Review Article

PRESENT STATUS OF ROTA VIRUS VACCINE

Shobhana D. Kelkar

Rotaviruses belonging to the family Reoviridae are recognized as the most important cause of severe viral gastroenteritis in humans and animals(l,2).

Groups, Subgroups and Serotypes of Rotaviruses

The genome of rotavirus is double stranded RNA having 11 segments. They are classified into seven distinct groups A through G. Rotaviruses belonging {o different groups can be differentiated by RNA electrophoretic patterns. Members within a group are classified further into subgroups and serotypes. Rotaviruses are devoid of envelope and have two capsides, inner and outer one. Among the major structural proteins of rotavirus, which are of antigenic importance are VP4, VP6 and VP7 *(Fig. 1)*. The major component of the inner capsid VP6, is coded by gene segment 6, and represents 60% of the total protein in an intact virion(3). Non neutralizing epitopes are located on this protein. Two of such epitopes are called subgroup antigens (Subgroups I and II).

The outer protein shell of rotavirus contains two distinct polypeptides, VP4 and VP7. These polypeptides induce antibodies with neutralizing activity. VP4 also induces cross neutralizing antibodies to certain extent. It is an important protein which is associated with infectivity of the virus. VP4 is coded by segment 4(4), protein VP7 is coded by gene segments 7, 8 and 9 in different strains of rotavirus(5). The predominant neutralizing antibody reactivity in hyperimmune serum is directed against the glycoprotein VP7 which makes up a greater percentage of the virion outer capsid. The number of VP7 molecules per virus particle are 780 as compared to 60 of VP4 per virus particle^). In addition, VP4 is labile and it is lost during storage of the virus. Thus, currently virus serotypes are defined on the basis of epitopes present on glycoprotein VP7 (6 specific)(7).

Group A rotaviruses have clearly been established as a leading cause of severe diarrhea and dehydration in infants and young children. Diarrhea caused by rotaviruses is unique in the sense, it occurs in equal proportions in developed and developing countries, although mortalities are higher in the latter(8-10). Many scientists believe that rotavirus associated diarrhea is unlikely to be controlled through improved sanitation, water supply or hygiene. Thus, the development of rotavirus vaccine has emerged as an important research priority. It is believed

From the National Institute of Virology, 20-A, Dr. Ambedkar Road, Pune 411 001.

Reprint requests: Shobhana D. Kelkar Senior Research Officer, National Institute of Virology 20-A, Dr Ambedkar Road, Pune 411 001.

Received for publication: January 4, 1993; Accepted: March 1, 1994

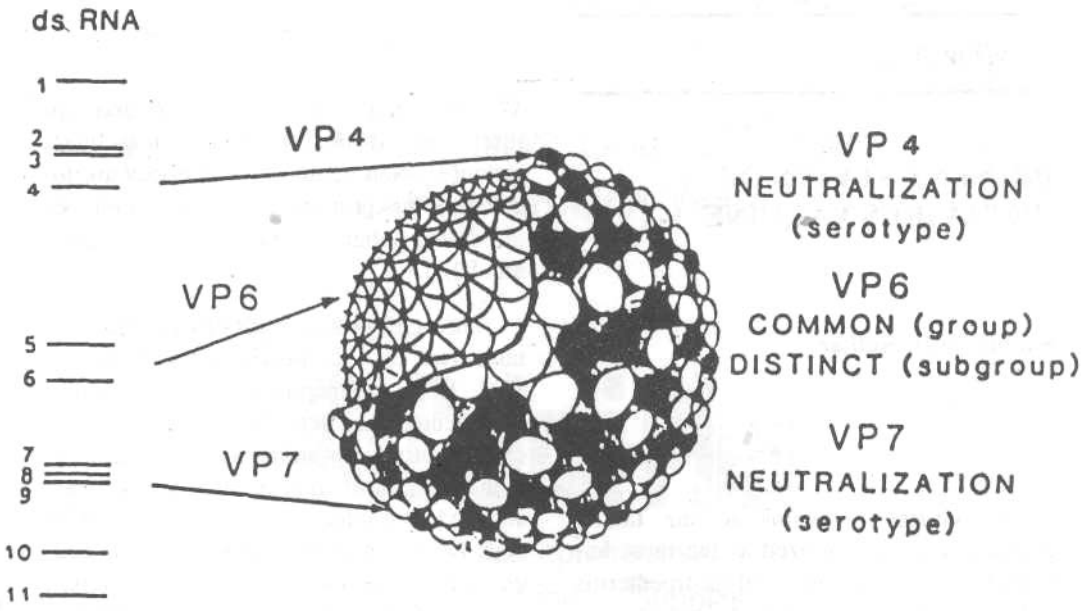

Fig. 1. Major Structural Proteins of Rotavirus (Reproduced from Kapikian et al.(81) modified as per Liu et al.(4)).

that an effective vaccine against group A rotaviruses administered to infants under 6 months of age in the developing countries may decrease the number of cases of diarrhea by more than 50 million episodes and prevent upto 800,000 deaths per year(9).

Prevention of rotavirus diarrhea has become potentially more complex because, there are atleast four epidemiologically, important distinct serotypes of human rotavirus(HRV)(11). Serotypes 1, 2, 3, 4 have been found to circulate at a variable rate in different geographic areas around the world(12-14). The relative importance of each has not been clearly known but serotype 1 appears to be the most common cause of disease worldwide(15). HRV strains belonging to serotypes 8 and 9 have been reported(16,17) but seem to circulate poorly. Recently, Urasawa *et al.*(18) described presumptive 7th HRV serotype.

Rotavirus Vaccines

An immunization strategy that is being evaluated in human field trials is mainly the Jennerian approach. The oral administration of live, attenuated rotavirus vaccines derived from following three animal rotavirus

strains have been evaluated:

(0 RIT 4237 vaccine (serotype 6) developed by Smith Kline - (Rixensart, Belgium).

(ii) WC 3 bovine strain (Serotype 6) developed at the Wistar Institute of Anatomy and Biology, Philadelphia:

(iii) The Rhesus rotavirus (RRV) simian strain of rotavirus, MMV-18006 (Serotype 3) developed at-National Institute of Health, USA.

(iv) Besides animal rotavirus strains, human rotavirus strains isolated from asymptomatic neonates are being tried as vaccine candidates.

(i) **RIT 4237 Vaccine**

The RIT 4237 vaccine strain was derived from Neonatal Calf Diarrhea Virus (NCDV).

In the early clinical trials carried out in Finland the RIT 4237 strain induced cross protection against clinically significant human rotavirus diarrhea(19,20). The protection rates were 88,% and 82%, respectively in these trials. However, the vaccine did not give significant protection against milder rotavirus diarrhea or asymptomatic infection. RIT 4237 vaccine was also administered to 54 infants in USA. The Vaccine appeared to be safe and immunogenic.

After two promising trials of RIT 4237 at Finland, it was necessary to see whether the same level of protection could be achieved in developing countries where the challenge dose of the virus could be much higher. To test this, Hanlon *et* a/.(21) conducted a trial of this vaccine in Gambia. The overall vaccine efficacy was 33%. Administration of the vaccine to Gambian children gave less protection against clinically significant diarrhea compared to that obtained in vaccine trials in Finland. The contrasting result in Gambian trials has been attributed to administration of vaccine at younger age, high frequency of enteric viruses, high levels of maternal antibodies, substances harmful for the vaccine virus in the breast milk of Gambian mothers. Besides this, the rotavirus responsible for Gambian outbreaks was serotype 2, whereas the predominant virus in Finland has been serotype 1. It is possible that RIT 4237 which is serotype 6 offers a better cross protection against rotavirus serotype 1 than against serotype 2. A similar finding was reported from a vaccine trial carried out at Rwanda(22).

Because the RIT 4237 trials failed in some of the developing countries, several strategies for rotavirus vaccination have been tried *viz,* multiple doses of vacqne(23), low passage level vaccine(24), influence of immunomodulatory substances on vaccine take up(25), expression of VP 7 antigen of bovine rotavirus in *E. coli* expression plasmid PEX(26), and use of synthetic peptides having immunogenic determinants().

(ii) WC-3 Vaccine

WC-3 (Wistar calf 3) is another bovine rotavirus strain which is a vaccine candidate. It was isolated in 1981 from a calf suffering from gastroenteritis in Pennsylvania.

Based on immunogenicity studies of Clark *et al.* (28), a placebo controlled double blind efficacy trial of the WC-3 vaccine was carried out in infants in suburban Philadelphia, during a predominantly serotype 1 rotavirus season in which three cases of mild rotavirus disease occurred among 49 vaccinees(29). Among the 55 placebo recepients, there were 14 cases of rotavirus

arrhea. Eleven of these were moderate to were. The study further revealed identical ites of natural rotavirus infection in vacciated and placebo groups. Results of serogical studies suggested that presence of N' is not the only mechanism of protection against rotavirus disease.

After the first successful trial of WC 3 raccine(29) and immunogenicity studies in "rench infants(30), a larger trial was taken jp by Bernstein *et* a/.(31). The authors also investigated the potential protective mechanisms against rotavirus infection and illness. In the double blind, placebo controlled trial, one group of 103 infants received one dose of the vaccine and same number of infants received placebo. Neither the number of symptomatic episodes of rotavirus diarrhea (21 vs 25) nor the number of moderate to severe rotavirus illness (9 vs 15) was significantly different in the vaccine or placebo recipients, respectively. Although antibody to WC 3 was induced in 97% of the vaccinees, only 9 infants (8,73%) of these developed antibody to human rotavirus serotypes 1 and 3. Majority of them had serum rotavirus IgA and serotype 1 neutralizing antibody acquired before immunization. Antibody response to VP4 and VP7 proteins of vaccine virus was als6 studied in infants who showed good ^{V}N' antibody response to vaccine virus(32). Thus, the vaccine was found to be ineffective, as against the report of Clark *et al.* (29). Circulating rotavirus in both the studies was serotype 1. However, the strains were different. The infants were of the similar ages in both the trials and were vaccinated only once with a similar vaccine preparation. The circulating strain difference may have accounted for the vaccine failure.

Despite an overall lack of efficacy, several correlates of protection could be found. Factors like previous rotavirus infection, high levels of WC 3 neutralizing antibody and preexisting maternal antibody with dilution 1:30 titres correlated with protection in the above vaccine trial. Results of further studies in this vaccine trail showed that VP7 appeared to be the dominant immunogen for the production of 'N' antibody, after intestinal infection of previously uninfected infants(32). Efficacy of the WC 3 vaccine was evaluated in another double blind placebo controlled trial involving 472 children in Bangui (Central Africa). Each child received two doses of the vaccine or a placebo. The vaccine failed to protect children from rotavirus diarrhea. The only positive effect was a significantly higher number of mild rotavirus diarrheal episodes in the vaccinated group(33).

Ward *et al*(34) determined the effect of WC 3 vaccination on serotype specific antibody responses in infants before and after natural infection with serotype 1 human rotavirus. Previous vaccination with WC 3 had littlie effect on the magnitude of these responses. In contrast, subjects infected with serotype, 1 strain before vaccination experienced large (average 12 fold) rises in neutralizing antibody to human serotype 1-4 when vaccinated with WC 3. Thus, although WC 3 and the natural strains are distinct serotypes their epitopes were sufficiently similar so that reinfection with WC 3 could boost neutralizing antibody titres to human serotypes in subjects primed by a previous natural infection.

Reassortant of WC-3 Rotavirus

A reassortant of WC-3 bearing gene 9 of human rotavirus serotype 1 strain designated as W 179 and all other genes derived from WC 3 (bovine strain) was constructed(35).

Clark *et al.* (36) evaluated the safety and efficacy of W 179 in a placebo controlled double blind efficacy trial in Philadelphia. Infants 2-11 months of age were given two doses of the vaccine (38 children) or placebo (39 children) 28 days apart. The immune response to serotype 1 was disappointing, but protective efficiency of the vaccine indicated that serotype-1 specific immune response may have been induced which is not detected by PRN antibody test. Perhaps this is a cellular response. Active surveillance during the subsequent rotavirus seasons revealed that there was no case of diarrhea in 38 vaccines but 8 cases of rotavirus gastroenteritis in 39 placebo control infants. Six cases of rotavirus gastroenteritis were caused by type 1 and two by type 3 virus.

(iii) **RRV 2 Vaccine**

Another animal rotavirus, rhesus rotavirus (RRV) strain also designated as MMU 18006 has been studied as a vaccine strain for the prevention of human rotavirus disease. RRV was isolated from a young monkey with diarrhea(37) and adapted to primate cell strain FRh-2 for use as a vaccine candidate in the 16th cell culture passage at NIH, USA(38). The major neutralization protein VP 7 of RRV is very closely related antigenically to the corresponding protein of the human rotavirus serotype which is the second most important rotavirus serotype with respect to human disease(39).

Reactogenicity and Immunogenicity of RRV

Early studies on RRV vaccine (developed at NIH) in adult human volunteers and young children showed that this strain was safe and immunogenic(40). However, the vaccine induced febrile reactions and diarrhea was observed in studies conducted in the pediatric population of Finland(40), Sweden(41) and USA(42,43). Therefore, reactogenicity and immunogenicity of RRV vaccine was evaluated at lower doses (10^4 PFU) by Perez Schael *et al.* in children(44). The vaccine did not cause any significant reactions among the vaccines in comparison with placebo recipients. The vaccine proved to be quite antigenic because 75.5% of the vaccines in the one to four month age group developed seroresponse. Fifteen per cent placebo recipients also developed a seroresponse. However, earlier trials were not very successful(45).

Protection Against Rotavirus Diarrhea with RRV

In an RRV vaccine trial in Venezuela(46). The efficacy of this vaccine against any rotavirus diarrhea was 68%.

RRV vaccine trial at 10^4 PFU was performed in 114 infants in Maryland. The vaccine efficacy was only 29%. The authors concluded that the vaccine was immunogenic and probably acceptably attenuated but this serotype 3 vaccine provided little heterotypic protection during serotype 1 outbreaks in the community(47). In another RRV trial reported by Vesikari *et al.* (48) vaccine protection rate of 38% was derived. The effect of concomitant breast feeding on seroconversion following oral administration of RRV, at 10^4 PFU dose was analysed(49). There was significant adverse effect of breast feeding with respect to RRV vaccine seroconversion.

A prospective study was undertaken by Perez-schael *et al.* (50) in Venezuela to evaluate the efficacy of RRV. The study suggested that resistance induced by the vaccine was type specific since significant protection was only evident against serotype 3 rotavirus and hence the need for polyvalent vaccine including at least four epide-

miologically important serotypes was felt.

Studies on RRV Reassortant Viruses

For protection against rotavirus diarrhea caused by serotypes, other than 3, reassortants between the rhesus and human rota virus 1,2 and 4 have been developed as potential vaccine candidates(51).

Vesikari *et al.* (52) tested immunogenicity and safety of the rhesus human rotavirus reassortants corresponding to serotypes 1 'and 2 in 2-4 month old Finnish infants. The candidate vaccines were tested individually and in combination with each other and in a trivalent combination with rhesus rotavirus. The authors concluded that VP-7 specific nutralizing antibody responses are likely to be lower after administering a combined vaccine -than that following vaccination with a single reassortant rotavirus.

Madore *et al.* evaluated the relative efficacy of RRV and human rhesus reassortant rotavirus vaccine (D ! RRV VP7 serotype) in infants from Rochester area(53). As serotype 1 was the prevalent rotavirus in this area. Efficacy of the vaccines was 66% and 77%, respectively in the first season after vaccination and 51.2% and 67.3%, respectively during following 2 rotavirus seasons.

Failure of rotavirus vaccines in developing countries has been partially answered by studies of Rimer *et al*.(54). Milk was collected from 56 New York and 70 Venezuelan mothers participating in Rhesus rotavirus pediatric vaccine trials. More Venzuelan milk samples had detectable RRV PRN antibody, RRV VP4 epitope-blocking antibody and higher RRV geometric mean titres than New York samples. Both milk and infants serum preimmunization PRN antibodies, RRV titres had a negative effect on seroconversion. The data suggested that VP4 specific milk antibodies may interfere with RRV seroconversion.

(iv) Neonatal Rotavirus Strain

A new rotavirus vaccine candidate, the M37 human strain was originally recovered from a symptom free newborn baby at a maternity hospital in Caracas, in Venezuela^). It is a naturally attenuated rotavirus with an altered VP 4 protein.

The rationale for developing the M 37 strain as a rotavirus vaccine stems from observations that the strain usually infects newborn infants without causing any symptoms and that asymptomatic neonatal infection has been associated with resistance to disease during subsequent rotavirus infection.

Recently, Midthun *et al,* carried out M 37 vaccine trial in adults, children and infants(56). Strain M 37 was both tolerated and immunogenic in young infants, but elicited neutralizitig antibody response primarily vaccine strain specific rather than serotype specific. This raises concern about the potential efficacy of this vaccine candidate.

Immune Response to Individual Polypeptides

During the vaccine trails, there was a lack of heterotypic immune response. In one study where post vaccination (RIT 4237 and RRV-1) immune responses to individual rotavirus polypeptide were studied, immune responses were directed mainly to VP 2 and VP 6 proteins(57), which induce non neutralizing antibodies.

In order to evaluate the efficacy of a vaccine, it is important to analyse antibody responses to defined epitopes on VP 4 and VP 7. Taniguchi *et al.* studied antibody responses to neutralizing epitopes on VP 4

and VP 7 in individuals infected or vaccinated with rotavirus(58). Antibody responses to VP 7 epi topes of the infecting serotype of virus were found at a hig'i frequency in both, infants and children. In contrast, the antibody responses to VP 4 and heterotypic VP 7 were observed only when the individuals possessed antibodies to any serotype of rotavirus in their acute phase or prevaccination sera. It seems that cross- reactive epitopes on VP 4 are less immunogenic and require priming for the antibody responses. A booster dose of vaccines may be effective in affording induction of antibodies to cross creative epitopes on VP 4.

Homotypic and Heterotypic Immune Responses

Green *et al.* reported that adult vaccinees exhibited both, a homotypic response to the immunizing antigen and heterotypic response to other serotypes(59). On the other hand, infant vaccinees developed homotypic responses but significantly fewer heterotypic response than adults (59% and 1.2%, respectively). From these results it appears that the inability to mount a heterotypic antibody response to the infecting serotypes in young infants may have been an important- factor in the failure of the vaccines to induce protection.

Other Approaches

Besides the conventional "Jennerian" approach to vaccination against rotavirus other approaches like use of non-infectious rotavirus(60), baculovirus expressed VP6 gene(61), and use of empty capsid(62) have been tried with limited success. Some of the most immunogenic antigens have been the cell surface expressed glycoproteins from enveloped viruses(63,64). In contrast, it has been difficult to generate good responses against antigens that are not normally expressed on the surface of the infected cells, *e.g.,* VP 7 of rotavirus(65). Rotaviruses are nonenveloped viruses that mature by budding into the endoplasmic reticulum (ER) of the infected cell(66). The serotype specific antigen VP 7 is directed to the ER and retained there as membrane associated protein for assembly in the maturing virus particles. Recently, however, a secreted version of VP 7 with correct N terminus was produced by exchanging the VP 7 signal peptide in the signal peptide sequence from influenza virus HA(67).

Subsequently Andrew *et al.* constructed a novel, cell surface expressed form of VP 7 to investigate the effect of cell surface localization on the immunogenicity of this glycoprotein when expressed in mice and rabbit by a recombinant vaccinia virus, the surface anchored Ag stimulated a level of rotavirus antibodies that was >100 fold above the level induced by the wild type VP 7. T cell responses to this antigen were also elevated in comparison with wild type intracellular protein(68).

Strategy with Respect to Rotavirus Immunization in India

Taking into consideration the vast number of rotavirus gastroenteritis cases, studies on rotavirus should be intensified. In India, rotavirus has been detected in stool specimens in about 20-30% of hospitalized diarrhea cases(69-72). However, epidemiological data is very scanty, this is mainly because commercial kits for the diagnosis of rotavirus are very expensive, the cost of testing each specimen being over Rs. 200. This has probably hampered studies on Rotavirus in India. Recently, ELISA test has been developed indigenously at National Institute of Virology, Pune, India for the rotavirus diagnosis which costs about Rs. 2/- per specimen(73).

There has been only one study(72) so far regarding prevalence of human rotavirus serotypes in India. Therefore, there is immediate need to take up work on monoclonal antibodies in order to have reagents for serotyping of rotaviruses prevalent in India. Such studies along with studies on antibody level to human and animal rotaviruses in pregnant women and children of urban and rural areas need to be done before rotavirus vaccine trials are taken up in India. Basic research on immune mechanism in rotavirus diarrhea is equally important.

At present, there is no clarity about correlates of protection against rotavirus diarrhea. However, it is known that good immunogenic strains are required as a vaccine candidate. The available data suggests that certain level of neutralizing antibody offers protection although protection in the absence of neutralizing antibody has been shown. Infants do not develop heterologous response whereas adults do generate good heterologous response(59). Moreover, if vaccinees possess pre immunization rotavirus antibody, they develop booster response after vaccination(28).

To achieve effective immune response in infants, it is worth trying to immunize mothers so that infants have passive immunity against rotavirus. This will certainly help in reducing morbidity and mortality due to clinically severe diarrhea in infants. Further, infants at 6 months may be immunized with one dose of any suitable animal rotavirus vaccine which may boost up the immune response in them. This kind of approach has been tried in dealing with bovine diarrhea. Calves can be protected by passive immunization taking advantage of the lactogenic immunity stimulated by maternal vaccination(74). A vaccine from suitable animal rotavirus can be prepared in India thereby reducing the cost of vaccination.

Another approach is to feed infants with hyperimmune colostrum obtained from vaccinated cows(75). It has been shown that calves can be protected by directly feeding them with hyperimmune colostrum obtained from vaccinated dams(76).

Recent Trends

After nearly one decade's work on vaccine development it has been realized that, there is need for research on the basic mechanisms of protective immunity against rotavirus disease. In 1992 more reports have started appearing on animal experiments with rotavirus vaccines, viz., mechanism of protection in mice(77), vaccinia-rotavirus recombinant vaccine in mice(78) efficacy of an inactivated oil-adjuvanted rotavirus vaccine(79) and rota *Escherichia* coli(80) have been tried. Significant success in morbidity and mortality reduction was achieved. A search from better vaccine candidate is continued by characterizing newer strains of rotaviruses.

Conclusion

Protection against rotavirus diarrhea has been so far inconsistant. Several problems have been identified related to vaccine development against rotavirus.

Efficacy of available vaccine candidates in different countries may be different. Therefore, more vaccine trails in heterogeneous populations are necessary. Also the search for better vaccine candidate strains need to be continued.

There is no clarity as yet about correlates of protection against rotavirus diarrhea. Therefore, there is a need for research on basic mechanisms of protective immunity against rotavirus illness.

Acknolwedgement

The author is grateful to Dr. K. Banerjee, Director, National Institute of Virology for useful suggestions during the preparation of the manuscript. I also thank Drs. S.R. Prasad, V.S. Padbidri and B.L. Rao for their critical comments.

REFERENCES

1. Estes MIC Palmer EL, Obijeski JF. Rotaviruses: a review. Curr Top Microbiol Immunol 1983, 105: 123-184.
2. Flewett TH, Woode GN. The rotaviruses. Brief review. Arch Virol 1978, 57: 1-23.
3. Novo E, Esparza J. Composition and topography of structural polypeptides of bovine rotavirus. J Gen Virol 1981, 56: 325-335.
4. Liu MO, Paul A, Estes MK. Identification of the Simian rotavirus SA11 genome segment 3 product. Virology 1988, 163: 26-32.
5. Estes MK, Cohen J. Rotavirus gene structure and function. Microbiol Rev 1989, 53: 410-449.
6. Prasad BVV, Wang GJ, Clerx JPM, Chiu W. Three-dimensional structure of rota viruses. J Mol Biol 1988, 199: 269-275.
7. Browning GF, Fitzgerald TA, Chalmers RM, Snodgrass DR. A novel group A rotavirus G serotype: serological and genomic characterization of equine isolate F123. J Clin Microbiol 1991, 29: 2043-2046.
8. Bartlett AV, Bednarz-Prashad AJ, DuPont IIL, Pickering LK. Rotavirus gastroenteri tis. Ann Rev Med 1987, 38: 399-415.
9. De Zoysa I, Feachem RG. Interventions for the control of diarrheal diseases among young children: Rotavirus and cholera immunization. Bull WHO 1985, 63: 569-583.
10. Black RE, Merson MH, Rehman ASMM, *et al.* A two-year study of bacterial, Viral, and parasitic agents associated with diarrhea in rural Bangladesh. J Infect Dis 1980, 142: 660-664.
11. Kapikian AZ, Chanock RM. Rotaviruses. In fields. Virology 1985, 2: 1353-1404.
12. Beards GM, Desselberger U, Flewett TH. Temporal and geographical distribution of human rotavirus serotypes, 1983 to 1988. J Clin Microbiol 1989, 27: 2827-2833.
13. Gerna G, Sarasini A, Arista S, *et al.* Prevalence of human rotavirus serotypes in some European countries 1981-1988. Scand J Infect Dis 1990, 22: 5-10.
14. Woods PA, Gentsch J, Gouvea V. Distribution of serotypes of Human rotavirus in different populations. J Clin Microbiol 1992, 30: 781-785.
15. Yokoyama T, Morita Y, Taniguchi K, Nakao T,, Clark HF. Rotavirus vaccine. *In:* Vaccines, Eds Plotkin SA, Mortina EA. Philadelphia W.B. Saunders Company, 1988, 517-525.
16. Matsuno S, Ilasegawa A, Mukoyama A, Inouye S. A candidate for a new serotype of human rotavirus. J Virol 1985, 54: 623-624.
17. Clark HF, Hoshino Y, Bell LM, *et al.* Rotavirus isolate WI61 representing a presumptive new human serotype. J Clin Microbiol 1987, 25: 1757-1762.
18. Urasawa S, Urasawa T, Wakasugi F, *et al.* Presumptive seventh serotype of human rotavirus. Arch Virol 1990, 113: 279-282.
19. Vesikari T, Isolauri E, Delem A, D Hondt E, Andre FE, Zissis G. Immunogenicity and safety of live oral attenuated bovine rotavirus vaccine strain RIT 4237 in adults and young children. Lancet 1983, H: 807-812.
20. Vesikari T, Isolauri E, D Hondt E, Delem A, Andre FE, Zissis G. Protection of infants against rotavirus diarrhea by RIT

4237 attenuated bovine rotavirus strain vaccine. Lancet 1984,1: 977-980.

21. Hanlon P, Marsh V, Shenton F, *et al.* Trial of an attenuated bovine rotavirus vaccine (RIT 4237) in Gambian infants. Lancet 1987, 1: 1342-1345.

22. Vesikari T. Clinical trials of rotavirus vaccines *In:* Viruses and the Gut. lid Forthing MJG Swan Press, London, 1985, pp 121-122.

23. Lanata CF, Black RE, Aguila, RD. Protec tion of Peruvian children against rotavirus diarrhea of specific serotypes by one,.two or three doses of the RIT 4237 attenuated bovine rotavirus vaccine. J Infect Dis 1989, 159: 452-459.

24. Vesikari T, Rautanen T, Isolauri E, Delem A, Andre FE. Immunogenicity and safety of a low passage level rotavirus candidate vaccine RIT 4256 in human adults and young infants. Vaccine, 1987, 5: 105-108.

25. Archambault-D, Morin G, Elazhary Y. Influence of immunodulatory agents on bovine humoral and cellular immune responses to parenteral inoculation with bovine rotavirus vaccines. Vet Microbiol 1988, 17: 323-334.

26. Francavilla M, Miranda P, Di matteo, A, Sarasini A, Gerna G, Milanesi G. Expres sion of bovine rotavirus neutralization an tigen in *Escherichia coli.* J Gen Virol 1987, 68: 2975-2980.

27. Borras-cuesta F, Petit Pery, P, A Fedon Y, Gamier J, Cohen J. Immunogenicity of synthetic peptides corresponding to re gions of the major inner capsid protein of bovine rotavirus (BRV). Ann Inst Pasteur/ Virol 1987, 138: 437-450.

28. Clark HF, Furukawa T, Bell LM, Offit PA, Perrella PA, Plotkin SA. Immune response of infants and children to low passage bovine rotavirus (strain WC3). Amer J Dis Child 1986, 140: 350-356.

29. Clark HF, Borian FE, Bell LM, Modosto K, Goulvea, Plotkin SA. Protective effect of WC3 vaccine against rotavirus diarrhea in infants during a predominantly serotype 1 rotavirus season. J Infect Dis 1988, 158: 570-587.

30. Garbag-Chenon A, Fontaine JL, Lasfargues G, *et al.* Reactogenicity and immunogenicity of rotavirus WC3 vaccine in 5-12-month old infants. Res Virol 1989, 140: 207-217.

31. Bernstein DI, Smith VE, Sander DS, Pax KA, Schiff GM, Ward RL. Evaluation of WC3 rotavirus vaccine and correlates of protection in healthy infants. J Infect Dis 1990, 162: 1055-1062.

32. Ward RL, Sander DS, Schiff GM, Bernstein DI. Effect of vaccination on serotype-specific antibody responses in infants administered WC3 bovine rotavirus before or after a natural rotavirus infection. J Infect Dis 1990, 162: 1298-1303.

33. Georges-Courbot MC, Monges J, Slopathis MR, *et al.* Evaluation of the efficacy of a low-passage bovine rotavirus (strain WC3) vaccine in children in central Africa. Re Virol 1991, 142: 405-412.

34. Ward RL, Knowlton DR, Greenberg HB, Schiff GM, Bernstein DI. Serum-neutralizing antibody to VP4 and VP7 proteins in infants following vaccination with WC3 bovine rotavirus. J Virol 1990, 64: 2687-2691.

35. Clark HF, Borian FE, Modesto K, Plotkin SA. Serotype 1 reassortant of bovine rotavirus WC3 strain WI79-9, induces a polytypic antibody response in infants. Vaccine 1990, 8: 327*332.

36. Clark HF, Borian FE, Plotkin SA. Immune Protection of Infants against Rotavirus Gastroenteritis by a Serotype 1 Reassortant of Bovine Rotavirus WC3. J Infect Dis 1990, 161: 1099-1104.

37. Stuker G, Oshiro LS, Schmidt NJ. Antigenic comparisons of two new rotaviruses from rhesus monkeys. J Clin Microbiol 1980, 11: 202-203.

38. Wallace RE, Vasinton PJ, Petricciani JC, Hopps HE and Lorenz DE. Diploid cell lines from subhuman primates as substrates for virus vaccine production. Prog Immunobiol Stand. 1972, 5: 181.

39. Kapikian AZ, Midthun K, Hoshino Y, *et al.* Rhesus rotavirus: A candidate vaccine for prevention of human rotavirus disease. Vaccines 85, Cold Spring Harbor Laboratory, 1985, 357-367.

40. Kapikian AZ, Hoshino Y, Flores J, *et al.* Alternative approaches to the development of a rotavirus vaccine. *In:* Development of Vaccines and Drugs Against Diarrhea. Eds Homgren J, Lindberg A, Mollby R. 11th Nobel Conference, Stock holm, 1985, London, Sweden: Student literature, 1986, pp 192-214.

41. Gothefors L, Wadell G, Juto P, Taniguchi K. Kapikian AZ, Glass RI. Prolonged efficacy of Rhesus rotavirus vaccine in Swedish children. J Inf Dis 1989, 159: 753-757.

42. Losonsky GA, Rennels MB, Kapikian AZ, *et al.* Safety, infectivity, transmissibility and immunogenicity of rhesus rotavirus vaccine (MMU 18006) in infants. Pediatric Infect Dis 1986, 5:25-29.

43. Anderson EL, Belshe BB, Bartram J, Crookshank-Newnan F, Chanock RM, Kapikian AZ. Evaluation of rhesus rotavirus vaccine (MMU 18006) in infants and young children. J Inf Dis 1986, 153: 823-831.

44. Perez-Schael I, Gonzalez M, Daoud N, *et al.* Reactogenicity and antigenicity of the rhesus rotavirus vaccine in Venezuelan children. J Inf Dis 1987, 155: 334-338.

45. Wright PF, Tajima T, Thompson J, Kokubun K, Kapikian A, Karzon DT. Candidate rotavirus vaccine (Rhesus Ro tavirus Vaccine) in children: An evalua tion. Pediatrics 1987, 80: 473.

46. Flores J, Gonzalez M, Perez M, *et al.* Protection against severe rotavirus diarrhea by rhesus rotavirus vaccine in Venezuelan infants. Lancet 1987, 1: 882-884.

47. Rennels MB, Losonsky GA, Young AE, Shindledecker CL, Kapikian AZ, Levine MM and Clinical Study Group. An efficiency trial of the Rhesus Rotavirus vaccine in Maryland. Amer J Dis Child 1990, 144: 601-604.

48. Vesikari T, Rautanen T, Varis T, Beards GM, Kapikian AZ. Rhesus rotavirus candidate vaccine.Amer J Dis Child 1990, 144: 285-289.

49. Pichichero ME. Effect of breast feeding on oral Rhesus Rotavirus Vaccine seroconversion. A meta analysis. J Inf Dis 1990, 162: 753-756.

50. Perez-Schael I, Garcia D, Gonzalez M, *et al.* Prospective study of diarrheal diseases in Venzulean children to evaluate the efficacy of rhesus rotavirus vaccine. J Med Virol 1990, 30: 219-229.

51. Kapikian AZ, Flores J, Midthun K. *et al.* Development of a rotavirus vaccine by a "Jennerian" and a modified "Jennerian" approach Vaccine 88. Cold Spring Harbor Laboratory USA, 1988, pp 151-159.

52. Vesikari T, Varis T, Green K, Flores J, Kapikian AZ. Immunogenicity and safety of rhesus-human rotavirus reassortant vaccines with serotype 1 or 2 VP7 specificity. Vaccine 1991, 9: 334-339.

53. Madore HP, Christy C, Pichichero M, *et al.* Panorama and Westfall Pediatric groups. Field trial of rhesus rotavirus or human-rhesus rotavirus reassortant vaccine of VP7 serotype-3 or serotype-1 specificity in infants. J Inf Dis 1992, 166: 235-243.

54. Rimer HC, Wassrman SS, Flores J, Pichichero ME, Losonsky GA. Rotavirus-specific breast milk antibody in two populations and possible correlates of interference with rhesus rotavirus vaccine

seroconversion. J Infect Dis 1992, 165: 826-830.

55. Perez-Schael I, Daoud G, White L, *et al.* Rotavirus shedding by newborn children. J Med Virol 1984, 1.4: 127-136.

56. Midthun K, Halsey NA, Jelt-Goheen M, *et al.* Safety and immunogenicity of human rotavirus vaccine strain M37 in adults; children and infants. J Infect Dis 1991, 164: 792-796.

57. Svensson L, Sheshberadaran H, Vesikari T, Norrby E, Wadell G. Immune response to rotavirus polypeptides after vaccination with heterologous rotavirus vaccines. (RIT 4237, RRV-1) J Gen Virol 1987, 68: 1993-1999.

58. Taniguchi K, Urasawa T, Kobayashi N, Ahmed MU, *et al.* Antibody response to serotype specific and cross reactive neutralization epitopes on VP4 and VP7 after rotavirus infection or vaccination. J Clin Microbiol 1991, 29: 483-487.

59. Green KY, Taniguchi K, Mackow ER, Kapikian AZ. Homotypic and heterotypic epitope-specific antibody responses in adults and infant rotavirus vacciness: Implications for vaccine development. J Infect Dis 1990, 161: 667-679.

60. Offit PA, Dudzik RI. Noninfectious rotavirus (strain RRV) induces an immune response in mice which protects against rotavirus challenge. J Clin Microbiol 1989, 27: 885-888.

61. Estes MK, Crawford SE, Penaranda ME, *et al.* Synthesis and immunogenicity of the rotavirus major capsid antigen using a Baculovirus expression system. J Virol 1987, 61: 1488-1496.

62. Brussow H, Bruttin A, Marc-Martin S. Polypeptide composition of rotavirus empty capsids and their possible use as subunit vaccine. J Virol 1990, 64: 3635-3642.

63. Blancou J, Kieny M, Lathe R, *et al.* Oral vaccination of the fox against rabies using a live recombinant vaccinia virus. Nature (London) 1986, 332: 373-375.

64. Yilma T, Hsu D, Jones L, *et al.* Protection of cattle against rinderpest with vaccinia virus recombinants expressing the HA or F gene. Science 1988, 242: 1058-1061.

65. Andrew ME, Boyle DB, Coupar BEH, Whitfeld PL, Both GW, Bellamy AR. Vaccinia virus recombinants expressing the SA 11 rotavirus VP7 glycoprotein gene induce serotype-specific neutralizing antibodies. J Virol 1987, 61: 1054-1060.

66. Bellamy AR, Both GW. Molecular biology of rotaviruses. Adv Virus Res 1990, 38: 1-43.

67. Stirzaker SC, Both GW. The signal peptide of the rotavirus glycoprotein VP7 is essential for its retention in the ER as an integral membrane protein. Cell 1989, 56: 741-747.

68. Andrew ME, Boyle DB, Whitfeld PL, *et al.* The immunogenicity of VP7 a rotavirus antigen resident in the endoplasmic reticulum, is enhanced by cell surface expression. J Virol 1990, 64: 4776-4783.

69. Maiya PP, Pereira SM, Mathan M, Bhat P, Albert MJ, Baker SJ. Ecology of acute gastroenteritis in infancy and early childhood in southern India. Arch Dis Child 1977, 52: 482-485.

70. Bhan MK, Kumar R, Khoshoo V, *et al.* Etiologic role of enterotoxigenic *Escherichia coli* and rotavirus in acute diarrhea in Delhi children. Indian J Med Res 1987, 85: 604-607.

71. Singh Bright P, Sreenivasan MA, Pavri KM. Viruses in acute gatroenteritis in children in Pune, India. Epidem Infect 1989, 102: 345-353.

72. Brown DWG, Mathan MM, Mathew M, Martin R, Beards GM, Mathan VI. Rotavirus epidemiology in Vellore, South India: Group, Subgroup, Serotype, and

Electropherotype. J Clin Microbiol 1988, 26: 2410-2414.

73. Kelkar SD. Development of indigenous ELJSA for rotavirus diagnosis and its comparison with commercial kit. Indian J Med Res (in press).

74. Snodgrass DR. Evaluation of combine rotavirus and enterotoxigenic *Escherichia coli* vaccine in cattle. Vet Rec 1986, 119: 39-42.

75. Brussow H, Hilpert N, Walter I, *et al.* Bovine milk immunoglobulins for passive immunity to infantile rotavirus diarrhea. J Clin Microbiol 1987, 25: 982-986.

76. Saif LJ, Redman DR, Smith KL, Theil KW. Passive immunity to bovine rotavirus in newborn calves fed colostrum supplements from immunized or nonimmunized cows. Infect Immun 1983, 41: 1118-1131.

77. Ward RL, McNeal MM, Sheridan JF. Evidence that active protection following oral immunization of mice with live rotavirus is not dependent on neutralizing antibody. Virol 1992, 188: 57-66.

78. Andrew ME, Boyle DB, Barbara EH, *et al.* Vaccinia-rotavirus VP7 recombinants protect mice against rotavirus induced diarrhea. Vaccine 1992, 10: 185-191.

79. Bellinzoni RC, Blackhall J, Baro N, *et al.* Efficacy of an inactivated oil-adjuvanted rotavirus vaccine in the control of calf diarrhea in beef herds in Argentina. Vaccine 1989, 7: 263-268.

50. Cornaglia EM, Fernandez FM, Gottschalk M, *et al.* Reduction in morbidity due to diarrhea in nursing beef calves by use of an inactivated oil-adjuvanted rotavirus-*Escherichia Coli* vaccine in Dams. Vet Microbiol 1992, 30: 191-202.

81. Kapikian AZ, Flores J, Hoshino Y, *et al.* Rotavirus: The major etiologic agents of severe infantile diarrhea may be controllable by a "Jennerian" approach to vaccination. J Infect Dis 1986, 153: 815-822.