* evidence has been obtained in favour of the antiviral efficacy of

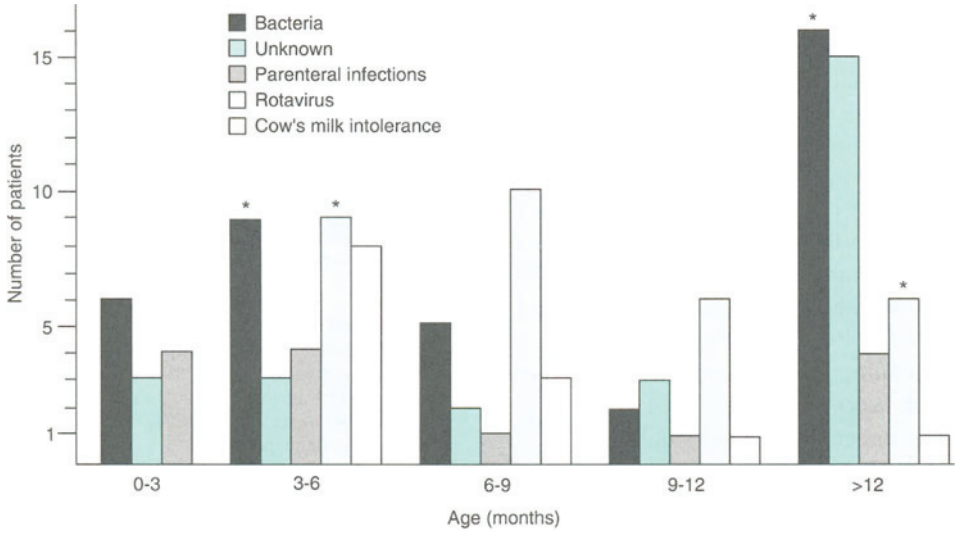

Fig. 1. Age distribution of 118 patients with diarrhoea classified by aetiology (reproduced from Capano et al.,[20] with permission). *Symbol:* * = these columns include 2 patients with the combined presence of *Salmonella* and rotavirus in the faeces.

immunoglobulin at the intestinal level. This will be discussed in detail in sections 3 and 4.

3. Administration of Oral Immunoglobulin to Patients with Rotaviral Diarrhoea

Rotavirus may be responsible for severe protracted diarrhoea, especially in immunodeficient children[34] and, less frequently, in immunocompetent children. The prominent role of rotavirus as agent of intractable diarrhoea in immunocompetent children has now been established. Indeed, rotavirus is the most common infectious agent of the so-called intractable diarrhoea syndrome, which often results in a fatal outcome.[35]

Data have been published supporting the efficacy of oral administration of serum human immunoglobulin to immunodeficient as well as immunocompetent children with persistent rotaviral diarrhoea. Losonsky et al.[33] reported that oral administration of human serum immunoglobulin to 3 immunodeficient patients resulted in the disappearance of free viral antigen from the stools, which was permanent in 1 case.

We have reported that administration of human serum immunoglobulin through a nasoduodenal tube to 2 children with rotaviral enteritis, lasting 5 and 7 months respectively and requiring long term total parenteral nutrition, resulted in the prompt and permanent clearance of the virus in both children. Neither patient had evidence of immunodeficiency and in both cases full and permanent recovery was obtained.[36] Following this preliminary report, other children with so-called intractable diarrhoea caused by rotavirus have been successfully treated with oral administration of immunoglobulin.[35] Furthermore, several children with protracted (>2 weeks) diarrhoea have also been successfully treated with oral immunoglobulin. In few cases, administration of a single dose of immunoglobulin was ineffective in clearing the virus, but a second dose was generally sufficient to eradicate the infection. However, data on the outcome of children with chronic rotaviral enteritis treated with oral immunotherapy are only preliminary, and case-controlled studies are needed in order to establish the efficacy of this treatment in patients with chronic viral diarrhoea.

Subsequently, a clinical trial was conducted to establish the efficacy of oral administration of serum human immunoglobulin in children admitted to hospital because of acute rotaviral gastroenteritis.[28] Children admitted with acute gastroenteritis associated with rotavirus positivity in the stools were enrolled and randomly assigned to group A (treated) or group B (controls).Those in group A received a single oral dose of human serum immunoglobulin 300 mg/kg, whereas children in group B received placebo. Parameters of efficacy were:

- clinical condition, as determined by a clinical assessment scoring system
- frequency and consistency of stools
- duration of diarrhoea
- duration of viral excretion
- length of hospital stay.

Children receiving immunotherapy had a better outcome than those in the control group, as judged by each of the parameters chosen prospectively. Interestingly, the duration of diarrhoea, of viral excretion and of hospital stay was reduced in a parallel fashion in children receiving human serum immunoglobulin compared with controls (fig. 2), suggesting that the efficacy was related to a direct antiviral effect. This hypothesis was supported by the presence of specific neutralising activity in the immunoglobulin preparation used.

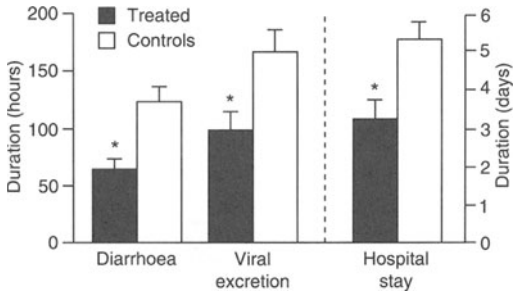

Fig. 2. Duration of diarrhoea and of viral excretion (in hours) and length of hospitalisation (in days) of children with rotavirus gastroenteritis treated either with a single oral dose of human serum immunoglobulin 300 mg/kg or with placebo. Part of these data have been reported previously.[28] *Symbol:* * = statistically significant decrease, $p < 0.01$.

4. Effect of Immunoglobulins on Rotaviral Infection of Enterocytes *In Vitro*

Preliminary data on the effect of human serum immunoglobulin on rotavirus infection of enterocytes have recently been obtained using an *in vitro* model.[37] Immunoglobulin was shown to counteract the cell damage induced by rotavirus in a dose- and time-dependent manner. Preincubation of rotavirus with immunoglobulin, prior to addition of the cells, was able to prevent infection. Furthermore, when immunoglobulin was added at early times after infecting the cells with rotavirus, the cytopathic damage was reduced and cell integrity was promptly restored.[37] This suggests that the efficacy of immunotherapy is, at least in part, related to its early administration.

5. Adverse Effects of Oral Administration of Immunoglobulin

Transmission of viral infections through the intravenous administration of immunoglobulin may occur.[38] However, several lines of evidence support the safety of oral administration of immunoglobulin. Human serum immunoglobulin preparations that are commercially available undergo specific treatment to inactivate potential pathogenic viruses, including those responsible for AIDS and hepatitis.[38,39] The oral route, compared with intravenous administration, is at low risk of adverse reactions. Indeed, no major adverse effects, nor transmission of viral infections, after oral immunoglobulin administration have been observed by us or by other groups.[28,40]

Finally, it is of interest to note that oral administration of immunoglobulin does not interfere with the normal immune response.[41]

6. Immunotherapy for Prevention of Rotaviral Diarrhoea

The efficacy of oral administration of antibody for the prevention of rotavirus disease has been demonstrated previously in animal models.[42,43] Local administration of bovine or human antibody

to rotavirus has proved capable of preventing rotavirus diarrhoea in infants.[44-46] A randomised trial of oral human gammaglobulin in the first week of life showed that immunotherapy was effective in decreasing the severity of symptoms in patients who had received the treatment as compared with controls.[45]

Furthermore, the addition of bovine antibody to the infant formula given to healthy infants was associated with a reduction of the number of days with diarrhoea per year in those receiving the supplemented formula compared with children receiving identical powdered formula without added antibody.[47] However, a recently published report describing a trial of an infant formula containing milk-derived antirotavirus antibodies from hyperimmunised cows showed that the incidence of rotaviral diarrhoea was not decreased in children receiving the enriched formula.[48] Furthermore, these investigators estimated that the increase of the price of enriched milk would be 20 to 30%. This cost, together with the lack of consistent beneficial results in terms of protection, make it difficult to envision a large scale use of immunoglobulin-enriched milk to prevent rotaviral diarrhoea.

7. Economic Effect of Immunotherapy

When choosing a therapy for such a common problem, the important point of cost effectiveness needs to be carefully considered. It has been calculated that the cost of oral treatment with human serum immunoglobulin at 300 mg/kg of an infant weighing 10kg is approximately $US200.[28] However, as an average, a 2-day reduction of hospitalisation may be expected for a child admitted with acute gastroenteritis (fig. 2), allowing a considerable reduction of the expenses related to hospital care.[28] In addition, a parallel reduction of working days lost by the parents of the children can also be expected, adding to the cost effectiveness of immunotherapy.

The shorter duration of viral excretion after immunotherapy can ultimately reduce the spreading of viral particles in the hospital setting. Therefore another potential benefit of immunotherapy for rotaviral diarrhoea is its likely effectiveness in reducing the incidence of nosocomial infections. It should be noted that rotavirus is among the major agents of nosocomial infections.[49]

8. Prospects for a Vaccine

Measures to prevent rotavirus spreading in the general population are only partially effective, as suggested by the similar incidence of rotavirus gastroenteritis in countries of varying sanitary standards.[50-52] The need for a vaccine is clear, and there have been many attempts at inducing active immunisation against rotavirus infection in infants and children.[53-55] However, in spite of the high priority given to development of vaccines for active immunisation, no satisfactory vaccines against rotavirus have yet been obtained.

8.1 Problems in Developing Rotaviral Vaccines

The principal problems that have been encountered in attempts to develop a vaccine capable of protecting against rotavirus infection are summarised in table II and discussed in sections 8.1.1 to 8.1.3.

8.1.1 Clinical Markers of Rotavirus Immunity

Serum antibodies are not good predictors of intestinal immune status, since transplacental transfer of maternal rotavirus-specific antibodies prevents discrimination of actively from passively acquired humoral immune response in infants.

Recently, a significant correlation between the presence of faecal antirotavirus immunoglobulin A and resistance to infection has been demonstra-

Table II. Problems in developing an effective vaccine against rotavirus

Serotype variability
Type-specific immunity
Immune response directed to stable epitopes that are not neutralising
Immune response directed to neutralising epitopes that are variable
Conserved neutralising epitopes are immunorecessive
Lack of agreement between systemic and local immune response
Lack of established markers of protection

ted.[56] Another important step has been the development of an assay to detect rotavirus-specific helper T cells among circulating mononuclear cells.[57] Several recent studies have documented the importance of these cells, as well as of CD8+ cytotoxic T lymphocytes, in the response to rotavirus infection in animal models.[58] Therefore, specific immunological markers will be available in the near future to establish the efficacy of vaccines.

8.1.2 Type-Specific Immunity

The immunity induced by live attenuated vaccines is generally type specific. This can be responsible for inadequate protection because of the several serotypes that circulate simultaneously, even within a single outbreak and in the same location.[59]

Furthermore, variations in the characteristics of rotaviruses may arise from mutations, genomic reassortments, genome rearrangements, or from modifications of viruses from animal sources.[60] This may select rotavirus strains that are resistant to vaccine-induced immunity.

8.1.3 Serological Varieties of Circulating Rotaviruses

The lack of a specific assessment of the serological varieties of circulating rotaviruses is a major problem. To overcome this, a new classification scheme was recently proposed. Each rotavirus strain was designated to have a G type (defined by VP7) and a P type (defined by antibodies to the protease-sensitive VP4), in a manner similar to the scheme used for influenza viruses. In humans, 8 rotavirus G types and 6 P types have been identified.[61] However, many viruses have yet to have their P types identified.[61] To facilitate this process, a convenient monoclonal antibody–based P typing immunoassay has recently been established.[62]

Overall, a more precise assessment of the serological varieties of circulating rotavirus remains essential to the development of an effective vaccine strategy.

8.2 Clinical Trials of Vaccines

Extensive vaccine field trials have now been performed in both developed and less-developed countries.[63-65]

Several vaccines so far have been developed by a jennerian approach, using a rotavirus strain derived from bovine or rhesus monkey, and by a modified jennerian approach with development of a quadrivalent vaccine composed of rhesus rotavirus and 3 reassortant rotavirus strains.[66] However, field trials of candidate vaccines have been disappointing so far. In only 1 study, conducted in Finland, have investigators found candidate oral rotavirus vaccines to be useful in clinical protection against severe gastroenteritis in the first 2 or 3 years of life.[67] These investigators recommended vaccination in infants aged <12 months.

Promising studies are being conducted using novel approaches such as:

- vaccines derived from naturally attenuated strains isolated from neonates[68]
- subunit vaccines[69]
- a recombinant adenovirus vector that expresses a modified rotavirus VP7 protein, a major neutralisation antigen.[70]

However, little success has been reported so far and no vaccine is currently licensed and available for immunisation of young infants. Furthermore, the cost effectiveness of such strategies is, at this time, only hypothetical and difficult to foresee.

9. Conclusions

Oral therapy with human serum immunoglobulin is presently available and represents, so far, a uniquely effective treatment for children with this debilitating and sometimes life-threatening disease.

Oral administration of human serum immunoglobulin matches most of the criteria for ideal drug therapy of rotavirus infection listed in section 2.1, and there is an increasing body of evidence to support the clinical efficacy of this treatment. The data obtained suggest that oral immunotherapy with immunoglobulins should be administered to children

admitted to hospital because of rotaviral diarrhoea, and indeed we now consider immunotherapy mandatory in children presenting with intractable rotaviral diarrhoea as it is thus far the only effective treatment for such a life-threatening disease.

A major problem is the expense and limited availability of human serum immunoglobulin in those countries where rotavirus infection is most dangerous for affected children. It is therefore an ethical commitment to provide resources to make this therapy widely available in developing countries. The cost of immunoglobulin for oral administration could be reduced by, for example, using less-purified product.

Vaccines show great promise for the future prevention of rotavirus gastroenteritis, but so far rotavirus vaccination has been demonstrated only to reduce the clinical severity of this type of infectious gastroenteritis in a number of small scale studies.

Acknowledgements

This work was supported in part by a grant from Consiglio Nazionale Ricerche Target Project 'Biotechnology and Bioinstrumentation', research program 91.01231. P.F. 70, and by a grant from Ministero della Sanità, AIDS research project (1994), program 9205-30.

References

1. Prospects for immunizing against rotavirus. In: Institute of Medicine. New vaccine development: establishing priorities. Vol. 1. Diseases of importance in the United States. Washington DC: National Academy Press, 1985: 410-23
2. Prince DS, Astry C, Vonderfecht S, et al. Aerosol transmission of experimental rotavirus infection. Pediatr Infect Dis J 1986; 5: 218-22
3. Matson OM, Estes MK. Impact of rotavirus infection at a large pediatric hospital. J Infect Dis 1990; 162: 598-604
4. Ho MS, Glass RI, Pinsky PF, et al. Rotavirus as a cause of diarrheal morbidity and mortality in the United States. J Infect Dis 1988; 5: 1112-6
5. Communicable disease report: rotavirus surveillance, 1991. Public Health Laboratory Service Communicable Disease Report. Vol. 20. London: Public Health Laboratory Service, 1991: 1
6. Le Baron CW, Lew J, Glass R. Annual rotavirus epidemic patterns in North America: results of a 5-year retrospective survey of 88 centers in Canada, Mexico and the United States. Rotavirus Study Group. JAMA 1990; 264: 783-8
7. Soriano BH, Avendano P, O'Ryan M, et al. Bismuth subsalicylate in the treatment of acute diarrhea in children: a clinical study. Pediatrics 1991 ; 87: 18-27
8. Ogawa K, Ben RA, Pons E, et al. Volatile fatty acids, lactic acid, and pH in the stools of breast-fed and bottle-fed infants. J Pediatr Gastroenterol Nutr 1992; 15: 246-7
9. Duffy LC, Zielezny M, Riepenhoff-Talty D, et al. Effectiveness of *Bifidobacterium bifidum* in mediating the clinical course of murine rotavirus diarrhea. Pediatr Res 1994; 35: 690-5
10. Isolauri E, Juntunen M, Rautanen T, et al. A human lactobacillus strain (*Lactobacillus casei* sp. strain GG) promotes recovery from acute diarrhea in children. Pediatrics 1991; 88: 90-7
11. Kaila M, Isolauri E, Soppi E, et al. Enhancement of the circulating antibody secreting cell response in human diarrhea by a human lactobacillus strain. Pediatr Res 1992; 32: 141-4
12. Isolauri E, Majamaa H, Arvola T, et al. *Lactobacillus casei* strain GG reverses increased intestinal permeability induced by cow milk in suckling rats. Gastroenterology 1993; 105: 1643-50
13. Majamaa H, Isolauri E, Saxelin M, et al. Lactic acid bacteria in rotavirus diarrhea. Microecol Ther. In press
14. Pizarro JM, Pizarro JL, Fernandez J, et al. Effect of nucleotide analogues on rotavirus transcription and replication. Virology 1991; 184: 768-72
15. Ludan AC. Current management of acute diarrhoeas: use and abuse of drug therapy. Drugs 1988; 36 Suppl. 4: 18-25
16. Powell DW, Szanter KE. Non antibiotic therapy and pharmacotherapy of acute infectious diarrhea. Gastroenterol Clin North Am 1993; 22: 683-707
17. Bishop R, Lund J, Cipriani E, et al. Clinical, serological and intestinal immune responses to rotavirus infection of humans. Med Virol 1990; 9: 85-109
18. Kapikian AZ, Chanock RM. Rotaviruses. In: Fields B, Knife DM, editors. Virology. 2nd ed. New York: Raven Press, 1990: 1353-404
19. Matsui SM, Mackow ER, Greenberg HB. Molecular determinant of rotavirus neutralization and protection. Adv Virus Res 1989; 36: 181-214
20. Capano G, Guandalini S, Guarino A, et al. Enteric infections, cow's milk intolerance and parenteral infections in 118 consecutive cases of acute diarrhoea in children. Eur J Pediatr 1984; 142: 281-5
21. Yolken RH, Wyatt RG, Zissis G, et al. Epidemiology of human rotavirus types 1 and 2 as studied by enzyme linked immunosorbent assay. N Engl J Med 1978; 299: 1156
22. LeBaron CW, Furutan NP, Lew JF, et al. Viral agents of gastroenteritis: public health importance and outbreak management. MMWR Morb Mortal Wkly Rep 1990; 5: 1-24
23. Wenman WM, Hinde D, Felthan S, et al. Rotavirus infection in adults: results of a prospective family study. N Engl J Med 1979; 301: 303
24. Offit PA, Clark HF. Protection against rotavirus-induced gastroenteritis in a murine model by passively acquired gastrointestinal but not circulating antibodies. J Virol 1985; 54: 58-64
25. Snodgrass DR, Well RW. Rotavirus infection in lambs: studies on passive protection. Arch Virol 1976; 52: 201-5
26. Woode GN, Bridges JC. Viral enteritis of calves. Vet Res 1975; 96: 85-8
27. Totterdell BM, Chrystie IL, Banatvale JE. Cord blood and breast-milk antibodies in neonatal rotavirus infection. BMJ 1980; 280: 828-30
28. Guarino A, Berni Canani R, Russo S, et al. Oral immunoglobulins for treatment of acute rotaviral gastroenteritis. Pediatrics 1994; 93: 12-6
29. Brussow H, Hilpert H, Walther I, et al. Bovine milk immunoglobulins for passive immunity to infantile rotavirus gastroenteritis. J Clin Microbiol 1987; 25: 982-6
30. Blum PM, Phelps DL, Ank BJ, et al. Survival of oral human immune serum globulin in the gastrointestinal tract of low birth weight infants. Pediatr Res 1981; 15: 1256-60

31. McClead RE, Gregory SA. Resistance of bovine colostral anti-cholera toxin antibody to *in vitro* and *in vivo* proteolysis. Infect Immun 1984; 44: 474-8
32. Petschow BW, Talbott RD. Reduction in virus-neutralizing activity of a bovine colostrum immunoglobulin concentrate by gastric acid and digestive enzymes. J Pediatr Gastroenterol Nutr 1994; 19: 228-35
33. Losonsky GA, Johnson JP, Winkelstein JA, et al. Oral administration of human serum immunoglobulin in immunodeficient patients with viral gastroenteritis. J Clin Invest 1985; 76: 2362-7
34. Saulsbury FT, Winkelstein JA, Yolken RH. Chronic rotavirus infection in immunodeficiency. J Pediatr 1980; 97: 61-5
35. Guarino A, Spagnuolo MI, Russo S, et al. Etiology and risk factors of severe and protracted diarrhea. J Pediatr Gastroenterol Nutr 1995; 20: 173-8
36. Guarino A, Guandalini S, Albano F, et al. Enteral immunoglobulins for treatment of protracted rotaviral diarrhea. Pediatr Infect Dis J 1991; 10: 612-4
37. Casola A, Bruzzese E, Berni Canani R, et al. *In vitro* effect of immunoglobulins on rotavirus-enterocyte interaction. J Pediatr Gastroenterol Nutr 1994; 19: 335
38. Schiff RI. Transmission of viral infections through intravenous immune globulin. New Engl J Med 1994; 331: 1649-50
39. Acquired immunodeficiency syndrome. WHO meeting and consultation on safety of blood and blood products. WHO Wkly Epidemiol Rec 1986; 18: 138-40
40. Bernhisel-Broadbent J, Yolken RH, Sampson HA. Allergenicity of orally administered immunoglobulin preparations in food-allergic children. Pediatrics 1991; 87: 208-14
41. Snodgrass DR, Madely CR, Weels PW. Human rotavirus in lambs: infection and passive protection. Infect Immun 1977; 16: 268
42. Schaller JP, Saif LJ, Cordle CT, et al. Prevention of human rotavirus-induced diarrhea in gnotobiotic piglets using bovine antibody. J Infect Dis 1992; 165: 623-30
43. Lecce JG, Leary HL, Clare DA, et al. Protection of agammaglobulinemic piglets from porcine rotavirus infection by antibody against simian rotavirus SA-11. J Clin Microbiol 1991; 29: 1382-6
44. Ebina T, Sato A, Umezu K, et al. Prevention of rotavirus infection by oral administration of cow colostrum containing anti-human-rotavirus antibody. Med Microbiol Immunol 1985; 174: 177-85
45. Barnes GL, Doyle LW, Hewson PH, et al. A randomized trial of oral gammaglobulin in low-birth-weight infants infected with rotavirus. Lancet 1982; 1: 1371-3
46. Davidson GP, Whyte PBD, Daniels E, et al. Passive immunization of children with bovine colostrum containing antibodies to human rotavirus. Lancet 1989; 2: 709-12
47. Turner RB, Douglas KH. Passive immunization for prevention of rotavirus illness in healthy infants. Pediatr Infect Dis J 1993; 12: 718-22
48. Brunser O, Espinoza J, Figueroa G, et al. Field trial of an infant formula containing anti-rotavirus and anti-*Escherichia coli* milk antibodies from hyperimmunized cows. J Pediatr Gastroenterol Nutr 1992; 15: 63-72
49. Ford-Jones E, Mindorff C, Gold R, et al. The incidence of viral-associated diarrhea after admission to a pediatric hospital. Am J Epidemiol 1990; 131 : 711 -8
50. Crawley JMS, Bishop RF, Barnes GC. Rotavirus gastroenteritis in infants aged 0-6 months in Melbourne, Australia: implications for vaccination. J Paediatr Child Health 1993; 29: 219-21
51. Kim HW, Brandt CD, Kapikian AZ, et al. Human reovirus-like agent (HRVLA) infection: occurrence in adult contacts of pediatric patients with gastroenteritis. JAMA 1977; 238: 404-7
52. Menon S, Santosham M, Reid R, et al. Rotavirus diarrhea in Apache children: a case control study. Int J Epidemiol 1990; 19: 715-21
53. Flores J, Kapikian AZ. Vaccines against viral diarrhea. Bailliere's Clin Gastroenterol 1990; 4: 675-93
54. Kapikian AZ, Flores J, Midthun K, et al. Strategies for the development of a rotavirus vaccine against infantile diarrhea with an update on clinical trials of rotavirus vaccines. Adv Exp Med Biol 1989; 257: 67-89
55. Wyatt RG, Kapikian AZ, Greenberg HB, et al. Development of vaccines against rotavirus disease. Prog Food Nutr Sci 1983; 7: 189-92
56. Matson DO, O'Ryan ML, Herrera I, et al. Fecal antibody responses to symptomatic and asymptomatic rotavirus infection. J Infect Dis 1993; 167: 577-83
57. Offit PA, Hoffenberg EJ, Pia ES, et al. Rotavirus specific helper T cell responses in newborns, infants, children, and adults. J Infect Dis 1992; 165: 1107-11
58. Dharakul T, Labbe M, Cohen J, et al. Immunization with baculovirus-expressed recombinant rotavirus proteins VP1, VP4, VP6, and VP7 induces CD8+ T lymphocytes that mediate clearance of chronic rotavirus infection in SCID mice. J Virol 1991; 65: 5928-32
59. Beards GM, Desselberger U, Flewett TH. Temporal and geographical distributions of human rotavirus serotypes from 1983 to 1988. J Clin Microbiol 1989; 27: 2827-33
60. Anon. Puzzling diversity of rotaviruses [editorial]. Lancet 1990; 335: 573-5
61. Dunn SJ, Greenberg HB, Ward RL, et al. Serotyopic and genotyopic characterization of human serotype 10 rotaviruses from asymptomatic neonates. J Clin Microbiol 1993; 31: 165-9
62. Coulson BS. Typing of human rotavirus VP4 by an enzyme immunoassay using monoclonal antibodies. J Clin Microbiol 1993; 31: 1-8
63. Flores J, Perez Schael I, Gonzales M, et al. Protection against severe rotavirus diarrhoea by rhesus rotavirus vaccine in Venezuelan infants. Lancet 1987; 1 : 882-4
64. Bernstein DI, Smith VE, Sander DS, et al. Evaluation of WC3 rotavirus vaccine and correlates of protection in healthy infants. J Infect Dis 1990; 162: 1055-62
65. Rennels MB, Losonsky GA, Yong AE, et al. An efficacy trial for the rhesus rotavirus vaccine in Maryland. Am J Dis Child 1990; 144: 601-4
66. Levine MM, Noriega F. Vaccines to prevent enteric infections. Bailliere's Clin Gastroenterol 1993; 7: 501-17
67. Vesikari T. Clinical trials of oral rotavirus vaccines: the Finnish experience. Vaccine 1993; 11: 255-61
68. Flores J, Perez-Schael I, Blanco M, et al. Comparison of reactogenicity and antigenicity of M37 rotavirus vaccine and rhesus-rotavirus–based quadrivalent vaccine. Lancet 1990; 2: 330-4
69. Streckert HJ, Brussow H, Werchaw H. A synthetic peptide corresponding to the cleavage region of VP3 from rotavirus SA11 induces neutralizing antibodies. J Virol 1988; 62: 4265-9
70. Both GW, Lockett LJ, Janardhana V, et al. Protective immunity to rotavirus-induced diarrhoea is passively transferred to newborn mice from naive dams vaccinated with a single dose of a recombinant adenovirus expressing rotavirus VP7sc. Virology 1993; 193: 940-50

Correspondence and reprints: Dr *Alfredo Guarino*, Department of Pediatrics, University 'Federico II' of Naples, Via S. Pansini 5, I-80131 Naples, Italy.