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Role of Coproantibody in Clinical Protection of Children during Reinfection with Rotavirus

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Rotavirus is the major cause of severe, dehydrating infantile gastroenteritis. Infection is limited to the gut, but the relative roles of serum and secretory copro-immunoglobulin A (IgA) in protection are unclear. Specific copro-IgA is predictive of duodenal antirotaviral IgA and correlates with virus-neutralizing coproantibody. Copro-IgA conversion is a more sensitive marker of rotavirus reinfection than seroconversion. We measured rotavirus reinfections by copro-IgA conversion prospectively in 35 children recruited at a time of severe rotavirus illness. The children were followed up longitudinally for 14 to 31 months to determine whether high coproantibody levels correlated with clinical protection against rotavirus disease. Ninety-four percent of the children experienced reinfection, and 38% developed persistent elevations in specific copro-IgA termed plateaus. Plateau children had a higher mean annual rate of rotavirus infection and a lower ratio of symptomatic to total number of rotavirus reinfections than did nonplateau children. The annual rates of rotavirus infection and disease were significantly higher outside the plateau than inside it in children experiencing antirotavirus copro-IgA plateaus. Frequent rotavirus infection of children appears to stimulate production of a specific copro-IgA plateau which correlates with protection against an excess of infection and symptomatic disease.

Rotaviruses are the single most important etiologic agents of severe diarrheal illness in infants and young children worldwide (17) and infect the young of most mammalian species. Rotaviruses are classified into serotypes based on the reaction of outer capsid proteins VP7 (G types) and VP4 (P types). In neonatal animals, the presence of virus-neutralizing antibody in the lumen of the small intestine correlates with resistance to rotavirus illness (23, 25). Immune protection in animals is often, but not always, serotype specific (5, 25, 27). Longitudinal studies of children indicate that primary rotavirus infection results in clinical immunity during later reinfection (4). The mechanisms responsible for immunity to rotavirus infection and illness in children are not clear. The presence of specific antibody in serum usually has not been associated with immunity (2, 8, 17).

In severe, acute rotavirus gastroenteritis in infants and young children, antibodies in sera and duodenal fluid are sensitive indicators of rotavirus infection (16). Specific fecal immunoglobulin A (IgA) shows a high predictive accuracy for specific duodenal IgA levels at 1 and 4 months postinfection (16). Fecal IgA can thus be used as a measure of intestinal IgA. Changes in antirotaviral copro-IgA correlate with virus-neutralizing coproantibody conversions and appear to be more sensitive than seroconversions as measures of rotavirus reinfections (11). The ability to detect rotavirus infection by repeated measurement of antirotaviral copro-IgA presented a nonintrusive means of monitoring rotavirus infection in young children and of correlating this with associated clinical symptoms. The aim of the study was to determine whether coproantibody levels were a correlate of clinical protection against rotavirus infection and symptomatic disease.

MATERIALS AND METHODS

Patients. We studied 44 children, 3 weeks to 39 months old, admitted with acute rotavirus diarrhea to the infectious disease ward between April 1984 and September 1985. The clinical, demographic, and laboratory findings of these children while hospitalized have already been described (16). No child was breast fed during the study. The study was approved by the Human Ethics Committees of the Royal Children's Hospital and the World Health Organization. The parents of the children gave informed, signed consent. Nine children did not complete the study, either because they were withdrawn by their mothers or because the families relocated and could not be traced. The data presented refer to the remaining 35 children.

Specimen collection. Acute, convalescent, and 4-monthly blood samples were collected from each child (11, 16). Fecal specimens were collected daily while each child was in the hospital and at 7- to 10-day intervals from all of the children for at least 14 months and from 20 (57%) of the children for 21 to 31 months. Each family was in telephone contact once each month with a research nurse, who visited the home 1 week later to obtain specimens and a clinical history of the child. Parents were asked to contact a pediatrician (K.G.) immediately if symptoms of enteritis occurred in the child. The grading system used to assess the severity of clinical disease was based on that described previously (4). A score of 0 to 4, describing level and duration, was assigned in each of three categories: fever, vomiting, and diarrhea. A score of 0 to 3 was assigned to the category of treatment supervision required: 0, parents alone; 1, infant health nurse; 2, general practitioner; 3, hospitalization. A total score of 1 to 8 indicated mild disease, and a score of 9 to 14 indicated

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moderate disease. No episodes classifiable as severe were reported. Extra fecal samples were collected during diarrheal episodes. Feces collected by mothers at home were stored frozen at −4°C for up to 1 month before collection and transport to the Royal Children's Hospital, where they were placed at −70°C until tested (16). Feces and sera collected in the hospital were stored at −70°C until tested.

Specific antibody estimation. Antirotaviral IgA, IgM, and IgG levels in all of the serum and fecal samples collected were estimated by enzyme immunoassay (EIA) using SA11 rotavirus as the antigen, as previously described (10). A seroconversion in IgG or IgA was defined as a fourfold increase in titer between two consecutive specimens such that the maximum value was greater than or equal to twice the positive-negative cutoff (10, 11). A positive IgM response in serum or feces was defined as a titer equal to or greater than 1:200. IgA coproconversion was defined as a threefold increase in EIA units per milliliter, to at least 100 U, occurring over a period of no more than 3 weeks, with no more than one coproconversion each month. This definition of conversions has been validated (10, 11). Rotavirus-neutralizing antibody titers in a subset of 422 fecal samples from 10 children were estimated by fluorescent focus reduction neutralization of cultivable rotaviruses (11, 12). A significant elevation in neutralizing antibody, indicating rotavirus infection, was defined as a fourfold increase in titer to at least 1:400 (11).

Fecal pathogen analysis. All fecal extracts were assayed for rotaviral antigen by monoclonal antibody EIA with EDTA treatment (13). Diarrheal feces were also examined for viruses by electron microscopy after concentration of fecal extracts. Where possible, the G type of the infecting rotavirus was determined by monoclonal antibody EIA (13). G type 1 rotaviruses were classified as monotype a, b, or c by reaction with a panel of monoclonal antibodies as described previously (9). Feces collected during diarrheal episodes were also cultured for the presence of salmonellae, shigellae, *Campylobacter jejuni*, *Yersinia enterocolitica*, and enteropathogenic *Escherichia coli* and examined by light microscopy for *Giardia lamblia* cysts.

Statistical methods. Chi-square, *t*, Mann-Whitney, and Wilcoxon statistical tests were performed by using the Minitab statistical software (20). Logit analyses were done on the Melbourne University VAX computer by using the GLIM 3.77 system (19, 22). In all analyses, statistical significance was achieved at the 5% level.

RESULTS

Relationship of rotavirus G type and monotype to severity of illness at hospitalization and on first reinfection. The G type and monotype of the rotavirus responsible for the severe, presumably primary (16), infection which precipitated hospitalization and recruitment to the study were determined in 30 (86%) of the children. During 1984, G type 1c predominated (10 [53%] of 19), with G type 4 common, especially from July to September (6 [32%] of 19). G type 1a was found in two May 1984 recruits (2 [13%] of 19) but predominated in 1985 (8 [89%] of 9). One child in each year was infected by rotavirus of dual G type: 1c and 4 in 1984 and 1a and 4 in 1985. These are not included in the following analysis. The infecting G type did not correlate with the severity of disease during hospitalization, as assessed by the need for intravenous treatment. However, there was a trend for children recruited in 1984 to be more likely than those recruited in 1985 to have symptoms on their first rotavirus reinfection

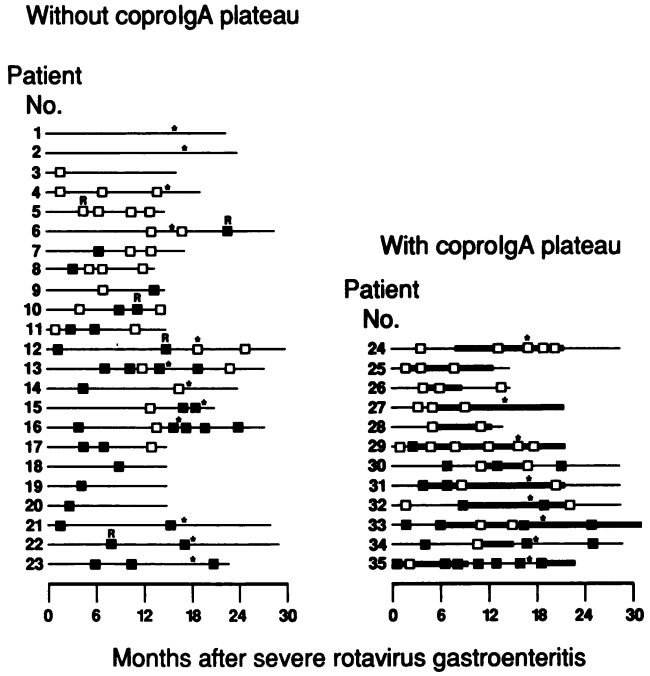

FIG. 1. Incidence of rotavirus infection and disease in children with and without an antirotaviral copro-IgA plateau. Symbols: ——, follow-up period; ▬▬, plateau period; □, asymptomatic copro-IgA conversion; ■, symptomatic copro-IgA conversion; R, rotavirus detected in stools; *, end of first rotavirus season after that in which the child was hospitalized with severe rotavirus gastroenteritis (November).

(Fisher's exact test, $P = 0.053$). Within the 1984 group, there was also a trend for children infected with G type 1a or 1c virus to be more likely than those infected with G type 4 virus to be symptomatic on their first rotavirus reinfection (Fisher's exact test, $P = 0.054$).

Detection of prolonged elevations in copro-IgA to rotavirus—the coproantibody plateau. Coproconversion following primary rotavirus infection lasted for up to 75 days before copro-IgA levels returned to the baseline (16). Copro-IgA profiles of 12 of the 35 study children showed prolonged elevations above the copro-IgA baseline that developed after the initial infection. A prolonged elevation was arbitrarily termed a copro-IgA plateau. This was defined as an elevation in copro-IgA to at least 100 U for at least 90% of a period of not less than 120 days.

Figure 1 represents plateau development in 12 children contrasted with the 23 children who did not develop a plateau. Plateaus appeared 2 to 11 months after recruitment and lasted from 139 to more than 773 days. In all but one case, the plateau began at the time of the first or second rotavirus reinfection. One child (no. 35) had two plateau periods. The geometric mean units of antirotaviral copro-IgA in each child showing a plateau ranged from 111 to 462 (median, 205) during the plateau period and from 3 to 99 (median, 53) outside the plateau period. The children without a copro-IgA plateau showed geometric mean units of 5 to 84 (median, 23).

Kinetics of responses of plateau and nonplateau children. One hundred sixteen coproconversions were recorded in 33 of the 35 children, including 62 coproconversions in children without a copro-IgA plateau and 54 in children before, during, and after a coproantibody plateau. Rotavirus copro-

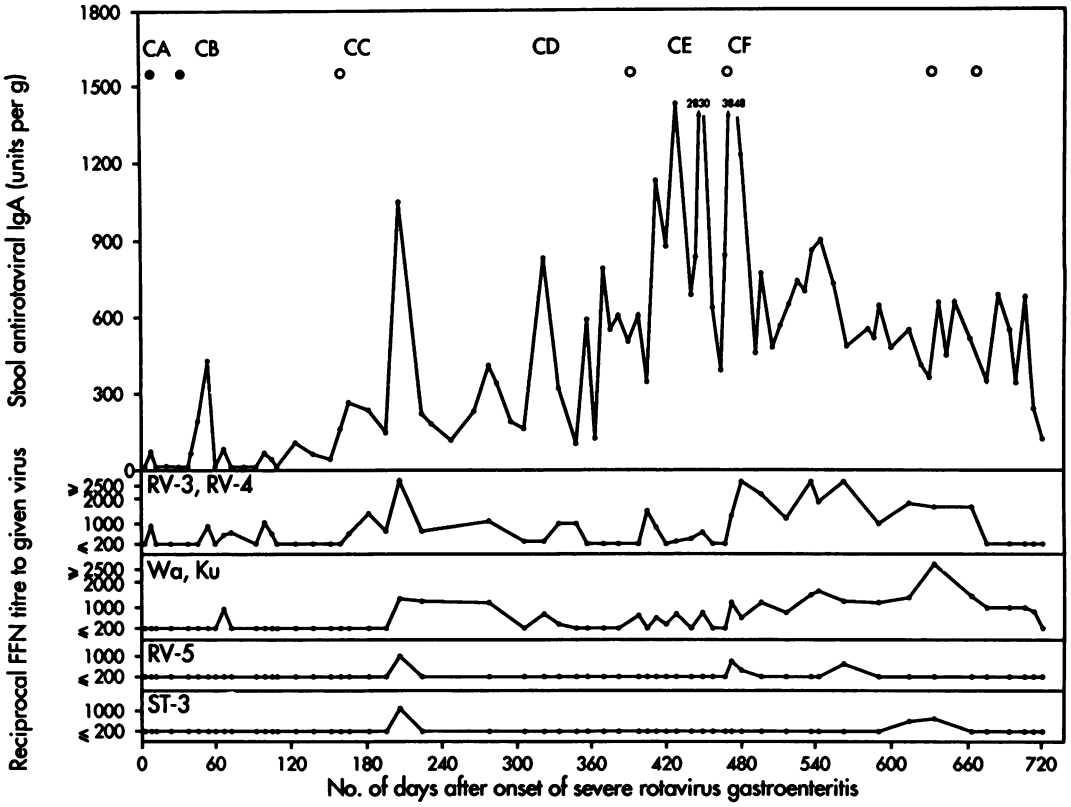

FIG. 2. Antirotaviral copro-IgA and neutralizing antibody profiles of a child with a specific copro-IgA plateau. Symbols: CA to CF, specific IgA coproconversion; ○, episode of gastroenteritis; ●, episode of gastroenteritis with rotavirus in stools. FFN, fluorescent-focus neutralization.

IgA and neutralizing coproantibody profiles illustrative of those measured in the children with and without IgA plateaus are shown in Fig. 2 (patient no. 33) and 3 (patient no. 21), respectively. Up to day 720, patient no. 33 experienced five copro-IgA conversions following the initial low-level rapid coproconversion (CA) during severe rotavirus infection at the age of 23 months. The second coproconversion (CB) was symptomatic and associated with rotavirus-specific copro-IgM and rotavirus excretion. Three of the later coproconversions (CB, CE, and CF) were concurrent with seroconversion in IgA or IgG. Three of the five rotavirus reinfections were associated with symptoms (CB, CE, and CF). With the exception of the fourth coproconversion (CD), all were of large and increasing amplitude. The plateau began with the second rotavirus reinfection (CC) and persisted at least to the end of the study, 930 days after recruitment. A seventh coproconversion at day 740, illustrated in Fig. 1 but not in Fig. 2, was accompanied by IgG seroconversion.

Nonplateau child no. 21 showed only IgA and IgM class serum responses to the initial severe rotavirus infection at the age of 2 months and delayed copro-IgA conversion (CA). Patient no. 21 experienced only two further IgA coproconversions (CB and CC), which were symptomatic, accompanied by specific copro-IgM or seroconversion, and of increasing amplitude. After each coproconversion, copro-IgA levels fell to below the positive-negative cutoff (28 U), so that a plateau was not formed.

In both plateau and nonplateau children, the copro-IgA profiles were closely paralleled by the neutralizing antibody profiles, particularly to rotaviruses RV-3 (G3 P2), RV-4 (G1 P1A), and ST-3 (G4 P2). Antibodies to RV-5 (G2 P1B), Ku (G1 P1A), and Wa (G1 P1A) appeared at the time of the IgA coproconversions. It was not possible to determine the virus serotypes responsible for the reinfections from the neutralizing antibody profiles because of these multiple responses.

Rotavirus reinfection and development of disease in relation to the presence or absence of a copro-IgA plateau. All of the children were followed up for at least one complete rotavirus season after recruitment, and 13 (37%) were followed up for a second complete season (Fig. 1). The data from the first season and from the combined seasons were considered separately. Too few children were followed up for a second season for separate analysis of that group. In the combined seasons, 22 (41%) of the rotavirus reinfections in plateau children were associated with symptoms and 34 (55%) of the reinfections in nonplateau children were symptomatic. In six (5.2%) of the rotavirus reinfections, symptoms were classified as moderate. Two of these were in plateau children, and four were in nonplateau children. In all other symptomatic reinfections (n = 50), symptoms were classified as mild. There was no statistically significant difference between the plateau and nonplateau groups in the severity of the symptoms recorded (mild or moderate).

There were no statistically significant differences between the plateau and nonplateau children for age of recruitment, sex, severity of rotavirus infection at presentation, or number of rotavirus seasons followed up (Table 1), although there was a trend for the rotavirus infection to be more severe at presentation in the nonplateau group (P = 0.06).

As shown in Table 2, plateau and nonplateau children

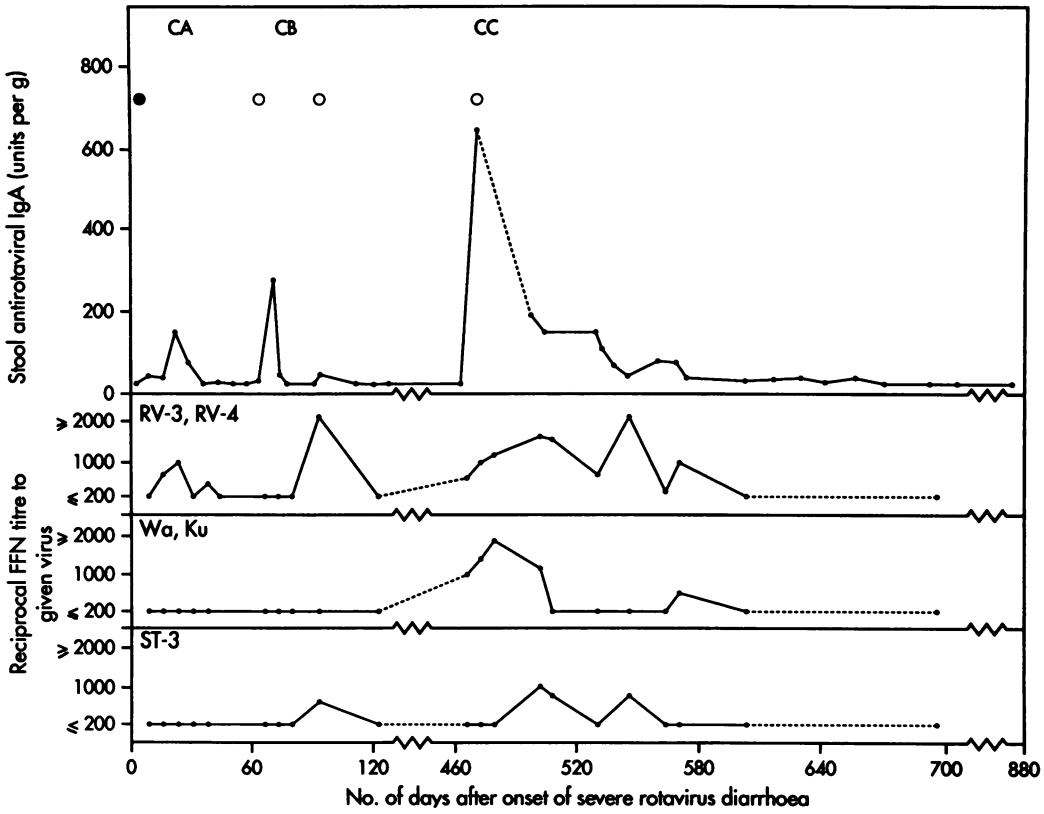

FIG. 3. Antirotaviral copro-IgA and neutralizing antibody profiles of a child without a specific copro-IgA plateau. For definitions of symbols, see the legend to Fig. 2. FFN, fluorescent-focus neutralization.

were comparable for number of days surveyed. Judged by IgA coproconversion, children who developed a plateau showed a significantly greater mean annual rate of rotavirus infection (2.88) than did nonplateau children (1.83) in the first season following recruitment ($P = 0.008$). During the combined seasons also, plateau children were infected with rotavirus at a significantly greater median annual rate (2.18) than nonplateau children (1.58; $P = 0.030$). However, the mean annual rates of symptomatic rotavirus infection were not significantly different between the plateau and nonplateau children in the first season (1.01 versus 0.99; $P = 0.96$) or the combined season (0.82 versus 0.92; $P = 0.78$).

In the season following their recruitment, the plateau children showed symptoms during a mean proportion of 0.33 of their rotavirus infections. This proportion was significantly less than that in the corresponding period in nonplateau children (0.60; $P = 0.042$). The same trend in proportions of symptomatic infection was evident when data for the second season following recruitment were included, but the difference was not statistically significant ($P = 0.061$). The antibody plateau continued into the second season in only one of the six plateau children studied (Fig. 1, no. 33).

Among plateau children during the season following their recruitment, the numbers of days to which each child was followed up inside and outside the plateau period were comparable (Table 3). These children showed a significantly lower median annual rate of infection inside the plateau period (2.14) than outside it (3.00; $P = 0.011$). This rate outside the plateau was also significantly greater than that in nonplateau children (1.49; Mann-Whitney test, $P = 0.001$). Rotavirus infections inside the plateau were significantly less likely to be symptomatic (0.51 per year) than those outside the plateau (2.14 per year; $P = 0.041$). This rate outside the plateau was not significantly different from the rate in

TABLE 1. Descriptive statistics of plateau and nonplateau children

Group (no. of children)	Mean age (mo) at recruitment (95% C.I.[a])	No. male/ no. female	Intravenous treatment		No. followed up for:	
			Yes	No	1 Season	2 Seasons
Plateau (12)	16.9 (11.4–22.4)[b]	7/5[c]	5[d]	7[d]	6[e]	6[e]
Nonplateau (23)	18.0 (13.9–22.2)[b]	14/9[c]	17[d]	6[d]	16[e]	7[e]

[a] C.I., confidence interval.
[b] $P = 0.73$ by the t test.
[c] $P = 0.9$ by the chi-square test.
[d] $P = 0.06$ between groups by logit analysis of proportions.
[e] $P = 0.12$ between groups by logit analysis of proportions.

TABLE 2. Comparison of rates of rotavirus infection and symptomatic disease in plateau and nonplateau children

Group (no. of children)	Mean no. of days followed up (95% C.I.[a]) during:		Annual rate of rotavirus infection[l] (95% C.I.) during:		Mean annual rate of symptomatic rotavirus infection[m] (95% C.I.) during:		Mean proportion of rotavirus infections with symptoms (95% C.I.) during:	
	1 Season	2 Seasons	1 Season[b]	2 Seasons[c]	1 Season	2 Seasons	1 Season	2 Seasons
Plateau (12)	474 (442–505)[d]	706 (582–830)[e]	2.88 (2.23–3.54)[f]	2.18 (1.78–2.53)[g]	1.01 (0.21–1.80)[h]	0.82 (0.16–1.48)[i]	0.33 (0.12–0.54)[j]	0.34 (0.12–0.56)[k]
Nonplateau (23)	490 (472–508)[d]	630 (554–706)[e]	1.83 (1.42–2.25)[f]	1.58 (0.88–2.27)[g]	0.99 (0.70–1.28)[h]	0.92 (0.65–1.19)[i]	0.60 (0.43–0.78)[j]	0.58 (0.43–0.74)[k]

[a] C.I., confidence interval.
[b] Mean values.
[c] Median values.
[d] $P = 0.35$ by the t test.
[e] $P = 0.27$ by the t test.
[f] $P = 0.008$ by the t test.
[g] $P = 0.030$ by the Mann-Whitney test.
[h] $P = 0.96$ by the t test.
[i] $P = 0.78$ by the t test.
[j] $P = 0.042$ by the t test.
[k] $P = 0.061$ by the t test.
[l] Mean number of rotavirus infections (number of copro-IgA conversions) each year for each child.
[m] Mean number of symptomatic rotavirus infections (number of copro-IgA conversions associated with gastrointestinal symptoms) each year for each child.

nonplateau children (0.99; t test, $P = 0.19$). The mean annual rates of rotavirus infection and symptomatic infection in plateau children during a plateau (Table 3) were not significantly different from those in nonplateau children (Table 2; t test, $P = 0.80$ and 0.11, respectively). Thus, during a plateau, plateau children showed lower annual rates of infection and symptomatic infection than in the period outside the plateau. These rates inside the plateau were comparable to the rates of the children who produced no antibody plateau at all.

Relationship of age at time of coproconversion to likelihood of symptomatic illness. Logit modelling of the probability of symptomatic rotavirus infection for each individual child showed that there was no significant effect of age on rate of symptomatic infection (beta = −0.18; standard deviation = 0.12; $P > 0.2$). Thus, the increasing age of each patient during the longitudinal study was not associated significantly with increased resistance to rotavirus disease, although a trend was evident. The lack of dependence of rate of symptomatic rotavirus infection on age was the same for both plateau and nonplateau children ($P > 0.15$).

Relationship of contact with other children to development of an antirotaviral copro-IgA plateau. During the study period, six (17%) of the children attended a day-care center full time for 1 to 2 years. Half of these children developed an antibody plateau. There was no significant difference in the attendance rates at day care between the plateau and nonplateau groups (Fisher's exact test, $P = 0.23$). Four plateau children had younger siblings at home (33%), a rate comparable to that of nonplateau children (13%; Fisher's exact test, $P = 0.13$). However, there was a trend for children with younger siblings or who attended day care full time to be more likely to develop plateaus (Fisher's exact test, $P = 0.054$). Among the children recruited in 1984 (9 plateau and 14 nonplateau), this trend reached statistical significance (Fisher's exact test, $P = 0.027$).

TABLE 3. Comparison of rotavirus infection and symptomatic disease in the periods inside and outside the plateau for children[a] with antibody plateaus in the first rotavirus season after their recruitment

Period	Median no. of days followed up (95% C.I.[b])	Annual rate of rotavirus infection[f] (95% C.I.)	Mean annual rate of symptomatic rotavirus infection[g] (95% C.I.)
Inside plateau	272 (211–332)[c]	2.14 (1.37–2.47)[d]	0.51 (0–1.04)[e]
Outside plateau	201 (137–266)[c]	3.00 (2.50–6.39)[d]	2.14 (0.33–3.96)[e]

[a] $n = 12$.
[b] C.I., confidence interval.
[c] $P = 0.23$ by the t test.
[d] $P = 0.011$ by the Wilcoxon test.
[e] $P = 0.041$ by the t test.
[f] Defined in Table 2, footnote *l*.
[g] Defined in Table 2, footnote *m*.

DISCUSSION

Frequent longitudinal measurement of antirotaviral copro-IgA levels in 35 children (after an initial severe rotavirus infection) showed that 33 (94%) experienced rotavirus reinfections and that 38% of the children developed persisting elevations of antirotaviral copro-IgA that lasted for up to 773 days. These persisting elevations have been designated coproantibody plateaus. Similar persisting elevations of copro-IgA levels were probably also present in five children studied by Nishio et al. (21). During the plateau, copro-IgA was associated with persisting rotavirus-neutralizing activity, mainly directed at the G types of rotavirus responsible for the initial severe infection. Boosts in neutralizing antibody titers to more than one G type were always observed on reinfection, so that it was not possible to assign a serotype to the infecting virus by this indirect method. Similar heterotypic neutralizing antibody responses have been found in sera obtained during rotavirus reinfection (7, 14).

In this study, symptomatic rotavirus reinfection was rarely associated with virus shedding detectable by electron microscopy or EIA. Detection and G typing of rotaviruses responsible for the reinfections observed are under way using the polymerase chain reaction technique described by Gouvea et al. (15). In preliminary testing of fecal samples from four reinfections in which virus was not detectable by other methods, G type 1 virus was present in two reinfections and no rotavirus was detected in two reinfections (18). This is consistent with our data showing that IgA coproconversion in the absence of detectable virus particles or antigen is a reliable and sensitive measure of rotavirus reinfection (11). It is possible that the copro-IgA conversions uncon-

firmed by any other antibody or antigen assay were not caused by rotavirus infection. However, we believe that the evidence presented in our previous publications (11, 16) strongly suggests that the copro-IgA conversions are rotavirus specific.

The observed trend for children recruited in 1984 to be more likely to show symptoms on their first rotavirus reinfection than those recruited in 1985 may be related to the prevailing rotavirus G types in children admitted to Melbourne hospitals with gastroenteritis from 1984 to 1986. G types altered from 1c (major), 4 (common), and 1a (minor) in 1984 to 1a (major), 1c (rare), and 4 (rare) in 1985 and to 1a only in 1986 (6). Children recruited in 1985 were all infected with G type 1a at that time and were likely to be reinfected with the same G type during 1985 and 1986, whereas those recruited in 1984 were likely to have been exposed to different G types during 1984 and 1985. The 1984 recruits with G type 4 infections also showed a trend to a lower incidence of symptoms on first reinfection. Further polymerase chain reaction typing of rotavirus from reinfections may help answer this question.

The children who developed a copro-IgA plateau had more frequent rotavirus reinfections than children who did not develop a plateau ($P = 0.03$). This increased frequency related to their period of follow-up outside the plateau period ($P = 0.011$). It is probable that frequent infection with rotavirus produced anamnestic gut IgA responses that led to plateau formation. Intestinal anamnestic responses to rotavirus have also been recorded elsewhere (28). It is likely that once a plateau was established, it was sustained by reinfection, which occurred at a rate lower than that outside the plateau but similar to that of nonplateau children. The plateau children, particularly those recruited in 1984, were more likely to have younger siblings or to attend day care than were the nonplateau children. It is therefore probable that the plateau children were exposed to rotavirus more often than were the nonplateau children, causing the higher frequency of rotavirus infection observed in the plateau group.

The appearance of a plateau could also be correlated with resistance to symptomatic infection. Once a plateau developed, children were less likely to show enteric symptoms during rotavirus reinfection than in the period without an antibody plateau. The duration of this protective effect (5 to 26 months) was similar to that observed against severe disease (at least 18 months) in children asymptomatically infected with rotavirus as neonates (4) and to that in children infected at 2 to 12 months of age (at least 2 years) (1). It is likely that similar mechanisms were involved in the protection against disease observed in these studies.

This study population was selected on the basis of severe rotavirus infection. In a birth cohort of randomly selected Melbourne children being followed longitudinally, rotavirus infections have been common and copro-IgA plateaus have occurred in a proportion of children on rotavirus reinfection (3). This preliminary finding suggests that the selection criteria did not bias this study towards unusually susceptible children. It is unlikely that immune antibody enhancement of infection occurred, as infection was most common in plateau children during the period when their coproantibody levels were low.

Our results demonstrate a direct correlation between high levels of intestinal IgA antibody to rotavirus and a significant degree of protection of children against natural rotavirus infection and disease. In stools, the presence of IgA antibody to rotavirus has been shown to correlate with the presence of rotavirus-neutralizing antibody (11, 12). These results are not in full agreement with data on adults challenged with human rotavirus CJN, when jejunal neutralizing antibody levels correlated inversely with the probability of illness and IgG levels in serum correlated inversely with the probability of infection (26). These responses may be peculiar to adults (26).

It is likely that immune mechanisms additional to neutralization of virus by antibody contributed to the protection observed in plateau children. Gastrointestinal cytotoxic T lymphocytes and intraepithelial lymphocytes are elicited in mice after oral rotavirus inoculation (24). Stimulation of specific memory T-cell clones on rotavirus reinfection may therefore enhance intestinal antibody production. Rotavirus-infected cells stimulate human leukocytes to produce a cytokine that enhances natural-killer cell activity (29). These cells can be mobilized quickly during infection and may play an important part in limiting acute viral infections (2).

On the basis of this study, we hypothesize that rotavirus infection of children at sufficiently frequent intervals stimulates production of a specific coproantibody plateau. This plateau is a correlate of resistance to natural rotavirus infection and disease in children who have experienced severe rotavirus gastroenteritis and frequent rotavirus reinfection previously. The resistance observed reduces the infection and disease rate of plateau children to that of nonplateau children, who have a lower frequency of exposure to rotavirus. Specific copro-IgA is predictive of duodenal antirotaviral IgA, and persisting levels of IgA in the small intestine could protect against infection and symptomatic disease. Further studies are under way to determine whether most children in a population produce antirotaviral copro-IgA plateaus and whether plateaus always result from reinfection or also from primary rotavirus infection. It will be most interesting to examine whether vaccination of children with rotavirus stimulates IgA coproconversion or plateau production and, if so, whether these are measures of vaccine efficacy.

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REFERENCES

1. **Bernstein, D. I., D. S. Sander, V. I. Smith, G. M. Schiff, and R. L. Ward.** 1991. Protection from rotavirus reinfection: 2-year prospective study. J. Infect. Dis. **164**:277–283.
2. **Bishop, R., J. Lund, E. Cipriani, L. Unicomb, and G. Barnes.** 1990. Clinical serological and intestinal immune responses to rotavirus infection of humans. Med. Virol. **9**:85–110.
3. **Bishop, R. F.** Unpublished data.
4. **Bishop, R. F., G. L. Barnes, E. Cipriani, and J. S. Lund.** 1983. Clinical immunity after neonatal rotavirus infection. N. Engl. J. Med. **309**:72–76.
5. **Bishop, R. F., S. R. Tzipori, B. S. Coulson, L. E. Unicomb, M. J. Albert, and G. L. Barnes.** 1986. Heterologous protection against rotavirus-induced disease in gnotobiotic piglets. J. Clin. Microbiol. **24**:1023–1028.
6. **Bishop, R. F., L. E. Unicomb, and G. L. Barnes.** 1991. Epidemiology of rotavirus serotypes in Melbourne, Australia, from 1973 to 1989. J. Clin. Microbiol. **29**:862–868.
7. **Brussow, H., J. Sidoti, D. Barclay, J. Sotek, H. Dirren, and W. Freire.** 1990. Prevalence and serotype specificity of rotavirus antibodies in different age groups of Ecuadorian infants. J.

Infect. Dis. **162:**615–620.
8. **Chiba, S., S. Nakata, T. Urasawa, S. Urasawa, T. Yokoyama, Y. Morita, K. Taniguchi, and T. Nakao.** 1986. Protective effect of naturally acquired homotypic and heterotypic rotavirus antibodies. Lancet **ii:**417–421.
9. **Coulson, B. S.** 1987. Variation in neutralization epitopes of human rotaviruses in relation to genomic RNA polymorphism. Virology **159:**209–216.
10. **Coulson, B. S., K. Grimwood, R. F. Bishop, and G. L. Barnes.** 1989. Evaluation of end-point titration, single-dilution and capture enzyme immunoassays for measurement of antirotaviral IgA and IgM in infantile secretions and serum. J. Virol. Methods **26:**53–66.
11. **Coulson, B. S., K. Grimwood, P. J. Masendycz, J. S. Lund, N. Mermelstein, R. F. Bishop, and G. L. Barnes.** 1990. Comparison of rotavirus immunoglobulin A coproconversion with other indices of rotavirus infection in a longitudinal study in childhood. J. Clin. Microbiol. **28:**1367–1374.
12. **Coulson, B. S., and P. J. Masendycz.** 1990. Measurement of rotavirus-neutralizing coproantibody in children by fluorescent focus reduction assay. J. Clin. Microbiol. **28:**1652–1654.
13. **Coulson, B. S., L. E. Unicomb, G. A. Pitson, and R. F. Bishop.** 1987. Simple and specific enzyme immunoassay using monoclonal antibodies for serotyping human rotavirus. J. Clin. Microbiol. **25:**509–515.
14. **Gerna, G., A. Sarasini, M. Torsellini, D. Torre, M. Parea, and M. Battaglia.** 1990. Group- and type-specific serologic response in infants and children with primary rotavirus infections and gastroenteritis caused by a strain of known serotype. J. Infect. Dis. **161:**1105–1111.
15. **Gouvea, V., R. I. Glass, P. Woods, K. Taniguchi, H. F. Clark, B. Forrester, and Z.-Y. Fang.** 1990. Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. J. Clin. Microbiol. **28:**276–282.
16. **Grimwood, K., J. C. S. Lund, B. S. Coulson, I. L. Hudson, R. F. Bishop, and G. L. Barnes.** 1988. Comparison of serum and mucosal antibody responses following severe acute gastroenteritis in young children. J. Clin. Microbiol. **26:**732–738.
17. **Kapikian, A. Z., and R. M. Chanock.** 1990. Rotaviruses, p. 1353–1404. *In* B. N. Fields and E. M. Knipe (ed.), Virology, 2nd ed. Raven Press, New York.
18. **Kirkwood, C., and S. Richardson.** Unpublished data.
19. **McCullagh, P., and J. A. Nelder.** 1983. Generalized linear models. Chapman & Hall, Ltd., London.
20. **Minitab, Inc.** 1989. Minitab (computer program). Release 7.2 DOS. Minitab Inc., State College, Pa.
21. **Nishio, O., Y. Ishihara, S. Isomura, H. Inoue, and S. Inouye.** 1988. Long-term follow-up of infants from birth for rotavirus antigen and antibody in the feces. Acta Pediatr. Jpn. **30:**497–504.
22. **Numerical Algorithm Group.** 1986. Generalised interactive modelling (GLIM) (computer program). Release 3.77. Numerical Algorithim Group, Oxford.
23. **Offit, P. A., and H. F. Clark.** 1985. Protection against rotavirus-induced gastroenteritis in a murine model by passively acquired gastrointestinal but not circulating antibodies. J. Virol. **54:**58–64.
24. **Offit, P. A., S. L. Cunningham, and K. I. Dudzik.** 1991. Memory and distribution of virus-specific cytotoxic T lymphocytes (CTLs) and CTL precursors after rotavirus infection. J. Virol. **65:**1318–1324.
25. **Snodgrass, D. R., and P. W. Wells.** 1976. Rotavirus infection in lambs: studies on passive protection. Arch. Virol. **52:**201–205.
26. **Ward, R. L., D. I. Bernstein, R. Shukla, M. M. McNeal, J. R. Sherwood, E. C. Young, and G. M. Schiff.** 1990. Protection of adults rechallenged with a human rotavirus. J. Infect. Dis. **161:**440–445.
27. **Wyatt, R. C., C. A. Mebus, R. H. Yolken, A. R. Kalica, H. D. James, and A. Z. Kapikian.** 1976. Rotaviral immunity in gnotobiotic calves: heterologous resistance to human virus induced by bovine virus. Science **203:**548–550.
28. **Yamaguchi, H., S. Inouye, M. Yamauchi, T. Morishima, S. Matsur o, S. Isomura, and S. Suzuki.** 1985. Anamnestic response in fecal IgA antibody production after rotaviral infection of infants. J. Infect. Dis. **152:**398–400.
29. **Yasukawa, M., O. Nakagomi, and Y. Kobayashi.** 1990. Rotavirus induces proliferative response and augments non-specific cytotoxic activity of lymphocytes in humans. Clin. Exp. Immunol. **80:**49–55.