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Prevalence of Neutralizing Antibodies against Different Rotavirus Serotypes in Children with Severe Rotavirus-Induced Diarrhea and Their Mothers

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Neutralizing antibody (NAb) responses to different rotavirus serotypes were compared in 64 convalescent-phase serum samples from hospitalized rotavirus-positive children less than 2 years of age and their mothers. Compared to the child patients, the mothers showed significantly higher NAb positivity to animal rotavirus serotypes G3 simian (96.88%), G6 bovine (85.94%), and G10 bovine (25.0%) and to human rotavirus serotypes G8 (79.69%) and G3 (57.81%) ($P < 0.01$ for each) but not to human serotypes G1, G2, G4, and G9 ($P > 0.05$). The overall prevalence of NAb among the child patients was low for human rotavirus serotypes G1 (20.31%) and G3 (21.8%). The comparative NAb response in individual mother-child paired serum samples was analyzed against each rotavirus serotype. A substantial number of child patients showed higher NAb titers than their mothers to serotypes G1, G2, G4, and G9, indicating that these serotypes are the major serotypes causing rotavirus diarrhea among the children of Pune, India. In these cases, the mothers were either negative or had lower titers of NAbs than their children. Correlation was observed between the infecting serotype and child patient serum that showed a homologous NAb response at a higher level than that of the mother. It appears that when the level of NAb to a particular serotype is higher among child patients than among their mothers, that serotype is the infecting serotype, and that low titers of NAb among the mothers predispose the children to infection with that serotype, if the serotype is in circulation.

Group A rotaviruses have been established as the most important etiologic agents of dehydrating gastroenteritis in infants and young children worldwide (27). Rotavirus serotypes are identified by two outer capsid proteins, VP7 and VP4, which elicit independently neutralizing antibodies (NAbs) and determine the virus G (glycoprotein) and P (protease sensitive protein) serotypes, respectively.

On the basis of VP7 protein, 14 different G types have been identified. Among them, 10 serotypes are associated with acute gastroenteritis in humans (28). Four G serotypes (G1 to G4), for which vaccines are being developed (33, 35), are the most frequently detected as etiologic agents of childhood diarrhea worldwide. Since VP4 is a poor immunogen, P serotyping is difficult due to nonavailability of typing sera or monoclonal antibodies (MAbs) directed against VP4 types. A VP4 classification system based on genotypes has been proposed, and presently 21 VP4 genotypes have been described (29, 41).

Studies have demonstrated that prechallenge titers of $\geq$20 NAbs against VP7 antigenic site A had a significant association with resistance to illness or shedding after rotavirus challenge (22). A similar association was observed between protection and the presence of a cross-reactive or strain-specific antibody to a VP4 epitope. These findings not only supported a correlation between serum antibodies and resistance to rotavirus disease or shedding but also indicated a protective role of epitope-specific antibody to VP7 or VP4 and, therefore, the importance of more than one viral protein for protection.

Although children can be infected with rotavirus several times during their lives, initial infection after 3 months of age is most likely to cause severe diarrhea and dehydration (16, 42, 50).

Velaquez et al. (50) reported in 1996 that after a single natural infection, 40% of children are protected against any subsequent infection with rotavirus, 75% are protected against diarrhea from subsequent infection, and 88% are protected against severe diarrhea. Second, third, and fourth infections confer progressively greater protection.

Rotavirus serotypes have been established on the basis of 20-fold or higher differences in reciprocal neutralizing titers with hyperimmune homologous and heterologous antisera (56, 57). Primary rotavirus infection usually results in production of NAb to the infecting serotype, although heterotypic NAb responses are also often detected (7, 13, 15, 19, 40, 45, 58). Subsequent rotavirus infection or inoculation with different serotypes has resulted in production of NAb to the new rotavirus strain and increased titers of antibody to other rotavirus serotypes, presumably due to anamnestic responses (4, 8, 15, 34, 51, 55).

To develop successful methods of immunoprophylaxis by active or passive immunization, seroepidemiological information is essential. Thus, the crucial question is how many rotavirus serotypes must be included in the vaccine to achieve protection against rotavirus disease, which may depend on the way the infant's immune system reacts to different rotavirus serotypes.

It is also important to document that the major rotavirus antigens in vaccines are representative of the most common strains prevalent in the country. However, strain prevalence patterns may vary regionally. Besides understanding the prev-

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alence patterns of rotavirus serotypes, it is important to understand the ability of vaccinees to react to candidate vaccines. Studies in the West have shown that serotypes G1, G3, and G4 in combination with genotype P (8) and G2 [P4] strains continue to be the four most prevalent rotavirus strains infecting humans (18). In recent years, serotype G9 has been reported in several countries. Serotype G9 may be the fifth-most-common human rotavirus serotype after G1 to G4. Therefore, vaccines under development should target G9 strains, and serotype G9 should be included as one of the common global serotypes (17, 30). The contributions of serological immune correlates of protection among children have not been identified for design and evaluation of effective rotavirus vaccines and have not yet been measured during vaccine trials.

Brussow et al. (10) (1988) reported an analysis of the persistence of maternal antibodies, serotype, and immunoglobulin class specificity of the immune response to rotavirus serotypes G1 to G4 and G6 among children in West Germany. Monospecific sera directed to serotypes G1 to G4 and the frequency of occurrence among Ecuadorian children have been reported (11). The presence of high levels of NAbs to G5, G6, G7, and G10 serotypes have been reported by Brussow et al. (9) (1991) in Ecuadorian children.

There are limited studies on NAb responses against several rotavirus serotypes among rotavirus-positive children less than 2 years of age. There is practically no data available from India.

Correlation of the serotype of the infecting virus with the specificity of the neutralizing immune response in primary rotavirus infection has also been shown (19). Arias et al. (3) (1994) characterized the neutralizing immune response to rotavirus serotypes G1 to G4, G8, and G9 in children with primary infections and reinfections and also correlated the immune response with the G serotype of the infecting virus. Classical neutralizing assays do not differentiate between neutralization mediated via VP4 and VP7. Reassortant viruses can be used to determine the relative importance of each VP4 and VP7 type in the immune response to rotavirus. Studies of the immunodominance of VP4 and VP7 in human hosts have provided conflicting results (14, 53, 54).

Gorell and Bishop (20) (1997) produced reassortants bearing VP4 of each of the major human rotavirus P types with VP7 of SA-11 origin. Further, using these reassortants, homotypic and heterotypic serum NAb to rotavirus proteins following natural primary infection and reinfection in children was studied. Increases in homotypic NAb titers following both primary and secondary infections were greater against VP7 than against VP4, with seroconversion against VP7 being significantly greater on reinfection than following primary infection.

The aim of the present study was (i) to study the prevalence of NAbs among child patients and their mothers and (ii) to understand the relationship between the NAbs to a particular serotype of mothers and their children who underwent severe rotavirus diarrhea.

MATERIALS AND METHODS

Patients and specimens. A group of 64 infants and children aged 1 month to 2 years was studied. The children belonged to the following age groups: <6 months (10.93%), >6 months to 8 months (21.87%), >8 months to 10 months (21.87%), >10 months to 12 months (17.18%), >12 months to 24 months (23.43%), and age group unknown (4.68%). All children were admitted to the diarrhea ward of Naidu Infectious Diseases Hospital, Pune, India, with acute gastroenteritis at some time during the years 1992 to 1994. The mothers of the children were not sick due to diarrhea at the time their children had active diarrhea. All infants were residents of Pune city, which is highly populated, or the surrounding rural areas. Fecal specimens from all the patients were tested by enzyme-linked immunosorbent assay (ELISA) for rotavirus and found to be positive. All the positive samples were serotyped directly from the patients' stool specimens, as clarified by a 20% suspension in phosphate-buffered saline. Serotyping was carried out using MAbs as capture antibodies (46). The capture of rotavirus from the specimens was probed by MAbs against G1 to G4, G6, G8, or G10 (method A). All the specimens were also tested by the procedure in which ELISA plates were coated with polyclonal antibody against the G1 to G4, G6, G8, or G10 serotypes, and rotavirus in fecal specimens was detected by MAbs against the respective rotavirus serotype (method B), the results of which are published (36). Serotyping using G9 MAb was recently done (unpublished data). All the MAbs employed are type specific; i.e., the reagents react specifically to only one serotype.

Convalescent-phase serum samples collected from all the above-mentioned rotavirus-positive children and their mothers by the Rotavirus Department, National Institute of Virology, Pune, at 1 to 5 months after the onset of diarrhea were frozen at −20°C until tested. Of the 64 serum samples, 41 (64.06%) were collected within 2 months, 12 (18.75%) were collected within 3 months, and 11 (17.18%) were collected between 3 to 5 months following diarrhea with rotavirus.

The sera from child patients were divided into two groups based on the typeability of the fecal samples obtained from the children. Group 1 contained serum samples of 25 child patients whose fecal specimens contained rotaviruses that were nontypeable by MAbs to G1 to G4, G6, G8, G9, or G10. Group 2 contained serum samples of 39 child patients whose fecal specimens were typeable with the above-mentioned MAbs.

The main limitation of the seroepidemiological study was that only a single serum sample was obtained from patients retrospectively, as this study was part of a rotavirus surveillance study.

Viruses. Reference strains of human rotavirus serotypes G1 (KU), G2 (S2), G3 (YO), G4 (ST-3), G8 (M-69), and G9 (F-45) and animal rotavirus serotypes G3 (SA-11), of simian origin; G6 (bovine Lincoln); and G10 (B-223) were used. All the virus strains were propagated in MA-104 cells in the presence of trypsin (48).

Neutralization assay. NAb titers to rotavirus serotypes were measured in 64 serum samples from rotavirus-positive child patients and 64 serum samples from their respective mothers. All serum samples were tested in duplicate for NAb to all the above-mentioned serotypes. The serum dilutions used were 1:50 and 1:100 for all serotypes. For the G2, G4, and G9 serotypes, a 1:200 dilution was also included.

The virus neutralization assay, based on a combined tissue culture and ELISA, was employed according to the procedure published earlier (38). In brief, the stock rotavirus of all the mentioned serotypes was titrated, the ELISA end point (EEP) was calculated by the neutralizing assay, and about 100 EEP of virus was used in each neutralizing assay. For every neutralizing assay, only one rotavirus serotype was used. To 100 EEPs of trypsin (SISCO, Mumbai, India)-treated rotavirus, equal volumes of serum dilutions were added. After being incubated for 1 h at 37°C, 30 μl of the mixture was inoculated in duplicate wells of 96-well microplates containing MA-104 monolayers after being washed with Dulbecco's phosphate-buffered saline. After 1 h of adsorption, maintenance medium, Earle's minimal essential medium (Hi-Media, Mumbai, India), was added and the plates were incubated at 37°C. The MA-104 cells in the plates were washed with Dulbecco's phosphate-buffered saline before addition of the test serum and virus mixture and after adsorption of the virus. The plates were frozen after 18 h of incubation, and the virus concentration was determined by ELISA. The results of the neutralizing test were expressed as serum dilutions, with those showing at least 50% virus neutralization considered positive.

Statistical methodology. Comparison of the prevalence of different rotavirus serotypes among children and their mothers was done by McNemar's test. Two independent proportions were compared by the chi-square test and the Fischer exact test. Two dependent proportions were compared by McNemar's test.

RESULTS

The prevalence of NAb to different human (G1 to G4, G8, and G9) and animal (G3 simian; G6 and G10 bovine) rotavirus serotypes in convalescent-phase serum samples from 64 Indian children with rotavirus diarrhea and their mothers was studied.

TABLE 1. Comparative prevalence of NAbs among rotavirus-positive children and their mothers

Group(s)	No. (%) positive for NAb to indicated rotavirus serotype								
	G1 (KU)	G2 (S2)	G3 (YO)	G4 (ST-3)	G8 (M-69)	G9 (F-45)	G3 (SA-11)	G6 (BOVLIN)[c]	G10 (B-223)
1 (n = 25)									
Child	2 (8.0)	14 (56.0)	6 (24.0)	10 (40.0)	8 (32.0)	18 (72.0)	12 (48.0)	7 (28.0)	3 (12.0)
Mother	12[b] (48.0)	8 (32.0)	16[a] (64.0)	18[b] (72.0)	20[a] (80.0)	18 (72.0)	24[a] (96.0)	21[a] (84.0)	6 (24.0)
2 (n = 39)									
Child	11 (28.21)	17 (43.58)	8 (20.51)	27 (69.23)	11 (28.21)	27 (69.23)	9 (23.08)	9 (23.08)	1 (2.56)
Mother	12 (30.76)	19 (48.72)	21[a] (53.85)	27 (69.23)	31[a] (79.49)	28 (71.79)	38[a] (71.79)	34[a] (87.17)	10[a] (25.64)
Both 1 and 2 (n = 64)									
Child	13 (20.31)	31 (48.44)	14 (21.8)	37 (57.81)	19 (29.68)	45 (70.31)	21 (32.81)	16 (25.0)	4 (6.25)
Mother	24 (37.5)	27 (42.19)	37[a] (57.81)	45 (70.31)	51[a] (79.69)	46 (71.88)	62[a] (96.88)	55[a] (85.94)	16[a] (25.0)

[a] The percent seropositivity for NAb to the rotavirus serotype among mothers compared to that for the child patients is highly significant ($P < 0.01$).
[b] The percent seropositivity for NAb to the rotavirus serotype among mothers compared to that for the child patients is significant ($P < 0.05$).
[c] BOVLIN, bovine Lincoln.

The serum samples were grouped as group 1 (n = 25), those from children who suffered from nontypeable rotavirus serotypes, and group 2 (n = 39), those from children who had diarrhea due to typeable rotavirus.

Prevalence of NAbs to different rotavirus serotypes among child patients and their mothers. In group 1, the percentages of prevalence of NAbs to rotavirus serotypes G3, G8, G3 simian, and G6 bovine ($P < 0.01$) and to G1 and G4 ($P < 0.05$) for the mothers were significantly higher that those for their children. The percentage of NAbs to serotype G2 was higher for the children (56.0%) than for their mothers (32.0%), and it was equal for both (72.0%) for the G9 serotype. The presence of higher levels of NAbs to G2 in the children than in their mothers and an equally high prevalence of G9 NAbs among both indicate that G2 and G9 are the main infecting serotypes in group 1. In this group, the infecting rotavirus serotype G2 could not be typed. Serotype G2, like other strains that have been reported in India, did not react to MAbs (S2-2G-10, IC10, 2FI) against the G2 serotype. In addition, some of the G9 strains in group 1 could not be serotyped, probably because of insufficient double-shelled particles (data not shown). Thus, NAbs to G2 and G9 appear to be acquired as a result of infection.

In group 2, the percentages of prevalence of NAbs to all serotypes among the mothers was significantly higher than those of their children ($P < 0.01$ for each) except for serotypes G1, G2, G4, and G9, for which an almost equal percentage of prevalence was observed among the children and their mothers, indicating a higher incidence of infection with these serotypes in group 2 (Table 1).

Overall, a low prevalence of NAbs to serotype G10 was found in the mothers (25.0%) as well as in the children (6.25%). This finding is probably due to lower circulation levels of G10.

Comparative NAb titers in each mother-child pair. NAb levels to different rotavirus serotypes were analyzed in each mother-child pair of serum samples, and the results are presented in Tables 2 and 3 (group 1) and Tables 4 and 5 (group 2).

In group 1, as expected, the mothers (M) had higher NAb titers than their children (P) (M > P) to several serotypes, viz., G1, G3, G4, G8, G3 simian, and G6 bovine. Some of the children also showed NAb titers equal to those of their mothers (M = P) (36.0% for G3 simian, 20.0% for G4, 24.0% for G9, 16.0% for G8), even above 8 months of age (Tables 2 and 3).

TABLE 2. Comparative NAb titers among group 1 mothers and child patients where M > P[a]

Category	Increase in N titer for indicated era[b]		No. of M serum samples with higher N titer to indicated serotype than that of P								
	P	M	G1 (KU)	G2 (S2)	G3 (YO)	G4 (ST-3)	G8 (M-69)	G9 (F45)	G3 (SA-11)	G6 (BOVLIN)[c]	G10 (B-223)
M > P	−ve	50	5	3	3	6	1	1	3	7	1
	−ve	100	7	1		1	12	1	10	7	4
	−ve	200			7	3		2		1	
	50	100				1	1	3	1	4	
	50	200			5		1			1	
	100	200						1			
Total (%)			12 (48.0)	4 (16.0)	15 (60.0)	11 (44.0)	15 (60.0)	8 (32.0)	14 (56.0)	20 (80.0)	5 (20.0)

[a] n = 25. P, child patients; M, mothers.
[b] N, neutralizing.
[c] BOVLIN, bovine Lincoln.

TABLE 3. Comparative NAb titers among group 1 mothers and child patients where P > M or M = P[a]

Category	Increase in N titer for indicated sera[b]		No. of P serum samples with higher N titer to indicated serotype than that of M								
	M	P	G1 (KU)	G2 (S2)	G3 (YO)	G4 (ST-3)	G8 (M-69)	G9 (F45)	G3 (SA-11)	G6 (BOVLIN)[c]	G10 (B-223)
P > M	−ve	50		2			1			1	1
	−ve	100	2	8		2			1		1
	−ve	200						4			
	50	100		1	1	2	1		1		1
	50	200						1			
	100	200						3			
Total (%)			2 (8.0)	11 (44.0)	1 (4.0)	4 (16.0)	2 (8.0)	8 (32.0)	2 (8.0)	1 (4.0)	3 (12.0)
M = P			(0.0)	3 (12.0)	0 (0.0)	5 (20.0)	4 (16.0)	6 (24.0)	9 (36.0)	1 (4.0)	0 (0.0)
M−ve, P−ve			11 (44.0)	7 (28.0)	9 (36.0)	5 (20.0)	4 (16.0)	3 (12.0)	0 (0.0)	3 (12.0)	17 (68.0)

[a] $n = 25$. P, child patient; M, mother.
[b] N, neutralizing.
[c] BOVLIN, bovine Lincoln.

The child patients of group 1 showed higher NAb titers than their mothers (P > M) to serotypes G2 and G9. Eight (32.0%) children had higher NAb titers than their mothers against G9, and 11 (44.0%) had higher NAb titers than their mothers against G2. The major infecting serotypes in group 1 seem to be G2 and G9. The statistical significance of the G2 and G9 serotypes showing higher NAb titers among child patients than the other serotypes was calculated, and it was found that the NAb titers of the child patients whose NAbs to serotype G2 were greater than those of their mothers were significantly higher ($P < 0.05$) than the NAbs to all other serotypes except G9. Similarly, the NAbs of the child patients whose NAbs to serotype G9 were greater than those of their mothers were also significantly higher ($P < 0.05$) than their NAbs to G1, G3, G8, G3 (SA-11), and G6 bovine.

In group 2, the sera of the child patients showed higher NAbs to serotypes G1, G2, G4, and G9, indicating that these serotypes are the main ones involved in infecting the children (Tables 4 and 5). NAb positivity to the G1 serotype of the child patients whose NAbs were greater than those of their mothers was significantly higher ($P < 0.05$) than that to other serotypes except for G2, G4, and G9. The NAbs to serotype G2 of the child patients whose NAbs were greater than those of their mothers were significantly higher ($P < 0.05$) than to all other serotypes except for G1, G4, and G9. The NAbs to serotype G4 of the child patients whose NAbs were greater than those of their mothers were significantly higher ($P < 0.05$) than to all other serotypes except for G1, G2, and G9. The NAbs to G9 of the child patients whose NAbs were greater than those of their mothers were significantly higher ($P < 0.05$) than to all other serotypes except for G1, G2, and G4.

For group 1 and group 2, the overall percentages of cases in which NAbs were absent in the mothers as well as the child patients (M−ve P−ve) for serotypes G10 and G1 were about 70 and 40%, respectively, indicating that these serotypes are at very low circulation levels. The levels of NAbs in the child patients whose NAb titers were less than or equal to those of their mothers in both groups seem to be maternal antibodies acquired either through placental transfer or through breast-feeding.

Correlation of infecting rotavirus serotype with the serum NAb response in the serotypeable group. The correlation of infecting serotype and NAb response (P > M and P ≤ M) was analyzed in group 2 children (Tables 6 and 7). Correlation of

TABLE 4. Comparative NAb titers among group 2 mothers and child patients where M > P[a]

Category	Increase in N titer for indicated sera[b]		No. of M serum samples with higher N titer to indicated serotype than that of P								
	P	M	G1 (KU)	G2 (S2)	G3 (YO)	G4 (ST-3)	G8 (M-69)	G9 (F45)	G3 (SA-11)	G6 (BOVLIN)[c]	G10 (B-223)
M > P	−ve	50	8	4	6	3	5	3	11	10	8
	−ve	100	3	7	2	6	18	3	18	11	1
	−ve	200			6			2		5	
	50	100	1		1	4	1	2	2	4	
	50	200			5	2					
	100	200			1	1		2			
Total			12 (30.76)	11 (28.20)	21 (53.84)	16 (41.02)	24 (61.53)	12 (30.76)	31 (79.48)	30 (76.92)	9 (23.07)

[a] $n = 39$. M, mothers; P, child patients.
[b] N, neutralizing.
[c] BOVLIN, bovine Lincoln.

TABLE 5. Comparative NAb titers among group 2 mothers and child patients where P > M[a]

Category	Increase in N titer for indicated sera[b]		No. of P serum samples with higher N titer to indicated serotype than that of M								
	M	P	G1 (KU)	G2 (S2)	G3 (YO)	G4 (ST-3)	G8 (M-69)	G9 (F45)	G3 (SA-11)	G6 (BOVLIN)[c]	G10 (B-223)
P > M	−ve	50	3		1	5	2				
	−ve	100	5	9		4	1	3			
	−ve	200	2					4		1	
	50	100		2		1	1	2		1	1
	50	200						2			
	100	200		2				6			
Total (%)			10 (25.64)	13 (33.33)	1 (2.56)	10 (25.64)	4 (10.25)	17 (43.58)	0 (0.0)	2 (5.12)	1 (2.56)
M = P			0 (0.0)	4 (10.3)	0 (0.0)	10 (25.64)	6 (15.38)	6 (15.38)	7 (17.94)	3 (7.69)	0 (0.0)
M−ve, P−ve			17 (43.58)	11 (28.20)	17 (43.58)	3 (7.69)	5 (12.82)	4 (10.25)	1 (2.56)	4 (10.25)	29 (74.35)

[a] $n = 39$. M, mothers; P, child patients.
[b] N, neutralizing.
[c] BOVLIN, bovine Lincoln.

the infecting serotype and the NAb response for the child patients whose NAb titers were greater than those of their mothers was found in 23 of 39 (58.97%) cases. Five more children showed NAb response to the infecting serotype, but the levels were lower than or equal to those of their mothers. It is possible that the children lacked maternal NAb and had developed low-level NAb to that serotype (Table 6, serial numbers [Sr. Nos.] 9 and 13 to 16). The sera of 28 out of 39 (71.79%) children showed a homologous NAb response (either P > M or P ≤ M) to the infecting serotype. In 7 of 39 cases where G1 was the only infecting serotype, a homologous NAb response (P > M) to the G1 serotype was observed, accompanied by a heterologous NAb response mainly to G2 and G4 and to a small extent to G6 and G9. In the 10 out of 39 (25.64%) cases with only G9 as the infecting serotype, only a homologous NAb response (P > M) was observed in 5 cases.

TABLE 6. Correlation of infecting rotavirus serotype and serum NAb response in children of group 2[a]

Sr. no.	Sp. no.[c]	Infecting serotype	NAb (P > M) to indicated serotype(s)	NAb (P ≤ M) to indicated serotype(s)[b]
1	931072	G1	G1, G2, G4	
2	931193	G1	G1, G2	G4
3	932047	G1	G1, G2	G3, G4, G8, G9
4	932577	G1	G1, G4	G9
5	932610	G1	G1, G6	G2, G3, G4, G3*
6	933190	G1	G1, G4	G3, G8
7	94402	G1	G1, G9	
8	9218967	G2 (method B)	G2	G9
9	9217865	G2	G4	G2
10	941095	G2	G2, G9, G10	G4, G3*
11	932567	G1, G2 (method B)	G2, G4	G8, G9, G6
12	934940	G1, G2 (method B)	G2, G9	
13	938529	G2, G4		G4, G9
14	9310309	G2, G4		G4
15	932579	G6	G1, G2	G3, G3*, G4, G8, G9, G6
16	935209	G6, G10		G3, G8, G9, G6, G3*
17	935522	G6, G10	G4, G6, G8, G9	G3, G3*
18	938048	G9	G9	G3, G4
19	938452	G9	G9	G4, G6
20	938499	G9	G9	G4
21	939031	G9	G9	
22	94403	G9	G2, G9	G8, G6
23	941534	G9	G2, G8, G9	G3*
24	941659	G9	G1, G8, G9	G4, G3*, G6
25	941885	G9	G9	
26	941991	G9	G4, G9	
27	942835	G9	G4, G9	
28	942838	G3, G9	G9	

[a] $n = 39$.
[b] G3*, G3 simian strain.
[c] Sp. no., specimen number.

TABLE 7. Lack of correlation of infecting rotavirus serotype and serum NAb response in children of group 2

Sr. no.	Sp. no.[b]	Infecting serotype by method A	NAb (P > M) to indicated serotype	NAb (P ≤ M) to indicated serotype[a]
1	931363	G1	G2	G4
2	941199	G1	G2, G9	G3*, G6
3	943096	G1 (method B)		G4
4	941647	G2	G1, G4, G9	G3*, G8
5	942309	G2	G4	G9
6	941645	G2, G3	G9	G6
7	931189	G6, G10	G3	G2, G4
8	931026	G6, G10		G2, G9
9	931163	G6, G10	G2, G8	G4
10	933188	G6, G10		G4, G9
11	935075	G6, G10		G4

[a] G3*, G3 simian strain.
[b] Sp. no., NIV specimen number.

The other five sera samples showed homologous as well as heterologous NAb responses to G2, G4, and G8. Eleven patients did not develop NAb to the infecting serotype but showed a heterotypic NAb response (Table 7). In conclusion, it appears from the results that children from group 2 were infected most frequently with serotypes G1, G2, G4, and G9.

The presence of NAbs to serotypes other than the infecting serotype among the children either may be a reflection of maternal NAbs or may be due to boosting of the antibodies.

Age of the child and number of infecting serotypes. It was expected that the sera of the children in the very young age groups would neutralize only one serotype but that sera from the older age group would neutralize more than one serotype because of the increased probability of repeated infections with rotavirus. However, such a phenomenon was not noticed. There was no correlation of the age of the children and the number of serotypes to which there was a NAb response ($P >$ M). In fact, even in the age group of ≥6 months to 8 months, 6 of 21 (28.57%) children showed NAb responses to 2 serotypes (Table 8).

The overall percentage of sera from children showing a monospecific NAb response was 14.06%, but that from child patients whose NAb titers were greater that those of their mothers (P > M) was 35.93%. Thus, a NAb response greater than that of the mother (P > M) seems to be a fairly accurate measure of homologous antibody response to rotavirus infection among child patients, because NAb levels less than or equal to those of the mother (P ≤ M) may reflect the presence of maternal antibodies.

Thirteen (20.31%) children, irrespective of age, did not show NAb response (P > M) to any of the serotypes tested (Table 8). Sera from these children need to be investigated further for the infecting serotypes.

DISCUSSION

The present investigation reports NAb responses to different rotavirus serotypes in the convalescent-phase serum samples of rotavirus-positive children hospitalized for diarrhea and their mothers. The presence of maternal antibodies in a child's serum may be due either to transplacental transfer of antibodies,

TABLE 8. Age group of child patients and their NAb response to different numbers of rotavirus serotypes

Age group (months)	Total no. of children	No. of children who had higher level of NAb response than their mothers to indicated no. of rotavirus serotypes (P > M)				No. of children who had NAb response to indicated no. of rotavirus serotypes			
		None	1	2	≥3	None	1	2	≥3
Group 1 (n = 25)									
≤6	3	1	2				1	1	1
>6–8	5	1	2	1	1		1		4
>8–10	4	2	1		1			1	3
>10–12	6	1	3	1	1		1	3	2
>12–24	6	1	3	1	1	1		2	3
Age of child not known	1			1					1
Total	25	6 (24.0)	11 (44.0)	4 (16.0)	4 (16.0)	1 (4.0)	3 (12.0)	7 (28.0)	14 (56.0)
Group 2 (n = 39)									
≤6	4		1	3				2	2
>6–8	9	4	2	2	1		3	3	3
>8–10	10	1	4	5			2	5	3
>10–12	5	1	1	2	1			1	4
>12–24	9	1	3	2	3			2	7
Age of child not known	2		1		1		1		1
Total	39	7 (17.94)	12 (30.76)	14 (35.87)	6 (15.38)		6 (15.38)	13 (38.33)	20 (51.28)
Grand total	64	13 (20.31)	23 (35.93)	18 (28.12)	10 (15.62)	1 (1.56)	9 (14.06)	20 (31.25)	34 (53.12)

mainly immunoglobulin G (IgG) or IgA antibodies, via mother's milk. A positive correlation between rotavirus antibodies in infants' gastrointestinal tracts and sera has been reported. Also, the presence of rotavirus IgG in a child's serum correlated with its presence in the mother (26). The benefits of maternal immunity are not lasting enough to give protection from rotavirus infection to children. Although it has been observed that children younger than 6 months have mild diarrhea which can be treated at home, children older than 6 months suffer from more-severe diarrhea requiring hospitalization (39). Thus, the protective effect of rotavirus antibodies may be limited to the period of lactation.

Since the study was conducted as part of a surveillance study of rotavirus, acute-phase serum samples were not collected. However, a study of the prevalence of NAbs to different rotavirus serotypes was carried out with the mothers' sera. Testing of the sera of the mothers of the child patients has provided very useful information regarding the relation of maternal immunity in terms of NAb to rotavirus diarrhea among children.

In 1988, Brussow et al. (10) reported that rotavirus antibody prevalence among children decreased from 4 to 12 months of age, indicating that even at the age of 1 year a child may have maternal antibodies, and we found that some children had titers of antibody against different serotypes equivalent to or less than those of their mothers. The presence of high titers of NAbs to a particular serotype in the mother's serum and the absence of NAbs in the child's serum indicate that the maternal antibody level has waned or that the child did not encounter infection with that serotype. It may also be that the serotype was not in circulation. The presence of lower titers of NAbs in the child than in the mother ($M > P$) reflects either circulating maternal NAbs in the child's serum or a heterotypic response induced by the infecting serotype. The absence of NAbs in the mother as well as the child patients may indicate either that the serotype was not in circulation or that infection with that serotype was not encountered.

The present study shows that the serum NAb responses of mothers as well as of children are useful measures of the prevalence of rotavirus serotypes in the community and that the presence of high levels of NAb to a particular serotype in the mother at the time of the child's illness is associated with a lower chance that the child is infected with that serotype. It has been noted that the mothers had high levels of NAbs to G3, G3 simian, G6 bovine, and G8. These serotypes were not much involved in causing diarrhea, although the children showed low levels of NAbs to these serotypes. Thus, it is likely that these serotypes were not circulating during the period of collection of the serum samples or that some other maternal factor was involved in the lower incidence of infection due to these serotypes. Recently, Burns et al. (12) (1996) reported the protective effect of factors like IgA antibody to VP6 in murine rotavirus infection.

In primary rotavirus infection, the NAb response tends to be specific for the infecting rotavirus serotype. From our data, it appears that 22 children who showed a monospecific NAb response higher than that of their mothers probably had primary infection and that the other 42 children may have undergone secondary or multiple infections. Brussow et al. (7) (1988) showed that only 20% of 71 infants seroconverted to a single rotavirus serotype, whereas 39% seroconverted to two or more rotavirus serotypes, even when possible secondary infections were excluded from the analysis. Thus, cross-neutralizing antibodies may be a rather common phenomenon in infants, especially among hospitalized infants. The cross-reactivity of NAbs in the serum samples from the children may not be due to two or more rotavirus strains causing the disease at the same time. The cross-reactivity observed may be because of the boosting effect of NAbs already present, the NAb response to VP4 antigen, or dual reactivity of the infecting rotavirus serotype. In the past, it was considered that in a particular child only the first episode of rotavirus infection was associated with severe symptoms (6, 25) or that the development of serum antibody followed the classical pattern of primary infection with early rises in antirotavirus IgM antibodies (24). Our data show that children 6 to 8 months of age also showed a higher antibody response than their mothers to more than one serotype, indicating that the children had already experienced rotavirus infection. Also, rotavirus-specific IgM antibodies detected in the asymptomatic mothers of rotavirus children (41a) indicated that IgM alone is not a marker for primary rotavirus infection. A high prevalence of IgM was observed in all age groups above 1 year of age up to adulthood, as reported by Brussow et al. (10) (1988). Therefore, the earlier notion that hospitalized diarrhea patients may have primary rotavirus infection may not be true. Thus, a child's showing a higher NAb response than his or her mother to a single serotype may indicate primary rotavirus infection, whereas a higher NAb to two or more serotypes may be a heterologous-anamnestic response elicited by the infecting serotype.

NAb studies by Gerna et al. (19) (1990) showed that in primary infections serotype 1 induced an antibody response to serotype 4 at least fourfold lower than the homotypic response but that serotype 2 elicited titers of antibody to serotypes 1 and 4 at least fourfold lower than the homotypic titer and serotype 4 infections produced a response to serotype 1 as high as the homotypic response. Ward et al. (52) (1990) reported that, after primary infection, NAb to serotype 1 had the highest titers, but NAb to serotype 3 had consistently high titers, and NAb to serotypes 2 and 4 had low titers ($\geq$20). Thus, there appears to be strain-to-strain variation as far as the immune response is concerned. In our study, there was a heterotypic response to several serotypes due to G1 strains, mostly to serotypes G2 and G4, and in some cases there was no homologous response. The infecting serotype G9 produced a heterotypic response to G2, G4, and G8 in some cases, in addition to the homotypic response.

Attempts were made to trace the infecting rotavirus serotype from the NAbs found in the sera of the children from group 1 who were infected with nontypeable rotavirus. Among the initial 35 serum samples of this group, 10 were proved to be G9 by isolation and/or reaction to G9 MAb (data not shown). However, it is expected that more cases due to G9 can be proved by isolation where detection of MAb to G9 was not possible, probably due to a lack of sufficient double-shelled particles. In these cases, the children showed higher titers of NAb to G9 than did their mothers and reacted monospecifically to G9; some sera showed reactions to serotypes G2 and G4 in addition to the infecting rotavirus, serotype G9, which appear to be due to a heterologous NAb response. Besides the G9 serotype, G2 was the major infecting serotype among group

1 child patients. However, G2 strains could not be serotyped. These strains appear to be similar to those reported by other Indian workers (1, 2). More work with these strains is indicated.

Similarly, from group 2, correlation has been shown between the infecting serotype and higher levels of NAb than those of the mothers in about 60% of children showing higher homologous NAb mainly to the G1, G2, G4, and G9 serotypes. Five children did not show higher levels of NAb than did their mothers to the infecting serotype, but they showed homologous NAb titers which were either less than or equal to that of their mothers. There are two possible reasons. Firstly, these children had no NAbs or had very low titers of NAbs to the infecting serotype; hence, the NAb titer did not exceed that of the mother. Secondly, we have observed that some mothers get infected with rotavirus while in close contact with their rotavirus-positive children, as indicated by development of rotavirus-specific serum IgM antibodies and seroconversion of rotavirus-specific IgA and IgG antibodies (41a). As a result of asymptomatic infection among the mothers, NAb titers generally tend to increase; hence, NAb levels among the children may not exceed that of their mothers.

Ryder et al. (44) have presented evidence that repeated episodes of rotavirus infection occur in adults at intervals as short as 1 year. Each episode appears to be accompanied by a transient rise in the titer of antibodies. Serotype G1 has been reported to be most common infecting serotype all over the world (5). However, our data shows that serotype G1 is circulating at comparatively lower rates in India (31, 37).

Two seroepidemiological surveys, in Japan in 1984 (49) and in Germany (10) showed that in primary infections the percentage of sera neutralizing only one rotavirus serotype was high in infants 8 to 12 months of age. With increasing age of the infants and thus an increased probability of repeated rotavirus infections, sera neutralizing more than one serotype became preponderant. The present study also showed more infants with higher levels of NAb to more than one rotavirus serotype. In developing countries like India, from the time children are 7 to 8 months old, their sera react to several serotypes, indicating early and multiple exposures to rotavirus.

Thirteen children's sera did not show higher titers of NAb than that of their mothers to any of the serotypes tested. Taniguchi et al. (47) reported rotavirus serotype G12 in the Philippines in 1990. Recently, Griffin et al. (23) (2002) identified the G12 serotype from nontypeable specimens. Moreover, although G5 has not been reported so far from India, it is quite likely that G5 circulates in India, as reported from South Africa (21). Therefore, it is important to test sera against the G5 and G12 serotypes and to attempt to isolate rotavirus from the fecal specimens of children whose serum samples were negative for NAbs to all the serotypes tested.

The results of the present investigation suggest that G1, G2, G4, and G9 are the most common serotypes causing diarrhea among children. Since the specimens were collected from 1992 to 1994, it would be interesting to know the present trend of rotavirus serotype circulation in Pune, India.

Our data show that the lack of NAbs to a particular serotype in a mother's serum may lead to infection with that serotype in her child. There has been controversy about the role of serum antibodies; however, recently the data from various studies was reviewed by Jiang et al. (32) (2002). It was suggested that serum antibodies at critical levels are either protective themselves or are important and powerful correlates of protection against rotavirus disease. It has been reported that NAbs which are most likely transplacental in origin were associated with protection against dehydration but not against mild disease or infection (44).

The most interesting findings of the studies are as follows. (i) Higher NAb response to a single serotype in child patients than in their mothers correlated well with the infecting serotype. Thus, in group 1, the major infecting serotypes were G2 and G9, whereas in group 2 they were G1, G2, G4, and G9. (ii) At a very early age, children were shown to exhibit NAb response ($P > M$) to more than one serotype. This finding may be attributed mainly to early exposure to one or more serotypes among these children.

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