EVIDENCE OF IMMUNITY INDUCED BY NATURALLY ACQUIRED ROTAVIRUS AND NORWALK VIRUS INFECTION ON TWO REMOTE PANAMANIAN ISLANDS

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of the Indian groups in Panama. We selected the islands of Tupile and Achutupo for study because they were highly traditional, had stable populations, and were quite remote. These features allowed us to study naturally acquired infection in an unchanged environment. Furthermore, the inhabitants had expressed an enthusiasm for participating in this study. Tupile, located one mile offshore, measures 1.0 km by 0.5 km and has ~1,200 inhabitants, all Cuna Indians, living in approximately 150 housing units made entirely of local materials. Achutupo, six miles from Tupile, is of similar size and demography. Both islands were devoid of fresh water sources, although Tupile had recently installed a centralized water distribution system made up of a series of plastic pipes that divert water from a mainland stream to each of the 150 dwellings. On both islands defecation is carried out in "privies" located on small platforms above the ocean; the average tide was two feet.

Each island had its own government-supported health center staffed by a resident nursing auxillary. The nearest hospital is at Aligandi; it is a well-equipped facility equidistant from the two islands and is staffed by a medical team headed by a well-trained physician. All health services were free. Oral rehydration solution was readily available on each island.

In August 1979, a door-to-door census was carried out on both islands. One month later, baseline sera were obtained by venipuncture from a 50% random sample of children younger than five years of age (if a guardian gave consent) on both islands and from all individuals five years of age or older in a 10% random sample of the 150 family units on each island. For the next seven months, specially trained Cuna Indian epidemiology field technicians conducted door-to-door surveillance for diarrheal illness six days a week. Their daily observations were recorded on a standard form. In March 1980 a second serum sample was obtained from all consenting members of the original cohort. We defined RV or NW infection by a fourfold or greater rise in serum antibody titer between September 1979 and March 1980. An episode of RV diarrhea was defined as the passage of more than three loose stools in which RV antigen was detected in a 24-hr period.

Of the 330 persons originally enrolled, 11 people from Tupile and 14 from Achutupo did not give a second blood sample and were considered study dropouts. Eight study dropouts were children younger than five years of age who died from the complications of diarrhea [6]; eleven were adults who refused to donate a second serum sample; and six were children whose parents forbade the second venipuncture. The present report includes only those subjects who were successfully studied and from whom two serum samples were collected.

Laboratory. Stool samples from patients with diarrhea were collected on the first day of symptoms and stored on each island at −10 C until monthly transport to our central laboratory in Panama, where they were maintained at −20 C until testing. Standard ELISA techniques were used to detect rotaviral antigen [7].

All serum samples were collected by aseptic technique, centrifuged within 6 hr of collection, and kept at 0 C for no more than 48 hr before they were entered into the Gorgas Memorial Laboratory Serum Bank, where they were stored at −20 C before being shipped, frozen, to Bethesda.

IgG antibodies to rotavirus were measured by standard complement fixing techniques [8] and the blocking radioimmunoassay [9] was used to detect antibody to Norwalk virus.

The Statistical Program for the Social Sciences (SPSS) was used for data analysis and the χ^2 (with the Mantel extension) or Fisher's exact test were used to test statistical significance.

Results

Rotavirus. On both islands, there was a strong positive linear association between increasing age

Table 1. Baseline titers of antibody to RV according to age among San Blas Indians from the islands of Tupile and Achutupo.

Age* (no. of subjects)	Baseline antibody titer† <1:4	1:4	>1:4	GMT‡
0–<2 (65)	56 (86)§	3 (5)	6 (9)	1.9
2–<5 (113)	65 (58)	17 (15)	31 (27)	4.6
≥5 (127)	30 (24)	26 (20)	71 (56)‖	6.7

NOTE. None of the differences between Tupile and Achutupo in prevalence of antibody at a particular titer in an age group are significant at the $P = .05$ level; therefore, the values for the islands have been combined.

* Age in years at baseline serum collection, September 1979.

† Titer of antibody to RV in September 1979.

‡ Geometric mean titer. Reciprocal dilution of sera with any detectable antibody.

§ No. of persons positive at each titer (%).

‖ Trend of increasing RV antibody titer with increasing age was significant ($P < .001$, by χ^2).

Table 2. Incidence of RV infection by age group in San Blas Indians on the islands of Tupile and Achutupo between September 1979 and March 1980.

Age (years)*	Island		*P* values†
	Tupile	Achutupo	
0–<2	23/39 (59)‡	1/26 (4)	<.001
2–<3	9/21 (43)	5/20 (25)	>.05
3–<4	12/18 (67)	0/12 (0)	<.004
4–<5	20/20 (100)	3/22 (14)	<.001
⩾5	17/77 (22)§	2/50 (4)§	<.03
Total	81/175 (46)	11/130 (8)	<.001

* Age in September 1979.

† Significance test (χ^2 or Fisher's exact) for differences in infection rates within a particular age group between Tupile and Achutupo.

‡ No. of persons with fourfold or greater rise in serum antibody titer between September 1979 and March 1980/no. of persons from whom sera was obtained and antibody titrations to RV carried out in September 1979 and March 1980 (% seroconverting).

§ No significant trend of decreasing infection rates with increasing age on a particular island. Difference between infection rates in Tupile residents less than five years old (64/98 = 65%) and more than five years old (17/77 = 22%) was significant (χ^2 = 27.18, P <.001). Difference between infection rates in Achutupo residents less than five years old (9/80 = 11%) and more than five years old (2/50 = 4%) was not significant (P = .08, Fisher's exact).

and increasing baseline rotaviral antibody titer (table 1). At the beginning of the study, 56 (86%) of 65 children less than two years of age had a RV antibody titer of <1:4, whereas 71 (56%) of 127 persons five years of age or older had RV antibodies in a titer of >1:4 (table 1). RV antibody titers on both islands were nearly three times higher in persons older than five years of age when compared with children younger than two years of age.

The incidence of RV infection on Tupile was significantly greater than that on Achutupo in nearly all age groups (table 2). On both islands, children younger than five years of age had higher infection rates than persons five years or older. Sixty-four (65%) of 98 Tupile residents younger than five years of age had at least a fourfold rise in titer compared with 17 (22%) of 77 persons five years of age or older (P < .001). Similarly, on Achutupo, 9 (11%) of 80 Achutupo residents less than five years of age had RV infection compared with 2 (4%) of 50 residents five years or older (P = .08).

Within a particular age group individuals with high baseline RV titers were significantly less likely to develop RV infections over the subsequent seven months of follow up than were persons of similar age with undetectable RV titers (table 3). Thirty-seven (57%) of 65 children between the ages of two and five years with a baseline RV antibody titer of <1:4 had a RV infection at some time over the next seven months, whereas only 5 (16%) of 31 children the same age with a baseline RV titer of >1:4 had RV infection over the same time period (P < .001). Among all 305 persons studied 151 (50%) had a baseline RV titer of <1:4, but this group accounted for 76% of all RV infections. Persons of any age with an initial RV antibody titer of >1:4 accounted for 35% of the study population but had only 10% of RV infections.

RV particles were detected in the stools of 40 (43%) of the 92 persons with RV infection, as determined by antibody studies, but in only 10 (5%) of

Table 3. Relation between baseline titer of antibody to RV and risk of subsequent RV infection by age in San Blas Indians on the islands of Tupile and Achutupo.

Age* (no. of subjects)	Baseline antibody titer†			*P* values‡
	<1:4	1:4	>1:4	
0–<2 (65)	24/56 (43)§	0/3 (0)	0/6 (0)	<.003
2–<5 (113)	37/65 (57)	7/17 (41)	5/31 (16)	<.001
5+ (127)	9/30 (30)	6/26 (23)	4/71 (6)	<.01
All ages (305)	70/151 (46)	13/46 (28)	9/108 (8)	<.0001

NOTE. Infection is defined as a fourfold or greater rise in antibody titer in sera collected in September 1979 and March 1980.

* Age (years) in September 1979.

† Titer of antibody to RV in September 1979.

‡ Significance test for trend in that age group for association of declining infection rate with increasing baseline RV antibody titer. Data for Tupile and Achutupo are combined.

§ No. of patients with fourfold or greater rise in RV antibody titer/total no. of patients in that age group with particular baseline antibody titer (% seroconverting). Data for Tupile and Achutupo are combined.

Table 4. Incidence of RV diarrhea in San Blas Indians according to age and baseline titer of antibody to RV.

Age* (no. of subjects)	Baseline antibody titer†		
	<1:4	1:4	>1:4
<2 (65)	20/56 (36)‡	1/3 (33)	1/6 (17)
2–<5 (113)	16/65 (25)	3/17 (18)	5/31 (16)
≥5 (127)	2/30 (7)	2/26 (8)	0/71 (0)
Total	38/151 (25)	6/46 (13)	6/108 (6)

NOTE. An episode of RV diarrhea is the passage of more than three loose stools (in which RV particles were identified) in a 24-hr period. Patients with more than one episode are listed only once.

* Age (years) in September 1979.

† Titer of antibody to RV in September 1979.

‡ No. of patients with an episode of RV diarrhea/no. of patients in that age group with particular baseline antibody titer (% seroconverting).

213 persons who did not have at least a fourfold rise in RV antibody titer ($P < .001$). Among 151 patients with a baseline RV antibody titer of <1:4, 38 (25%) had at least one episode of RV diarrhea, whereas only 12 (8%) of 154 patients with a baseline RV antibody titer of ≥1:4 had an episode of RV diarrhea (table 4; $P < .001$).

Norwalk virus. Sixty-five percent of 65 persons less than two years of age had baseline NW antibody titers of <1:100, while 87% of 111 persons five years of age or more had a baseline NW antibody titer of >1:100 (table 5). Baseline NW antibody prevalence was similar on both islands. Unlike RV, NW infection on both islands showed a marked trend of decreasing infection rate with increasing age (table 6). Subjects less than three years of age represented only 35% of the study population but accounted for 71% of NW infections. NW infection rates were similar on both islands.

Table 5. Baseline titers of antibody to NW virus according to age among San Blas Indians from the islands of Tupile and Achutupo.

Age* (no. of subjects)	Baseline antibody titer†		
	<1:100	1:100	>1:100
0–<2 (65)	42 (65)‡	12 (18)	11 (17)
2–<5 (113)	23 (20)	16 (14)	74 (65)
≥5 (127)	3 (2)	13 (10)	111 (87)§

* Age (years) in September 1979.

† Titer of antibody to NW in September 1979.

‡ No. of persons positive at each titer (%).

§ Trend of increasing NW antibody titer with increasing age is significant ($\chi^2_1 = 53.2$, $P < .001$).

Table 6. Incidence of NW infection by age group in San Blas Indians on the islands of Tupile and Achutupo between September 1979 and March 1980.

Age* (years)	Island		Total incidence for both islands
	Tupile	Achutupo	
0–<2	20/39 (51)†	9/26 (35)	29/65 (45)
2–<3	9/21 (43)	6/20 (30)	15/41 (37)
3–<4	4/18 (22)	2/12 (17)	6/30 (20)
4–<5	1/20 (5)	3/22 (14)	4/42 (10)
≥5	5/77 (6)	3/50 (6)	8/127 (6)
Total	39/175 (22)‡	23/130 (18)‡	62/305 (19)‡

* Age in September 1979.

† No. of persons with fourfold or greater rise in serum antibody titer between September 1979 and March 1980/no. of persons on whom titrations for antibody to NW were performed with sera collected in September 1979 and March 1980 (% seroconverting).

‡ Trend of decreasing infection rates with increasing age is significant (χ^2_1 for trend with Mantel extension) on Tupile ($P < .001$) and Achutupo ($P < .01$) and for both islands combined ($P < .001$). None of the differences in infection rates within a particular age group between Tupile and Achutupo are significant.

A high baseline titer for antibody to NW was associated with protection from subsequent NW infection only in children less than five years of age (table 7). Forty-eight percent of 65 children less than five years of age had a baseline NW titer of <1:100 but they had 57% of this age group's NW infections.

Discussion

The purpose of this investigation was to learn more about the protective role of humoral immunity that occurs after naturally acquired infection with rotavirus and Norwalk virus. Infection with these viruses before our period of surveillance (as measured by presence of antibody in baseline sera) and during the seven-month period of surveillance was common: nearly half of the 305 patients enrolled in the study had RV and/or NW antibodies at the start of the investigation, and during the seven-month surveillance period 46% of Tupile residents and 8% of Achutupo residents had RV infection. Approximately 20% of the study population on both islands had NW infection during the surveillance period.

A high baseline titer of antibody to either RV or NW was associated with protection against subsequent infection. Presently, we are not able to determine if these serum antibodies per se were protective or if they were just closely associated with

Table 7. Relation between baseline titer of antibody to Norwalk virus and risk of subsequent NW infection by age in San Blas Indians on the islands of Tupile and Achutupo.

Age* (no. of subjects)	Baseline antibody titer† <1:100	≥1:100	*P* values‡
<5 (178)	31/65 (48)§	23/113 (20)	$\chi_1^2 = 14.59$, $P < .001$
≥5 (127)	1/3 (33)	7/124 (6)	Fisher's exact, >.05

NOTE. Infection is defined as a fourfold or greater rise in antibody titer in sera collected in September 1979 and March 1980.

* Age in September 1979.

† Titer of antibody to NW in September 1979.

‡ Significance test in that age group for association of declining infection rate with increasing baseline NW antibody titer.

§ No. of patients with fourfold or greater rise in titer of antibody to NW/total no. of patients in that age group with particular baseline antibody titer (% seroconverting). Data for Tupile and Achutupo are combined.

another unmeasured factor (perhaps local antibody on the surface of the gut) that, in itself, provided protection from reinfection. Within each of the age groups we studied, a person with a baseline titer of antibody to RV of >1:4 was approximately five times less likely to develop RV infection over the subsequent seven-month surveillance period than was a subject of comparable age with a baseline titer of < 1:14. Similarly, an individual with a baseline titer of antibody to NW of ≥1:100 was at least half as likely to develop NW infection than was an individual of the same age with a baseline titer of <1:100.

The little data that exists on the protection afforded by humoral RV antibodies against naturally acquired RV infection is conflicting. Bishop [10] studied a cohort of newborns for a three-year period and did not find any evidence of protection against subsequent reinfection (which was usually asymptomatic). Early infection/illness in Bishop's patients did confer relatively persistent clinical protection. Mata et al. [11], in Guatemala, found no evidence of clinical immunity occurring after infection. Black et al. [12] used sensitive antibody detection methods to allow detection of antibody in nearly all patients and found striking evidence of humoral immunity. Our data are consistent with those of Black et al., although in our study far fewer children of less than two years of age had detectable RV antibody at the start. Forty-six percent of our 151 patients with no initial titer of antibody to RV experienced a subsequent RV infection compared with 8% of 108 who had an initial titer of >1:4 ($P < .001$). That only 10% of the 92 RV infections in our study occurred in subjects with an initial titer of >1:4 suggests that a vaccine producing durable high titer RV serum antibody will probably prevent the majority of RV infections. The lack of any detectable antibody in a large portion of persons older than five years of age is probably explained by the relative insensitivity of the complement-fixation assay we used. Serological tests developed more recently have been shown to be more sensitive [13].

Rotaviral infection rates were surprisingly high in persons more than five years old included in our study. However RV disease was primarily restricted to children less than five years of age. Bishop et al. [10] have demonstrated that neonatal RV infection does not confer immunity against reinfection but does protect young children against the development of clinically severe disease during reinfection. Therefore, our findings extend those of Bishop et al. and suggest that the protection against development of clinically severe disease during reinfection is long lasting.

We found large differences in RV infection rates between Tupile and Achutupo among study subjects of all ages. This finding may be partially explained by the greater prevalence of high baseline titers of antibodies to RV in Achutupo residents of all ages. Forty-four percent of Achutupo residents had a baseline titer of antibody to RV of >1:4, compared with 29% of Tupile residents ($P < .01$). Alternatively, Tupile, despite its proximity to Achutupo, may have merely experienced a chance outbreak of RV infection. Previous studies of highly communicable viruses in isolated but adjacent villages in the developing world have shown marked differences in infection rates [14].

Our finding an increased prevalence of NW antibody with increasing age fits the pattern documented in previous work involving young children from less-developed countries. Cukor et al. [15] demonstrated that by age six nearly 60% of children from Taiwan or the Phillipines had antibodies to NW. Black et al. [12], in Bangladesh, found that 100% of their study population had antibodies to NW by the age of four years. Our results demonstrating that less than half of our study children of less than two years of age had detectable antibody to NW, whereas, by age five, 98% of the population had antibodies to

NW, places Cuna Indian children between Asian and Bangaladeshi children in age-specific prevalence rates of antibody to NW.

The paucity of longitudinal studies of NW infection in children from the developing world allows a comparison of our incidence data with only the similarly collected Bangladeshi data of Black et al. [12], in which 369 children <50 months of age were studied. These investigators found that 46% of the children developed NW infection over a 12-month surveillance period. Adjustment of our seven-month surveillance rates to an annual basis for comparison reveals that 63% of San Blas Cuna Indian children <48 months old had NW infection.

The high level of protection that an NW antibody titer of ≥1:100 provided against infection in the present study confirms Black's similar findings [12]. In our study, 10% of subjects of all ages with baseline titers for antibody to NW of >1:100 developed NW infection, compared with 7% of subjects <50 months of age with the same titer in Black's study. Similarly, 48% of our patients less than five years old and with a baseline titer for antibody to NW of <1:100 subsequently developed NW infection, in contrast to the 37% figure in Black's study of children of a similar age.

Challenge studies in adult North American volunteers [16] have been interpreted to suggest that factors other than serum antibodies or other closely associated antibodies (such as intestinal antibody) are important in providing immunity to gastroenteritis caused by Norwalk virus. Our findings and those of Black et al. [12] are in sharp contrast to these data. The differences in the age at which the first infection occurred in developing- and developed-world populations and differences in the sensitivity of the immunoassays used in these studies may partially explain the different findings. Additional prospective studies in developing- and developed-world populations of all ages will help clarify this important point.

Protection against reinfection with NW appeared to be highly associated with having detectable baseline antibodies to NW; in RV infections, the level of protection conferred by the CF antibodies was not as absolute. Although these differences could easily be explained by differences in the sensitivity of the assays used to detect RV and NW, they also suggest that immunity against each of these agents may be induced through different mechanisms.

The similarity of our findings in this community-based study in a remote area of Latin America and those of Black et al. [12] in a similarly rural underdeveloped area in Asia emphasizes the global importance of RV and NW as agents of enteric disease. The concordance of the Bangladeshi and Panamanian findings concerning the importance of humoral immunity or another closely associated factor in preventing infection should stimulate efforts to develop a safe vaccine that will induce long-lasting immunity to RV and NW.

A vaccine against RV has already been described [4]. While efforts to improve the quality and quantity of water available in the developing world, and thereby prevent diarrhea, are laudable, the enormous capital expenditure required for installation of these systems will likely guarantee that large sections of the developing world will continue to have high incidences of diarrheal disease. Establishment of effective, low-cost vaccine programs in underdeveloped countries, such as The Gambia and the Ivory Coast [1, 2], suggests that in the immediate future the development of effective, safe vaccines against enteric disease may be a more effective and realistic approach to the prevention of diarrheal disease.

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