PRESENT STATUS OF
ROTA VIRUS VACCINE

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Rotaviruses belonging to the family Reoviridae are recognized as the most important cause of severe viral gastroenteritis in humans and animals(1,2).

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 to 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(6). 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
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 up to 800,000 deaths per year (9).

Prevention of rotavirus diarrhea has become potentially more complex because, there are at least 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:

(i) 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 al.(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 vaccine, low passage level vaccine, influence of immunomodulatory substances on vaccine take up, expression of VP 7 antigen of bovine rotavirus in E. coli expression plasmid PEX, 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 recipients, there were 14 cases of rotavirus
arrhea. Eleven of these were moderate to severe. The study further revealed identical episodes of natural rotavirus infection in vaccinated and placebo groups. Results of serological studies suggested that presence of N' is not the only mechanism of protection against rotavirus disease.

After the first successful trial of WC 3 vaccine(29) and immunogenicity studies in French infants(30), a larger trial was taken up by Bernstein et al.(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 also studied in infants who showed good 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 titles correlated with protection in the above vaccine trial. Results of further studies in this vaccine trial 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 little 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 10^3 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 rotavirus 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 neutralizing 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 Venezuelan 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(55). 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 neutralizing 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 trials, 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 epitopes of the infecting serotype of virus were found at a high frequency in both, infants and children. In contrast, the antibody responses to VP 4 and heterogeneous 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-reactive 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 inconsistiant. 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.
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