

## Comparison of Serum and Mucosal Antibody Responses Following Severe Acute Rotavirus Gastroenteritis in Young Children

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The development of mucosal immunity is presumed to be the most important marker of rotavirus infection. The practical difficulties of obtaining small-bowel secretions stimulated this study of the antibody response to acute rotavirus infection at other sites. Forty-four infants admitted to the hospital with rotavirus gastroenteritis had serum, saliva, and feces collected at the acute phase (median, 5.5 days), during convalescence (median, 33.5 days), and 4 months later (median, 12.2 weeks). A subgroup of 19 children also had duodenal juice collected in parallel. Rotavirus-specific immunoglobulin G (IgG), IgA, secretory immunoglobulin, and IgM were measured and compared in all samples. The results showed that the estimation of antirotavirus serum IgM, serum IgG, duodenal juice IgA, and duodenal juice IgM by an enzyme immunoassay indicated an immune response to severe primary rotavirus infection in all children. Four months later, the levels of serum IgG and IgA served as the most sensitive markers of the preceding rotavirus infection. The predictive accuracies of immune responses at different sites in relation to a positive IgA immune response in the duodenum were calculated. Fecal IgA predicted duodenal IgA rotavirus antibodies with accuracies of 86% at 1 month and 92% at 4 months. The high sensitivity of serum IgM and IgG in detecting rotavirus infection and the high predictive accuracy of fecal IgA as an indicator of duodenal IgA abrogates the need for duodenal intubation to detect (or monitor) an immune response to rotavirus infection. This finding has important practical implications for epidemiological studies of acute diarrhea in children and in rotavirus vaccine trials.

Rotaviruses are the single most common cause of severe acute diarrhea in infants and young children. The mortality and morbidity associated with infection in developing and developed countries have led to the recognition of the need for an effective rotavirus vaccine (15). Rotaviruses infect mature epithelial cells lining the upper portions of the villi of the small intestine (5), and antibodies present in the intestinal lumen have been shown to play a primary role in passive protection of young animals (18, 22).

It is assumed that antibodies produced by cells in the gut mucosa in response to infection and secreted into the gut lumen are the most important markers of an immune response. However, it is not clear whether the immune response can be detected equally well in serum and in gut secretions. Initial assessments of candidate oral rotavirus vaccines have used an enzyme immunoassay (EIA) of serum antibodies to detect immune responses (15, 26), even though animal experiments have indicated that serum antibody levels generally cannot be correlated with protection (18, 22). Studies of the immune response to natural infection in young children have mainly focused on the measurement of rotavirus antibodies in serum or in feces (8, 12, 19, 20, 23, 24). Two studies have measured rotavirus antibodies in small intestinal secretions (6, 13). Davidson et al. (6) compared immunoglobulin M (IgM), IgG, and IgA antirotavirus antibody responses in serum and duodenal juice obtained from eight children (2 to 24 months old) at 1 week and 4 to 5 weeks

after the onset of infection. Hjelt et al. (13) compared IgG, IgA, and secretory immunoglobulin antirotavirus antibody responses in serum, duodenal juice, and feces obtained from eight children (36 days to 7.2 years old) 1 to 2 days and 5 to 9 days postadmission to the hospital with rotavirus gastroenteritis. The results of both studies combined indicate that specific IgM, IgA, and secretory immunoglobulin in serum from some children may show patterns of response identical to those measured simultaneously in intestinal secretions.

The aim of this study was to document in the most comprehensive manner possible the development and persistence of rotavirus IgG, IgM, IgA, and secretory immunoglobulin antibodies in serum and secretions (saliva, duodenal juice, and feces) obtained simultaneously from infants and young children following severe acute rotavirus infection. In particular, the study was designed to determine the specimen(s) and the assay(s) best suited to the detection of an immune response to rotavirus infection. The results show that EIA measurement of antirotavirus IgM or IgG antibodies in sera obtained 7 and 30 days after the onset of symptoms is a reliable indicator of a recent rotavirus infection. In addition, the presence of antirotavirus IgA in stools 4 weeks after infection indicates the presence of the same antibody in duodenal fluid, with a predictive accuracy of 86%.

### MATERIALS AND METHODS

**Patients.** Patients were 44 children (26 male), 3 weeks to 39 months old, admitted to the infectious diseases ward of the Royal Children's Hospital, Melbourne, Victoria, Australia, with acute rotavirus diarrhea (less than 6 days from onset)

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between April 1984 and September 1985. Vomiting was present in all children, and 42 had fever. Dehydration was assessed as 5% (16 children), 6 to 9% (15 children), or  $\geq 10\%$  (13 children), and parenteral rehydration was undertaken in 28 of 44 children. The duration of symptoms was 4 to 13 days (median, 6 days), and hospitalization occurred for a median of 4 days (range, 2 to 13 days). Recovery in five children was complicated by the development of sugar malabsorption.

Patients were divided into two groups with similar demographic and clinical features. Group A comprised 19 children (11 male), 2 to 39 months old (mean, 19.5 months), from whom saliva, duodenal juice, feces, and serum were collected. Group B comprised 25 children (15 male), 0.75 to 36 months old (mean, 16 months), from whom saliva, feces, and serum were collected. None of the children were breast-fed during the course of the study.

Rotavirus infection was diagnosed within a few hours of admission to the hospital by electron microscopy of negatively stained concentrated fecal homogenates. Results were confirmed by an EIA with monoclonal antibodies developed in our laboratory to identify group and serotype antigens (4). A total of 27 children were infected with serotype 1 rotaviruses, and 10 were infected with serotype 4 rotaviruses. No serotype could be assigned to rotaviruses infecting seven of the children.

Ethical approval for the study was obtained from the Human Ethics Committees of the Royal Children's Hospital and of the World Health Organization. Informed consent was obtained from the parents of the children prior to enrollment in the study.

**Sample collection.** Three specimens each of blood, saliva, and feces were collected from the 44 children studied. In addition, three specimens of duodenal juice were collected from the 19 children in group A. The sets of specimens from each child were collected at 2 to 7 days (median, 5.5 days), 24 to 66 days (median, 33.5 days), and 100 to 282 days (median, 122 days) after the onset of symptoms. Although collection intervals varied from child to child, for each individual child the sets of specimens (serum and secretions) were collected on the same day. Additional fecal specimens were collected daily while each child was in the hospital and weekly thereafter for the duration of the study. Blood specimens were collected by venipuncture or finger pricking. Blood was allowed to clot at room temperature, centrifuged at  $750 \times g$  for 5 min, and stored at  $-70^\circ\text{C}$ . Unstimulated whole saliva was obtained by placing rolls of cotton gauze in the mouth between the cheek and clenched teeth, close to the opening of the parotid ducts. When moist, the rolls were removed, and the sublingual pool of saliva was also collected. The gauze rolls were centrifuged at 3,000 rpm for 5 min, and the saliva was stored at  $-70^\circ\text{C}$ . Feces were collected and stored at  $-70^\circ\text{C}$  within 4 h if collected in the hospital. Feces collected at home were immediately stored at  $-20^\circ\text{C}$  initially, transferred to the hospital in a car freezer, and then stored at  $-70^\circ\text{C}$ . Duodenal juice was collected in the hospital by using the technique described by Ford (7). Children fasted and were premedicated with oral quinalbarbitone (12 mg/kg) and metoclopramide (0.2 mg/kg). Once the child was sedated, a Watson pediatric intestinal biopsy capsule (minus the knife block) with an attached French five-gauge feeding tube was introduced, and progress to the third or fourth part of the duodenum was monitored by fluoroscopy. Small portions of air were instilled via the biopsy capsule tube to encourage aspiration of fluid up the feeding tube. Duodenal fluid (5 to 10 ml) was aspirated within 5 min, immediately centrifuged at  $750 \times g$  for 5 min, and

stored at  $-70^\circ\text{C}$ . Following duodenal aspiration all children were observed in a recovery room for 2 to 4 h until awake.

**Antibody assays.** Serum, saliva, duodenal juice, and feces were all examined for the presence of rotavirus antibodies by an EIA modified to quantitate class-specific antibodies (IgA, IgM, and IgG) and secretory immunoglobulin. IgA, IgG, and secretory immunoglobulin in all specimens and IgM in saliva, duodenal juice, and feces were measured by modifications of a direct EIA (2, 4). IgM in serum was measured by a capture EIA.

Briefly, the technique of direct EIA involved coating microdilution trays with SA11 antigen (grown in MA104 cells), followed by the addition of a sample of serum, saliva, duodenal juice, or fecal homogenate. After the trays were washed, specific antibody was detected by using rabbit antihuman IgA, secretory immunoglobulin, IgM, or IgG conjugated to horseradish peroxidase (DAKO Immunoglobulins) and by using 3,3',5,5'-tetramethyl benzidine (TMB) substrate. The optical density at 450 nm was measured by using a Titertek EIA reader. All samples were assayed in duplicate against SA11 antigen and against uninfected MA104 cells as the control antigen. The specificity of the reaction was controlled by including wells containing SA11 or control antigen incubated in the absence of the sample. Sensitivity was checked by including wells containing SA11 or control antigen incubated in the presence of samples known to contain high titers of a particular rotavirus-specific immunoglobulin class. A test well was considered positive if its optical density at 450 nm was greater than or equal to two times that of its own control. The capture technique used for the serum IgM assay included affinity-purified goat antihuman IgM (TAGO Immunoglobulins) as the initial coating on the microdilution plates. Serial doubling dilutions of each test serum specimen (beginning at 1:50) were added. After the plates were washed, SA11 and MA104 cells were added to test and control wells for each serum specimen. The plates were washed again, and monoclonal antibody to rotavirus group antigen (developed in our laboratory) conjugated to horseradish peroxidase was added, followed by TMB substrate. The sensitivity and specificity of the assay were checked by the inclusion of known positive and negative specimens. No rheumatoid factor was detected in any of the serum specimens by latex agglutination with RapiTex R.F. (Behring Institute).

Rotavirus antibodies of each class were quantitated by endpoint titrations by using doubling dilutions. The positive/negative (P/N) cutoff values established for each immunoglobulin class at each site are shown in Fig. 1. The data establishing these values will be published elsewhere. An immune response for IgA, secretory immunoglobulin, and IgG was defined as a fourfold increase in titer in two consecutive specimens such that the maximum value was greater than or equal to two times the P/N value. An immune response for IgM was defined as a titer greater than or equal to two times the P/N value in a single specimen.

**Statistical methodology.** The major statistical tests used were the McNemar test for correlated proportions and a generalization of the Wilcoxon signed-rank test (21). These tests allowed pairwise and multiple comparisons to be made between different antibodies at a given site and between different sites for the same antibody by using, respectively, the proportions of elevated immune antibody response (McNemar test) and the actual antibody titers or the logarithmic (to the base 2) antibody titers (Wilcoxon-type test). The reliability of extraintestinal sites as indirect measures of immune response in the small intestine was tested by assess-

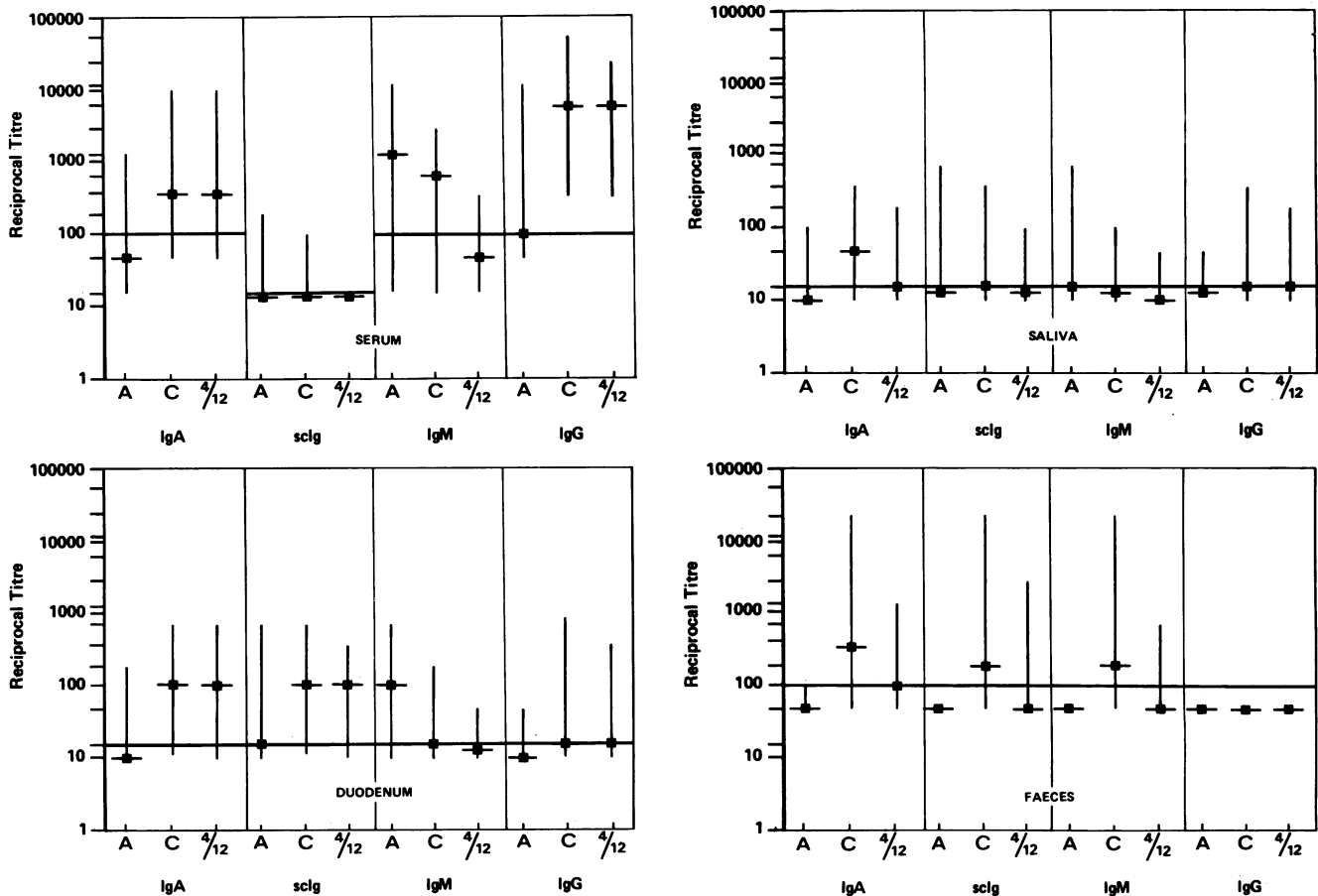


FIG. 1. Rotavirus antibody titers in serum and intestinal contents after rotavirus enteritis in infants during the acute phase (A), during the convalescent phase (C), and after 4 months ( $\frac{1}{12}$ ). ■, Median value. Horizontal lines represent P/N cutoff values. Vertical lines represent ranges of values. scIg, Secretory immunoglobulin.

ments of sensitivity and positive predictive accuracy. The Kendall tau value (16) was calculated to assess the association between antibody titers in duodenal fluid and other sites. All statistical computations were carried out by using the Minitab (Minitab Manual, 1985) and custom-written Minitab macros.

## RESULTS

**Occurrence of immune response during month 1 after infection.** The percentages of patients demonstrating antirotavirus immune responses in duodenal juice, saliva, feces, and serum are shown in Table 1. The total number of patients showing an immune response in at least one subclass of antibody at each site sampled is also shown, since many patients showed an immune response in more than one subclass of antibody. The medians and ranges of values for each immunoglobulin class in each specimen are shown in Fig. 1.

All patients studied showed an immune response in duodenal juice. The dominant antibody responses were in IgA (84%) and IgM (84%). An immune conversion to secretory immunoglobulin was detected in 58% of patients and was always associated with a response in IgA or IgM antibody (or both). IgG responses were seldom detected (26%). An immune response (predominantly in IgA and IgM antibodies) was observed in saliva and feces from 80 and 84% of

patients, respectively. Titers of all antibody subclasses in duodenal juice and saliva (Fig. 1) were low, ranging from  $<1:12.5$  to  $1:800$ . Titers in feces were often high and ranged from  $1:50$  to  $1:25,600$ , with a median of  $1:400$  in convalescent-phase specimens. No IgG antibodies were detected in feces from any children.

All children showed an IgM immune response in serum, and 91% showed an IgG immune response. Only 3 of the 44 children (all older than 6 months) had elevated IgG titers in acute-phase sera. Of the 44 children, 4 showed no seroconversion to IgG antibodies. Three of these were aged 3 months or less. The fourth child had a high titer of IgG antibodies in acute-phase serum ( $1:6,400$ ) that did not rise further. An IgA immune response in serum was detected in 68% of children, but secretory immune responses occurred in only 14% of patients. The ages of patients who failed to show an IgA immune response ranged from 2 to 37 months (mean, 16.2 months).

**Rotavirus antibody levels 4 months after infection.** The percentages of children with detectable levels of immunoglobulin subclasses (greater than or equal to two times the P/N cutoff values) at each of the four sites 4 months after rotavirus gastroenteritis are listed in Table 2. Few children showed detectable antirotavirus IgM antibodies at any site. IgA antibodies were detected frequently in duodenal juice, saliva, feces, and serum. The McNemar test for correlated

TABLE 1. Immune responses in serum and mucosal sites following rotavirus gastroenteritis

Specimen	No. of patients examined	% of patients with immune response to:				Total immune response (% of patients)
		IgA	Secretory immunoglobulin	IgM	IgG	
Duodenal fluid	19	84	58	84	26	100
Saliva	44	55	20	50	30	80
Feces	44	77	66	61	0	84
Serum	44	68	14	100	91	100

proportions showed that the IgA immune response 4 months after rotavirus gastroenteritis was not significantly different whether measured at the duodenal or fecal site ( $P > 0.10$ ). Pairwise comparisons of the median antibody titers (4 months after rotavirus gastroenteritis) done with the Wilcoxon signed-rank test showed that median IgA antibody titers in the duodenum were not significantly different from median IgA antibody titers in feces ( $P > 0.10$ ) and serum ( $P > 0.80$ ). The Maritz generalized Wilcoxon test for trends showed that the IgA antibody titers were not significantly different 4 months after rotavirus gastroenteritis in the duodenum, feces, and serum ( $P > 0.05$ ). IgG antibodies were present in all serum samples. Low positive levels of IgG antibodies were detected in approximately 60% of saliva and duodenal juice specimens. There was a statistically significant association ( $P < 0.015$ ) between IgG titers of  $\geq 1:12,800$  in serum and the detection of positive levels of antirotavirus IgG (titer,  $\geq 1:50$ ) in acute- and convalescent-phase specimens of saliva and duodenal juice.

**Kinetics of response.** The development and changes in titers of rotavirus antibodies of each immunoglobulin subclass in duodenal juice (19 children) and saliva, feces, and serum (44 children) are shown in Fig. 1. The P/N cutoff values for antibody measurements are indicated.

For duodenal and saliva specimens, antirotavirus IgA titers were highest in specimens obtained approximately 30 days after onset and were also elevated in most children 4 months later. The earliest detection of antirotavirus IgM was on day 4 after the onset of symptoms, but most positive specimens were obtained on days 6 and 7 after onset. Secretory immunoglobulin was detected in some patients during the acute phase, increased in titer during convalescence, and remained elevated in titer 4 months later.

The collection of numerous fecal specimens from each child allowed a closer examination of the timing of the appearance of rotavirus antibodies. Rotavirus-specific IgA, IgM, and secretory immunoglobulin appeared as early as day 3, reached maximum values between days 11 and 15, and decreased to approximately P/N cutoff values by week 5.

The antirotavirus antibody responses in serum differed from the secretory responses in saliva, duodenal juice, and feces. Positive serum IgM levels were detected as early as

day 2 after onset, but the highest titers were generally found in specimens obtained more than 6 days after onset. Serum IgG and IgA titers reached maximum levels during convalescence, and these were maintained with little change at 4 months. Median levels of IgG (1:6,400) in convalescent-phase specimens and specimens taken at 4 months were significantly different from median levels of IgA (1:400) ( $P < 0.0001$ ).

**Prediction of duodenal IgA response by responses at more accessible sites.** Assuming that the IgA immune response in the duodenum is of critical importance, the data were examined to assess the sensitivity and positive predictive accuracy of responses at more accessible sites.

Sensitivity is a measure of the likelihood that a particular site will show a positive response when the response in the duodenum is known to be positive, e.g., if a test on specimens from an extraduodenal site reveals a positive response in 8 cases out of 10 in which the duodenal response is positive, then the sensitivity of the test at that site is 80%.

Predictive accuracy is a measure of the likelihood that an immune response known to be positive at an extraduodenal site is associated with a positive immune response in the duodenum; e.g., if an immune response at an extraduodenal site in 10 cases is associated with an immune response in the duodenum in 7 cases, the predictive accuracy of the test at that site is 70%.

Tables 3 and 4 list these indices for immune conversion at different sites at 4 weeks and for elevated antibody levels at different sites at 4 months, respectively.

At 4 weeks, a serum IgM response was always found when a duodenal IgA response occurred, i.e., the sensitivity of serum IgM was 100%. The sensitivity of serum IgG was 87.5%. Four months after rotavirus infection, the sensitivity of serum IgG and IgA as indicators of elevated duodenal IgA antibodies was 100%. The most accurate predictors of a duodenal IgA response during the first 4 weeks after onset were salivary IgA (86%) and fecal IgA (86%). After 4 months, predictive accuracy was highest for fecal IgA (92%) as compared with other sites. The Kendall tau value was calculated to measure the association between IgA antibody titers in duodenal fluid and in saliva, feces, and serum. There was a significant association only between duodenal and

TABLE 2. Elevated specific antibodies in serum and mucosal sites 4 months after rotavirus gastroenteritis

Specimen	No. of patients examined	% of patients with positive antirotavirus antibody:				Total positive antibody (% of patients)
		IgA	Secretory immunoglobulin	IgM	IgG	
Duodenal fluid	19	79	74	32	63	79
Saliva	43	60	40	9	70	70
Feces	43	63	20	7	0	63
Serum	43	93	0	14	100	100

fecal IgA antibody titers (Kendall tau, 0.392;  $P = 0.036$ ). The Kendall tau value for duodenal fluid and saliva was 0.091; that for duodenal fluid and serum was 0.227. This test indicates that only the IgA titers in duodenal fluid and feces are concordant ( $P > 0.05$ ).

### DISCUSSION

Young children with rotavirus gastroenteritis severe enough to warrant hospitalization simultaneously exhibited specific antibody responses in duodenal fluid, saliva, feces, and serum. The responses documented were probably characteristic of responses to a primary rotavirus infection. There are three reasons for this assumption. Firstly, all children studied had severe symptoms, and it now seems likely that in a particular child, only the first rotavirus episode is associated with severe symptoms (1, 10). Secondly, the development of serum antibodies followed the classical pattern of a primary infection, with early rises in antirotavirus IgM antibodies. As corroborative evidence, only 3 of the 44 children showed elevated serum antirotavirus IgG titers in acute-phase sera. It is possible that in these three children the antibodies may not have preceded the onset of disease but may have represented a rapid and disproportionate increase in the levels of IgG3 subclass antibody, which has kinetic properties similar to those of IgM (9). Finally, during longitudinal surveillance, many of the children experienced further proven rotavirus infections, but these were not associated with the development of serum antirotavirus IgM (unpublished observations).

There was considerable variation in the immune responses of individual children. These variations may have been due to differences in the ages of the children or in the severity of their illness or to minor differences in sampling times after onset, as has been observed in calves (11, 25). The variable responses detected in this study were not due to inaccuracies inherent in the use of SA11 as an antigen in the assay system, since the substitution of rotavirus serotypes 1 and 4, which are homologous with the rotavirus serotypes excreted by individual children, did not alter the results of the assays (unpublished observations).

The isotypes involved most frequently in immune conversions in duodenal juice, saliva, and feces were antirotavirus IgM and IgA, even in the four infants who were less than 3 months old, confirming the results from other studies (6, 13, 19, 20, 23). Most increases in IgM were detected by days 6 to 7 after the onset of symptoms. The appearance of IgM in gut secretions supports the belief that it may have a protective role in the early elimination of antigens from mucosal surfaces before the development of an IgA immune response (3). Peak titers of rotavirus-specific IgA were usually de-

TABLE 3. Sensitivity and predictive accuracy of immune responses at different sites in relation to a positive IgA immune response in the duodenum during the first 4 weeks after rotavirus infection

Site	Immunoglobulin	% Sensitivity <sup>a</sup>	% Predictive accuracy <sup>a</sup>
Serum	G	87.5 (14/16)	82 (14/17)
	A	62.5 (10/16)	83 (10/12)
	M	100 (16/16)	84 (16/19)
Saliva	A	75 (12/16)	86 (12/14)
Feces	A	75 (12/16)	86 (12/14)

<sup>a</sup> Data in parentheses represent number positive/number tested.

TABLE 4. Sensitivity and predictive accuracy of elevated antibody levels at different sites in relation to elevated duodenal IgA levels 4 months after rotavirus infection

Site	Immunoglobulin	% Sensitivity <sup>a</sup>	% Predictive accuracy <sup>a</sup>
Serum	G	100 (15/15)	79 (15/19)
	A	100 (15/15)	79 (15/19)
Saliva	A	73 (11/15)	85 (11/13)
Feces	A	73 (11/15)	92 (11/12)

<sup>a</sup> See Table 3, footnote a.

tected in feces between days 11 and 15 after onset. This may explain why relatively low peak titers of rotavirus-specific IgA were detected in duodenal contents sampled on days 5 to 7 (early) or days 28 to 35 (late) as compared with fecal samples. The measurement of duodenal fluid secretory immunoglobulin was not as useful as the measurement of IgA or IgM in the detection of an immune conversion after rotavirus infection. Low titers of antirotavirus IgG were detected in duodenal fluid from some children. These may have been derived by passive transfer from serum (3), since there was a statistically significant correlation ( $P < 0.01$ ) between IgG antibodies in duodenal juice and the occurrence of serum IgG antibody titers of  $\geq 1:12,800$ . Rotavirus-specific fecal IgG antibodies were never detected in the present study, even in the presence of rotavirus-specific IgG antibodies in duodenal fluid and saliva. Partially degraded rotavirus IgG coproantibodies have been reported in previous studies (13, 23).

Immune conversions were observed with a similar frequency of duodenal contents and in serum. Serum IgM and IgG were more sensitive measures of response than was serum IgA. Rotavirus-specific IgA responses have been less well characterized than those of IgG. Hjelt et al. reported a significant increase in IgA levels in serum in 10 of 12 patients 7 to 14 days after the onset of diarrhea, and 80% of samples were positive after 6 months (12). IgG also persisted for months in serum in their patients (8, 12). A total of 68% of our patients showed an acute-phase serum IgA response, and 93% had persisting antibody at 4 months. Hjelt et al. also reported antirotavirus secretory immunoglobulin in 17 of 20 patients at 7 to 14 days that virtually disappeared by 1.6 to 3.0 months. In our study the early secretory immunoglobulin response was very low (14%), and none of 43 sera were positive at 4 months. Differences in the definition of secretory immunoglobulin responses may account for these conflicting results. Our results do not support a role for serum secretory immunoglobulin as a marker for a recent rotavirus infection.

Only one of the four infants younger than 3 months demonstrated an IgG seroconversion between acute- and convalescent-phase specimens. However, when serum IgG measurements were repeated 4 months later in these four children, the expected fall in rotavirus antibody titer was not observed. The development of serotype-specific neutralizing antibodies could not be demonstrated (unpublished observations). This suggests that there was an initial active IgG immune response that was obscured by the preexisting maternally derived IgG. IgM seroconversions were detected in all four infants, indicating the importance of measuring IgM antibody in sera after a rotavirus infection in neonates or young infants.

The isotypes most frequently detected 4 months after a severe acute rotavirus infection were IgA in intestinal con-

tents and IgA and IgG in serum. Coproantibodies detected in some children 4 months after severe rotavirus gastroenteritis may have been due to a relapse or to reinfection and not to the persistence of these antibodies, as previously suggested (8, 12, 13). Marked weekly fluctuations in rotavirus-specific coproantibodies were observed in individual children, and these were often associated with transient diarrhea (with or without rotavirus excretion). These observations will be discussed elsewhere in more detail. They lead to the conclusion that the presence of rotavirus-specific IgA in feces 4 months after a rotavirus infection need not suggest the persistence of antibody from the original infection but may represent a response to repeated infection.

A recent paper studying cholera and toxigenic *Escherichia coli* concluded that serum antibodies were the best indirect measure of intestinal immunity after the clinical disease (14). Having similarly documented the antibody responses at a number of sites in children with rotavirus infections, we found it possible to compare and to make judgements as to whether measurements of rotavirus antibodies in an easily accessible specimen such as serum, saliva, or feces would accurately reflect the levels of antibodies secreted into the small intestine at the site of infection. Although serum IgA showed a high predictive accuracy at 1 and 4 months in relation to duodenal IgA, the presence of rotavirus-specific fecal IgA showed an even stronger correlation ( $P = 0.036$ ) in the children studied. This may indicate that a longitudinal study of fluctuations in antirotavirus IgA coproantibodies reflects the fluctuating production of antibodies in the small intestine, perhaps in response to a recurrent asymptomatic infection or to a persisting infection.

The results of this study indicate that estimations of antirotavirus serum IgM and serum IgG are sensitive indicators of an immune response to severe primary rotavirus gastroenteritis and reflect the response in duodenal fluid. In specimens taken 4 months later, the most reliable markers for a previous rotavirus infection are serum IgG and serum IgA. IgA coproantibody is a useful predictor of antirotavirus IgA secretion in the duodenum. Thus, the measurement of serum antibodies or coproantibodies accurately reflects duodenal antibodies, and there appears to be no advantage to be gained by measuring duodenal fluid antibodies. This removes the need for duodenal intubation to detect an immune response to rotavirus infection. This advantage has important practical implications for epidemiological studies of acute diarrhea in children and in trials testing the immunogenicity and efficacy of candidate rotavirus vaccines.

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