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Traveler's Diarrhea Associated with Rotavirus Infection: Analysis of Virus-Specific Immunoglobulin Classes

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An enzyme-linked immunosorbent assay for the detection of rotavirus-specific immunoglobulin G (IgG), IgM, and IgA antibodies was used in a serological study of Traveler's diarrhea. The antigenically related simian rotavirus (SA-11) was used as antigen in this study. Serum was obtained from two groups of volunteers before travel (pre-travel) and at various times after arrival at their destination (post-travel). One group consisted of 47 American Peace Corps volunteers who traveled to Honduras, and the second group consisted of 66 Panamanian travelers who visited Mexico. An association between rotavirus and Traveler's diarrhea was found in each group with 36% of American Peace Corps volunteers and 30% of Panamanians with diarrhea demonstrating a ≥4-fold increase in rotavirus antibody titers in the post- as compared to the pre-travel specimens. While no rotavirus-specific IgM antibody was detected in any serum tested, increases in specific antibody were found in both the IgG and IgA immunoglobulin classes.

Human rotavirus is recognized as a major pathogen causing acute diarrheal disease in children (2, 8, 20, 24). It is widely believed that in adults it usually causes asymptomatic infections. This conclusion rests on four observations: (i) most children are seropositive for rotavirus antibody by 3 years of age (19), (ii) the highest incidence of symptomatic infections, characterized by virion and viral antigen-positive stools (13, 16, 33), occurs between 6 months and 1 year of age (19), (iii) a high percentage of adult sera have rotavirus-specific antibody (19), and (iv) rotaviruses, serologically related to the human virus (18, 34), cause diarrhea only in young animals (1, 14, 39, 40).

Consistent with the conclusion that adult infections are asymptomatic, rotavirus was demonstrated only once (3) in a significant proportion of stools from patients with Traveler's diarrhea (TD). This syndrome occurs frequently (attack rate, 20 to 60%) in adult travelers visiting developing countries and may be caused by various agents (3, 22, 30, 31). Although it has been primarily associated with enterotoxigenic *Escherichia coli*, rare evidence of rotavirus involvement has been described (26) using the complement fixation (CF) assay with Nebraska calf diarrhea virus (NCDV) as antigen. More recently, Echeverria et al. (10) reported a ≥4-fold rise in antibody titers to simian rotavirus (SA-11) in 8% of patients with TD entering South Korea. However, in another study of this population (11), rotavirus particles were found in only 1 of 42 stools and a significant rise in antibody titer was not associated with disease.

In this report, we describe the adaptation of an enzyme-linked immunosorbent assay (ELISA) for the detection of antibody to rotavirus and demonstrate the association of rotavirus infection with 30 to 36% TD in two population groups, respectively, consisting of American Peace Corps volunteers (PCV) traveling to Honduras and of Panamanian travelers to Mexico. Rotavirus-specific antibody is found in both immunoglobulin G (IgG) and IgA classes.

MATERIALS AND METHODS

Cells and virus. Primary African green monkey kidney (AGMK) cells were obtained from Flow Laboratories, Rockville, Md., and were passaged three times in minimum essential medium (MEM) supplemented with 10% fetal calf serum (FCS) (MEM-10% FCS). The continuous line of fetal Rhesus monkey kidney (MA-104) cells was kindly donated by E. Bohl, Ohio State University, Columbus, and was grown in MEM-10% FCS. SA-11 virus was obtained from S. S. Kalter, Southwest Foundation for Research and Education, San Antonio, Tex. It was grown in secondary AGMK or MA-104 cells in MEM containing 0.5% bovine serum albumin (MEM-BSA).

Antigens. Confluent monolayers of secondary AGMK cells were exposed to SA-11 virus at a multiplicity of infection (MOI) of 0.5 plaque-forming units (PFU) per cell in MEM-1% FCS containing 2.5 μg of trypsin (Difco Laboratories, Detroit, Mich.) per ml. After adsorption for 2 h at 37°C, MEM-BSA containing 2.5 μg of trypsin per ml was used to overlay the cells, and virus was extracted 48 h later by five cycles

of freezing and thawing. The cell lysate was cleared of debris by centrifugation at 10,000 × *g* for 15 min and was used as the antigen in the serum survey. For the immunoglobulin isotype assays, virus was pelleted by centrifugation at 70,000 × *g* for 90 min at 4°C and resuspended in phosphate-buffered saline, pH 7.4 (PBS) containing 0.05% Tween 20 (PBS-t). Antigen protein concentration was determined by the method of Lowry et al. (23). Mock antigen was prepared from extracts of AGMK cells cleared of cell debris by centrifugation at 10,000 × *g* for 15 min and adjusted to protein concentrations identical to those used with SA-11 antigen. Influenza virus (A/Vic/3/75) antigen was kindly donated by B. Brooks, Department of Neurology, The Johns Hopkins University. It had been grown in the allantoic fluid of embryonated chicken eggs and was purified by two cycles of sucrose gradient centrifugation. The virus stock contained 2000 chick cell agglutinin and 530 μg of protein per ml. Before use in ELISA, it was inactivated by exposure to 0.5% Formalin for 48 h at 4°C and extensively dialyzed against PBS.

Antisera and immunoglobulins. Anti-SA-11 serum was prepared in guinea pigs (Hartley strain) by subcutaneous inoculation (1 ml) of virus emulsified in Freund complete adjuvant (50%, vol/vol). Animals were given one booster injection 2 weeks after inoculation and were bled 2 and 4 weeks later by cardiac puncture. Anti-human IgG (heavy and light chain) used in the serum survey was prepared to display the broadest possible specificity. Briefly, pooled human serum was incubated with agarose beads bound to the IgG fraction of goat anti-human IgG (Miles-Yeda, Israel) for 12 h at 4°C. The bound IgG (16.25 μg/ml) was eluted with 0.5 M acetic acid and was shown to be free of IgM and IgA by immunoelectrophoresis (28) against goat anti-whole human serum (Cappell Laboratories, Cochranville, Pa.). Anti-IgG serum was prepared in a normal goat by subcutaneous inoculation of 50% (vol/vol) emulsion in complete Freund adjuvant followed by one subcutaneous and one intravenous booster injection, respectively, at 4 and 7 weeks post-primary inoculation. The specificity of this antiserum was determined by immunoelectrophoresis against pooled human serum, and the globulin fraction was precipitated with sodium sulfate. IgG fractions of goat antisera to human IgM and IgA, both heavy-chain specific, and the IgG fraction of goat antiserum to the Fc fraction of the γ-chain of human IgG were purchased from Cappell Laboratories. Together with the globulin fraction prepared in our laboratory, they were conjugated to alkaline phosphatase (Sigma type VII) by the method of Voller et al. (37). The activity and specificity of the alkaline phosphatase-conjugated antisera were tested by assaying for reactivity with various amounts of human IgG, IgM, and IgA (37).

Study groups. Two groups of sera were studied. The first consisted of specimens obtained from 47 American PCV aged 23 to 68 who traveled to Honduras in August 1978. Clinical information was obtained as previously described (32) by means of a diary maintained by each individual during the first 6 weeks after arrival in Honduras and by questionnaires and interviews at the beginning and end of the study period. Three serum specimens were collected from each individual: one before departure (pre-travel) and two (post-travel) collected at 3 and 6 weeks after arrival in Honduras. TD was defined as three or more watery stools per day or at least one watery stool with cramps, vomiting, fever, or prostration. Loose stools (LS) was defined as only one or two watery stools per day without other symptoms (32). The second set of sera studied in this series was collected from Panamanian volunteers traveling to Mexico in March 1979. In this study, TD was defined as the passing of more than two watery stools in a 24-h period. Serum specimens were obtained from 66 individuals before departure for Mexico (pre-travel) and within 10 days after returning home from a 15-day trip (post-travel). Henceforth, we will designate as positive a pair of sera displaying ≥4-fold higher antibody titers post- as compared to pre-travel. Antibody titers in the negative pairs are identical or less than fourfold higher in the post- as compared to the pre-travel sera.

Paired sera obtained from two normal individuals before immunization with a subunit A/Vic/3/75 vaccine and 3 weeks thereafter, were obtained from B. Brooks, The Johns Hopkins Medical Institutions. They were shown to be, respectively, negative (titer 1:10) and positive (titer 1:640) for antibody to A/Vic by hemagglutination inhibition (29).

ELISA procedure. Antibody assays were performed by a modification of the procedure of Voller et al. (37). Briefly, for rotavirus antibody, round-bottom polyvinyl microtiter plates (Cooke Engineering Co., Alexandria, Va.) were pre-coated overnight (4°C) with guinea pig anti-SA-11 serum (150 μl/well) diluted 1:10,000 in carbonate buffer, washed with PBS-t, and coated with 27 μg of protein per ml of SA-11 antigen (150 μl/well) at 37°C for 2 h. Antigen-coated plates were washed with PBS-t and exposed to patient sera (150 μl/well) diluted in PBS-t containing 1% FCS and 0.5% guinea pig serum (GPS). After overnight incubation at 4°C, the appropriate enzyme-linked anti-immunoglobulin was added (150 μl/well) and the plates were incubated for 2 h at 37°C. They were exposed for 30 min at room temperature to 150 μl of *p*-nitrophenyl phosphate substrate (Sigma 104) in diethanolamine buffer (pH 9.6) per well, and the reaction was stopped by the addition of 50 μl of 3 M NaOH. The amount of yellow color produced by the reaction of the enzyme on the substrate was measured in a Beckman DBG spectrophotometer at 400 nm. A/Vic antibody assays were done in similar fashion but on plates directly coated with 150 μl of A/Vic/3/75 antigen (2.7 μg of protein per ml) per well and with diluent consisting of PBS-t supplemented with 1% BSA instead of FCS and GPS. Antigen (SA-11 or A/Vic)-coated wells exposed to the appropriate enzyme-linked antiglobulin in absence of patient serum served as background control. Reactivity levels higher than this background were not observed in wells containing test serum and enzyme-linked anti-immunoglobulin in the absence of antigens, nor in wells exposed to the enzyme-linked anti-immunoglobulin in the absence of antigen or test serum. ELISA antibody titers were expressed as the reciprocal of the highest serum dilution that yielded an optical density at 400 nm (OD_{400})

of greater than 2 standard deviations over the mean background values. Statistical analyses were performed on the log transformation of these reciprocal titer values (6). Stool specimens in the Panamanian study group were analyzed for rotavirus antigen as described by Yolken (43).

RESULTS

Assay specificity. Sera with established rotavirus reactivity were obtained from R. Yolken, The Johns Hopkins Medical Institutions, and were assayed in ELISA with SA-11 antigen. These included: (i) five sera (no. 1 to 5) positive for rotavirus antibody at titers of 1:8 to 1:32 as determined by complement fixation (CF) with NCDV or O antigen, (ii) two sera negative for rotavirus antibody in CF and collected from a 6-year-old child (no. 6) and a newborn (no. 7), and (iii) two sera (no. 8 and 9) collected from individuals positive for rotavirus antigen in their stool specimens as independently determined by ELISA with antiserum prepared against virion-positive human stool filtrates (43).

The results of these studies, expressed as OD_{400} readings, are summarized in Fig. 1. The CF-positive sera (no. 1 to 5) and those (no. 8 and 9) obtained from the two patients positive for rotavirus antigen in the stools reacted with SA-11 in ELISA. Consistent with previous observations (15), comparison of the ELISA titers with those independently obtained by CF confirmed the greater sensitivity of the ELISA. Thus, in ELISA the antibody titers of the CF-positive (no. 1 to 5, 8, and 9) sera ranged between 128 and 32,768 as compared to titers between 8 and 32. Furthermore the CF-negative serum obtained from a 6-year-old child (no. 6) had low-level reactivity (titer = 32). However, the newborn's serum (no. 7) was negative in both assays (Fig. 1A). Six to ten wells on each plate were used to determine background. It was never higher than 0.2 (Fig. 1A) and in most experiments it ranged between 0.1 and 0.18 (Fig. 1B).

Reactivity with SA-11 was virus specific. Thus, sera previously shown to be positive for rotavirus antibody (no. 10 to 12) reacted with SA-11 but not with mock antigen preparations (Fig. 1B). Furthermore, when 11 paired (pre- and post-travel) sera from diarrheal patients known to display increased (≥4-fold) rotavirus antibody titers post-travel were assayed against SA-11 and A/Vic, only one (no. C14), showed a concomitant increase in antibody titers to SA-11 and A/Vic. In all others (10/11), the increase in antibody titer post-travel was only against SA-11. Geometric mean titers (GMT) to SA-11 in the post-travel specimens were 4,705 (95% confidence interval, 2,438; 9,077) as compared to 445 (95% confidence interval, 223; 891) in the pre-travel sera, whereas against A/Vic they were 675 (95% confidence interval, 518; 877) and 588 (95%) confidence interval, 345; 1,002), respectively, in the post- and pre-travel sera (Table 1). Conversely, sera obtained post-immunization with A/Vic had significantly higher titers (2,048) than the pre-immunization ones (128) when assayed against A/Vic and did not demonstrate a concomitant increase in titer to SA-11 (titer = 32, both pre- and post-immunization) (data not presented).

Assay variables. Experimental variables examined in these series included: (i) diluent composition, (ii) serum storage and handling, (iii) antigen preparation and concentration, and (iv) plate preparation. The diluents tested on paired sera of previously established rotavirus reactivity (no. 8 and 9) consisted of PBS-t supplemented with 1% FCS and 0.5% GPS and PBS-t supplemented with 1% BSA. Although in both cases, rotavirus antibody titers were ≥4-fold higher in the post- than in the pre-travel sera, lower background (OD_{400} = 0.2 as compared to 0.3), absence of nonspecific reactivity in the pre-travel specimen, and a relatively higher reactivity in the post-travel sera were observed with FCS-GPS supplemented PBS-t (Fig. 2A). Virtually identical responses were obtained with sera exposed to 1, 3, and 9 cycles of freezing and thawing (Fig. 2B). Plates precoated with guinea pig anti-SA-11 serum could be stored at 4°C for 6 days. Irreproducible results were obtained when antiserum-precoated plates were stored for intervals longer than 6 days and when SA-11 antigen-coated plates were stored for any time interval. Unlike the SA-11 situation, optimal assay conditions for A/Vic antibody included direct antigen coating of the microtiter plates and the use of PBS-t supplemented with 1% BSA.

The effect of the SA-11 antigen preparation and concentration was studied with two sets (no. 10 and 11) of paired sera. Two series of experiments were performed. In the first series, sera were assayed on plates coated with 26, 13, 6, 5, or 3.25 μg of protein per ml of AGMK-grown SA-11 antigen. Titers ≥4-fold higher in the post- as compared to the pre-travel specimens were observed at all but the lowest (3.25 μg of protein per ml) antigen concentrations studied in this series (Fig. 3A). It should be pointed out that similar results were obtained with SA-11 partially purified by centrifugation at 70,000 × *g* for 90 min (data not presented). In the second series of experiments, plates were coated with SA-11 grown in AGMK cells in presence of unsupplemented MEM, MEM with 0.5% BSA, or MEM

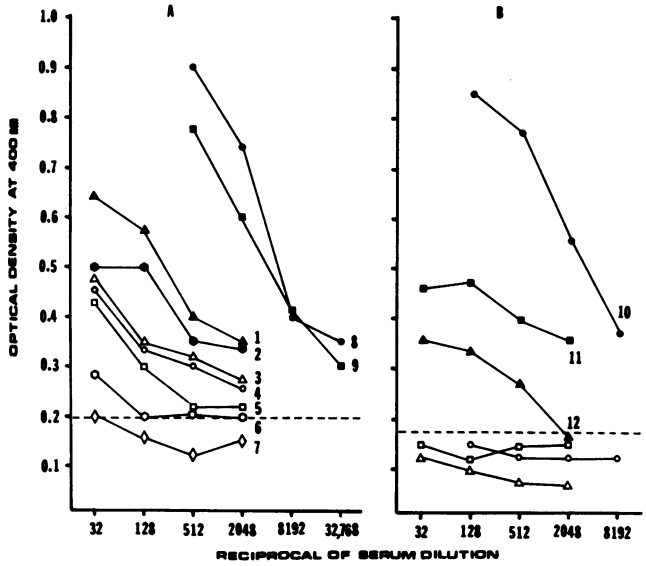

FIG. 1. *ELISA assay of sera with known rotavirus specificity. Background level (- - -). (A) Positive (no. 1 to 5) or negative (no. 6 and 7) sera and sera (no. 8 and 9) collected from individuals with rotavirus antigen-positive stools assayed against SA-11 antigen with anti-human IgG (heavy- and light-chain specific). (B) Sera (no. 10 to 12) previously determined to be positive for rotavirus antibody assayed against SA-11 antigen (solid symbols) or mock antigen (open symbols).*

TABLE 1. *SA-11 and influenza A/Vic/3/75 antibody in pre- and post-travel sera from Panamanian travelers*

Patient	Antibody titers[a] to:			
	SA-11		A/Vic/3/75	
	Pre-travel serum	Post-travel serum	Pre-travel serum	Post-travel serum
C37	512	32,768	512	512
C50	128	2,048	512	512
C38	512	2,048	2,048	2,048
C20	128	2,048	2,048	2,048
C13	2,048	8,192	512	512
C69	512	2,048	512	512
C70	2,048	8,192	512	512
C46	512	2,048	128	128
C54	512	8,192	512	512
C14	128	8,192	512	2,048
GMT	445 (223; 891)	4,705[b] (2438; 9077)	588 (345; 1002)	675[c] (519; 877)

[a] Titers are expressed as the reciprocal of the highest serum dilution that gives a response 2 standard deviations over the mean background.

[b] GMT of SA-11 antibody are significantly ($P < 0.001$ by Student's t test) higher in the post- than pre-travel sera. Values in parentheses represent 95% confidence interval (6).

[c] GMT of A/Vic antibody are not significantly different ($P > 0.3$ by Student's t test) in the pre- and post-travel sera.

with 1% boiled FCS or with SA-11 grown in MA-104 cells. All antigens were equally reactive (Fig. 3B).

Reproducibility of the ELISA procedure. Reproducibility was assessed by assaying 49 paired sera at three different times. The sera consisted of 19 positive and 30 negative pairs. Identical titers in the pre-travel samples were observed in 20 (41%) of the cases. Nineteen (39%) of the pre-travel specimens displayed a fourfold difference in antibody titer (Table 2). Significantly, the differential response of the post- as compared to the pre-travel serum specimens (equal or ≥4-fold-increased titers) was

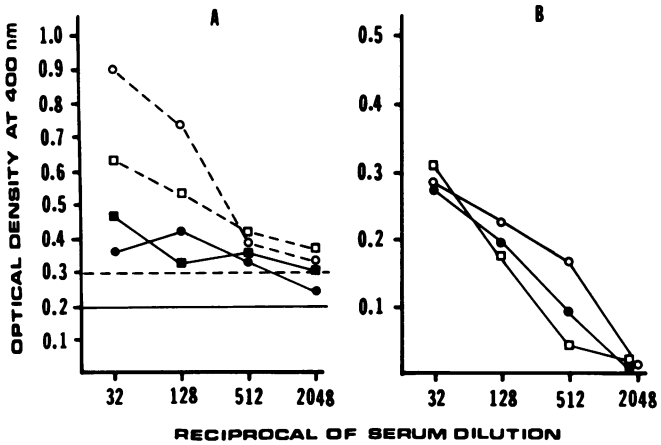

FIG. 2. *Assay variables. (A) Effect of diluent composition on SA-11 antibody titers. Pre (● ■)- and post (○ □)-travel sera from patient no. 8 (rotavirus antigen-positive stool in acute phase of disease) were assayed in diluent containing either PBS-t with 1% BSA (squares) PBS-t with 1% FCS and 0.5% GPS (circles). (-----) Background for BSA containing diluent. (——) Background for FCS and GPS containing diluent. (B) Effect of freezing and thawing on antibody titers after 1 (●), 3 (○), and 9 (□) cycles of freezing and thawing. Results plotted after background substraction.*

reproducible in 48 of the 49 (98%) pairs. Of the 19 positive pairs in the series, 9 displayed the same increase in the anti-SA-11 post-travel titer, whereas in the other 10 pairs, the titer of the post-travel specimen was at least fourfold higher than that of the pre-travel, but the titers were not consistently reproduced.

Rotavirus antibody in TD patients. Indicative of a recent rotavirus infection, 21 of the 47 American PCV studied in this series had a significant (≥4-fold) increase in rotavirus-specific antibody titers in the post- as compared to the pre-travel sera. Of these, 17 (36%) were symptomatic. Thirteen experienced at least one TD episode, and 4 experienced at least one LS episode. Significantly, in all but one (no. H61) of the symptomatic individuals, the increase in the antibody titers followed the development of the clinical symptoms. Furthermore, in those patients who experienced repeated episodes of TD or LS or both (no. H29, H30, H33, and H41) the increase in antibody titer was observed after the TD rather than the LS episodes (Table 3). Geometric mean titers (GMT) in the symptomatic patients were 128 (95% confidence interval, 58; 288) and 835 (95% confidence interval, 242; 1,640) in the pre- and post (42 days)-travel specimens, respectively. Examination of the individual pairs suggests that the relatively lower (2.9-fold) increase in antibody titers at 21 days post-travel may be due to: (i) disease onset after 21 days post-travel (viz., no. H52 and H30) or (ii) disease onset close to 21 days with or without a relatively longer interval before a significant increase in antibody titers (viz., no. H20 and H25) (Table 3) or both (i) and (ii).

It should be pointed out that increased antibody titers post-travel were observed in those individuals with relatively low levels of rotavirus-specific antibody before travel onset (GMT, 128; 95% confidence interval, 75; 218 and GMT, 128; 95% confidence interval, 16; 1,022 for symptomatic and asymptomatic patients, respectively). Antibody titers in the pre-travel sera of patients that did not have serological evidence of recent rotavirus infection were significantly ($P < 0.001$ by Student's t test) higher (Table 4).

Similar results were obtained in the Panama series (Table 5); however, overall, rotavirus-specific antibody titers were higher than in the American PCV group. Briefly, 6 of the 20 volunteers who developed TD (30%) demonstrated a significantly higher (GMT, 4,096; 95% confidence interval, 1,018; 16,475) rotavirus antibody titer in the post- as compared to the pre-travel (GMT, 181; 95% confidence interval, 80; 407) sera. Furthermore, pre-travel antibody titers in the individuals who did not become infected with rotavirus were significantly higher ($P < 0.001$ by Student's t test) than those of individuals who developed infection. It may be significant that of the four individuals positive for rotavirus antigen in the stools, two (C37 and C58) displayed symptomatic disease and increased post-travel antibody titers, whereas two others (C15 and C51) remained asymptomatic and did not show an increase in post-travel antibody titers. The pre-travel titers of the

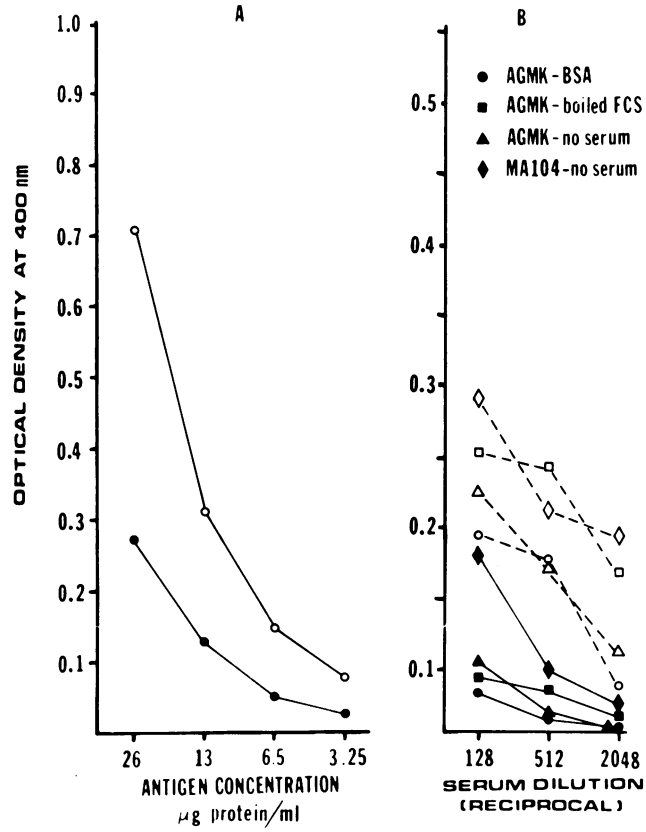

FIG. 3. *Effect of antigen concentration and preparation on antibody titers. (A) Serum 10 (pre [●]- and post [○]-travel samples) diluted 1/512 in PBS-t with FCS and GPS was assayed on microtiter plates coated with increasing concentrations of SA-11 antigen. (B) Antibody titer of serum 11 (pre [solid symbols]- and post [open symbols]-travel) was determined against SA-11 antigen prepared under the various listed conditions and adjusted to a constant (13 μg of protein per ml) concentration. Results were plotted after background substraction.*

symptomatic patients were 512, whereas those of the asymptomatic subjects were 2,048.

Immunoglobulin isotype of rotavirus antibody. In the first set of experiments, two known positive (no. C9 and C14) and one negative (no. C15) pairs of sera were fractionated by centrifugation on a 40% sucrose gradient as previously described (12), and the immunoglobulin fractions were independently assayed for virus-specific IgG, IgM, and IgA. Titers ≥4-fold higher in the post- as compared to the pre-travel 7-9S immunoglobulin fractions were observed with both anti-human IgG and anti-human IgA sera (Table 6). The 19S fractions (data not presented) were negative (titer, <32). In a second series of experiments, 34 serum pairs from both study groups were assayed for rotavirus-specific IgG and IgA. The anti-IgG was specific for the Fc fragment of IgG, whereas the anti-IgA was heavy-chain specific. Increased (mean, 3.8-fold) antibody titers in the post- as compared to the pre-travel sera were observed in 24 of the 34 (71%) pairs of sera when assayed with anti-IgG. Significantly, increased antibody titers, (mean, 7.7-fold) in the post- as compared to the pre-travel sera were also observed in 26 of 34 pairs (76.5%) assayed with anti-IgA. Eleven paired sera assayed with anti-human IgM (heavy-chain specific) were virtually negative (titer, <32; Fig.

TABLE 2. *Reproducibility of ELISA anti-SA-11 titers*

Change in titer[a]	Frequency	% Frequency
0	20	41
1	4	8
2	19	39
3	6	12

[a] Expressed in logs to base 2 for 49 pre-travel sera assayed at three separate intervals. Increased (≥4-fold) or similar antibody titers in the post- as compared to the pre-travel sera were reproducible in 48 of the 49 pairs.

TABLE 3. *Rotavirus antibody titers in symptomatic american PCV*

Patient	Dx	Day	SA-11 antibody titers[a]		
			Pre-travel serum	Post-travel serum	
				21[b]	42[b]
H5	LS	13	256	1,024	1,024
H9	TD	19	256	1,024	1,024
H10[c]	TD	15	0	0	32
H22	TD	10, 17	128	512	128
H25	TD	19	128	128	2,048
H29[c]	TD	17, 21, 25, 36	128	128	2,048
H30	TD	37	512	512	2,048
H33	TD	40	128	128	512
H36	TD	6, 11	128	ND	2,048
H37	LS	31	32	ND	128
H41[c]	TD	6	512	2,048	2,048
H47[c]	TD	7, 14	32	ND	128
H50	TD	17	128	2,048	2,048
H52	LS	25	128	128	2,048
H61	LS	22	128	2,048	2,048
H62	LS	26	512	512	2,048
H39	TD	34	128	ND	2,048
		GMT	128 (56; 288)	371 (148; 933)	835 (424; 1640)

[a] Titers are expressed as the reciprocal of the highest serum dilution that gives a response two standard deviations over mean background levels. GMT are higher for post- than pre-travel sera. Values in parentheses represent 95% confidence intervals (6).

[b] Day post-travel.

[c] H10 and H41 were concomitantly diagnosed as positive for ETEC. TD episode of patient H29 at 36 days post-travel and that of patient H47 at 14 days post-travel were associated with ETEC and with *E. histolytica*, respectively (32). H30, H33, and H41 experienced LS episodes at 6, 4, and 30 days post-arrival in Honduras, respectively. These were not accompanied by increase in antibody titers.

4). Temporal analysis of the development of the IgA response in the American PCV study group indicated that the increase in rotavirus-specific IgA antibody was obtained at 42 but not at 21 days post-travel (Fig. 5).

DISCUSSION

The salient feature of these studies is the observation that 30 to 36% of adults with TD demonstrate an increase in rotavirus-specific circulating antibody, indicative of recent infection. The virus-specific antibody is of both IgG and IgA classes. The studies merit discussion from the standpoint of: (i) the specificity and sensitivity of the serological assay used in these series, (ii) the association of rotavirus with disease in adult humans, and (iii) the significance of circulating virus-specific IgA antibody with regard to infection of the gastrointestinal tract.

ELISA: specificity and sensitivity. Consistent with the conclusions of Ghose et al. (15), virtually 100% of adult sera studied by ELISA in this series had relatively high (128 to 32,768) rotavirus-specific antibody titers before travel. This compares to a 45% prevalence (17) and relatively low (8 to 32) titers as determined by the CF assay in similar population groups (17)

TABLE 4. *Rotavirus antibody in sera from American PCV*

Clinical status	Sera	SA-11 antibody titers (GMT) in paired sera[a]	
		Positive[b]	Negative[c]
Symptomatic	Pre-[d]	128 (75; 218)	630 (321; 1,234)
	Post-	906 (478; 1,714)	337 (113; 856)
Asymptomatic	Pre-[e]	128 (16; 1,022)	463 (56; 3,848)
	Post-	812 (74; 8,902)	312 (69; 1,402)

[a] GMT of paired sera obtained pre- and 6 weeks post-travel. Titers are significantly (≥4-fold) higher in the post- as compared to pre-travel specimens of positive but not of negative pairs. Values in parentheses represent 95% confidence interval (6).

[b] There were 17 symptomatic and 3 asymptomatic patients.

[c] There were 20 symptomatic and 7 asymptomatic patients.

[d] Pre-travel titers are significantly lower ($P < 0.001$ by Student's t test) in positive than in negative pairs. Post-travel titers do not differ significantly ($P > 0.1$).

[e] Pre-travel and post-travel titers are not significantly different ($P > 0.1$ by Student's t test) in positive and negative pairs.

and in some of our sera (unpublished data). The data suggest that SA-11 is an efficient substitute for human rotavirus. This is particularly significant in view of the difficulties associated with the preparation of large quantities of human rotavirus antigen stocks with reproducible im-

TABLE 5. *Rotavirus antibody in Panamanian sera*

Clinical status	Sera	SA-11 antibody titers (GMT) in paired sera[a]	
		Positive[b]	Negative[c]
Symptomatic	Pre-[d]	181 (80; 407)[e]	2,496 (1,283; 4,861)
	Post-	4,096 (1,018; 16,475)	1,130 (457; 2,793)
Asymptomatic	Pre-[f]	827 (303; 2,258)[e]	1,237 (811; 1,884)
	Post-[g]	4,319 (1,648; 11,329)	795 (537; 1,179)

[a] GMT of paired sera obtained pre- and post-travel. Titers are significantly (≥4-fold) higher in the post- as compared to the pre-travel specimens of positive but not of negative pairs.

[b] There were 6 symptomatic and 13 asymptomatic patients.

[c] There were 14 symptomatic and 33 asymptomatic patients.

[d] Pre-travel titers are significantly lower ($P < 0.001$ by Student's t test) in positive than in negative pairs. Post-travel titers do not differ significantly ($P > 0.1$).

[e] Difference in the pre-travel titers of symptomatic and asymptomatic individuals is not statistically significant ($P > 0.1$ by Student's t test).

[f] Pre-travel titers are not significantly different ($P > 0.1$ by Student's t test) in positive and negative pairs.

[g] Post-travel titers are significantly higher ($P < 0.001$ by Student's t test) in the positive than in the negative pairs.

munological properties. Indeed, human or gnotobiotic calf fecal virus preparations still possess tightly bound virus-specific antibodies even after purification (9, 38), thus giving rise to background reactions in ELISA conjugate controls and to false-negative results (44). Furthermore, the extent of antibody contamination cannot be controlled from one preparation to another causing irreproducible results. Although adapted to tissue culture (42) sufficient quantities of high-titer HRVL (Wa strain) are still difficult to prepare. It should be pointed out that studies describing this adaptation revealed virtually identical increases in antibody to SA-11 and Wa virus in convalescent as compared to acute sera from Wa-infected gnotobiotic piglets (42). The data underscore the close antigenic similarity between SA-11 and human rotavirus, supporting the conclusion that SA-11 is an efficient substitute antigen for human rotavirus.

Among the assay variables studied in these series, antigen concentration and the method of coating the plates appeared to be of the utmost significance. Consistent with previous observations by Yolken and co-workers (44), plate precoating with hyperimmune antibody is required to bind SA-11 virus even when the virus is partially purified by pelleting at 100,000 × g for 1.5 h (unpublished data). Furthermore, the greatest increase in post-travel antibody titers was observed on plates coated with the highest (26 μg of protein per ml) SA-11 antigen concentration studied. At the lowest SA-11 antigen concentration (3.25 μg of protein per ml) antibody titers were similar in both pre- and post-travel specimens. This finding underscores the limitations of serum survey studies using single serum dilutions, particularly if antibody-coated virus preparations are used as the antigen (44).

TABLE 6. *Rotavirus antibody in 7-9S immunoglobulin fractions*

Subject[a]	Antiserum to[b]	SA-11 antibody titer in			
		Whole serum		7-9S immunoglobulin	
		Pre-	Post-	Pre-	Post-
C14	IgG	8,192	32,768	1,024	4,096
	IgM	<16	<16	<16	<16
	IgA	32	2,048	<16	256
C9	IgG	512	2,048	64	256
	IgM	<16	<16	<16	<16
	IgA	16	2,048	<16	256
C15	IgG	2,048	2,048	128	128
	IgM	ND[c]	ND	<16	<16
	IgA	ND	ND	<16	<16

[a] C14 and C19 are known positive pairs, and C15 is a known negative pair as determined with anti-human IgG (heavy and light chain).

[b] Anti-human IgM and IgA were heavy-chain specific. Antiserum to IgG was prepared against the Fc portion of the γ-chain.

[c] ND, Not done.

Differential response of pre- as compared to post-travel specimens was reproducibly observed in 48/49 (98%) sera studied in these series.

The rationale for using anti-human IgG prepared against the whole IgG molecule (heavy and light chain) in the TD serum surveys was the expectation that by virtue of its reactivity against both κ and λ light-chains it would possess a broad specificity. However, to determine whether IgA or IgM light-chain reactivity was contributing to the activity detected by this anti-IgG antiserum, 34 paired sera were assayed for antibody immunoglobulin class with heavy-chain-specific anti-IgA and IgM and with a new

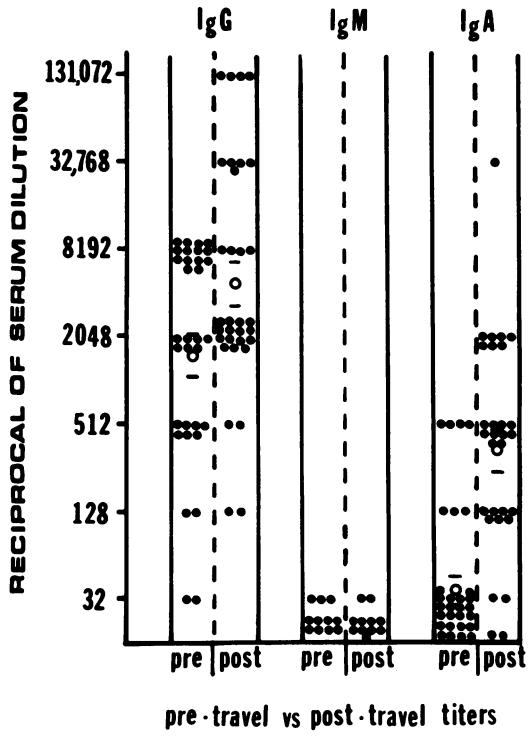

FIG. 4. *Immunoglobulin class of rotavirus antibody. Titers in the IgG, IgA, and IgM class of pre- and post-travel sera are presented. Bars represent ±1 standard error about the GMT* (○).

reagent that was specific for the Fc portion of the γ-chain of human IgG. The activity of the anti-Fc reagent was higher than that of the anti-IgG that was heavy- and light-chain specific and that had been used in the serum survey (Tables 3 to 5). Indeed pre-travel GMT obtained with this reagent were significantly (14-fold) higher than those obtained for the same sera with anti-human IgG with heavy- and light-chain specificity. This increase may be attributed to greater enzyme activity of the Fc fragment-specific conjugate. Alternatively, it may have resulted from its specificity for the Fc fragment of the γ-chain, as this portion of the antibody would be more available as a binding site for antiglobulin than the Fab fragments that are involved in antigen binding.

Rotavirus and TD. Based on their results with electron microscopy and ELISA with SA-11 antigen, von Bonsdorff et al. (36) concluded that rotavirus can cause severe gastroenteritis in a proportion (25%) of adult individuals comparable to that (40%) observed in children. Bolivar et al. (3) reported TD secondary to the detection of rotavirus in stool specimens in 25% of United States summer students newly arrived in Mexico. However, serological data were not presented and therefore the possibility that infection was coincidental and unrelated to disease cannot be excluded. Our studies confirm the association of rotavirus with TD by demonstrating a significant increase in rotavirus-specific antibody titers after disease (Table 3). A similar incidence of rotavirus-associated disease was observed in American PCV (17/47 = 36%) traveling to Honduras and in Panamanians (6/20 = 30%) traveling to Mexico. Pre-travel antibody titers were generally higher (GMT, 1,185) in the Panamanian than in the American (GMT, 337) study group (Tables 4 and 5). However, in both groups the pre-travel titers were significantly (5- to 13-fold) lower in those individuals that experienced rotavirus infection (increased post-travel titers) than in those that did not sustain such infections (Tables 4 and 5).

Rotavirus antigen was detected in the stools of only 4 of the 19 Panamanian subjects with increased virus-specific antibody titers in the post- as compared to the pre-travel sera. This is not unexpected considering that: (i) virus in the stool is antibody bound (9, 38), (ii) the sensitivity of the antigen detection assay is equivalent to that of electron microscopy and therefore a positive result requires 10^6 to 10^7 virus particles (43), and (iii) virus detection even in radioimmunoassay is virtually impossible in stools containing

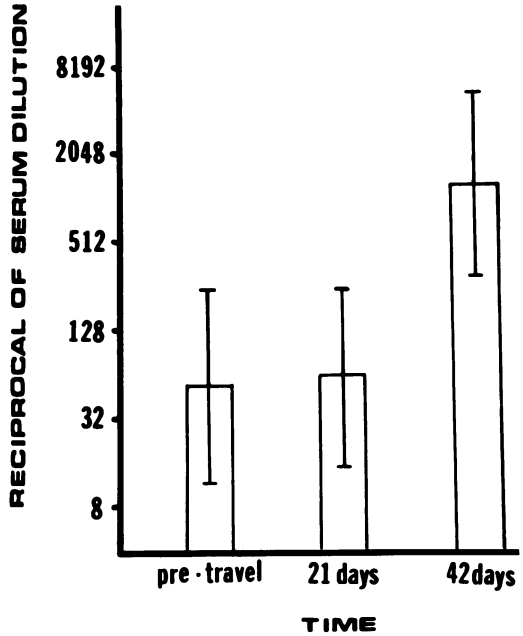

FIG. 5. *Development of anti-rotavirus serum IgA antibody (GMT) in American PCV studied pre-travel and at 21 and 42 days after arrival in Honduras. Bars represent 95% confidence interval.*

virus-specific IgA antibodies (38), a most probable response after infection (21, 41; Fig. 5). Significantly, pre-travel antibody titers in the two Panamanian subjects that did not develop TD (C15 and C51), although they were positive for viral antigen in the stool, were 4-fold higher (2,048) than those of the two individuals (C37 and C68) that were symptomatic while positive for viral antigen in the stools. Final conclusions cannot be reached from these numbers. However, the data are consistent with the interpretation that rotavirus infection and disease preferentially occurs in individuals with relatively low pre-travel antibody titers.

It should be pointed out that consistent with previous observations (22, 26, 30, 31), we also found that the most common pathogen isolated during diarrheal episodes (21%) was enterotoxigenic *E. coli* (ETEC). Other bacterial (*Salmonella*, *Shigella*, and *Vibrio*) and parasitic (*Entamoeba*) pathogens were an uncommon cause of TD (32). Furthermore, there was evidence for dual infections. Thus four PCV had serological evidence of recent infection with Norwalk agent associated with TD, and ETEC was isolated from two of them (32). ETEC was also isolated from three PCV and *Entamoeba histolytica* was isolated from one PCV with serological evidence of recent infection with rotavirus (Table 3).

Immunoglobulin class of rotavirus-specific responses. A significant increase in the IgG and IgA rotavirus-specific post-travel antibody titers was observed, respectively, in 24 (71%) and 26 (73.5%) of the 34 sera assayed in these series. The increase in rotavirus-specific IgG was observed by day 21 post-travel, whereas significant levels of rotavirus specific IgA antibody were observed on day 42. Consistent with previous observations indicating that IgM response is present only in the acute sera of children infected with human rotavirus (44), IgM was not found in any of the 11 sera tested (Fig. 4).

An IgG response followed by an IgA one was previously reported for infection of the gut with attenuated poliovirus. In that study, virus-specific serum IgG was observed 7 days after immunization whereas virus-specific IgA was observed only after 42 days (27). Indeed, in the gastrointestinal tract, which is the site of rotavirus replication, there is a predominance of IgA plasma cells containing α chains and capable of binding secretory component (4). Perfusion studies of human intestines demonstrated that newly synthesized IgA is found in the venous effluent and is oligomeric (5), containing monomeric, dimeric, and secretory IgA molecules (21). Studies of IgA in the serum of dogs, mice, guinea pigs, rats, and sheep demonstrated that IgA produced in the walls of the gut eventually reaches the blood via the lymphatic system (35). The overall conclusions that can be drawn from these data are that oral immunization (with rotavirus for example) results in an immune response in the gut-associated lymphoid tissue that is predominantly IgA in nature (21), and that IgA produced by plasma cells in the gut will ultimately reach the serum (7). Consistent with these interpretations and the previously reported protective role played by neutralizing antibody in the gut (41), we found that reinfection occurs in individuals with very low titers (pre-travel) of circulating virus-specific IgA (Fig. 4 and 5) at the time of exposure to the virus. Levels of circulating serum IgG do not appear to be protective from reinfection, as indicated by the relatively high pre-travel titers of IgG antibody in these individuals (Fig. 4). Studies presently in progress in our laboratory are designed to inquire into the role of local and circulating virus-specific IgA antibody in secondary infection using an animal model of rotavirus-induced diarrheal disease.

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