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Persistence of Antibodies to Rotavirus in Human Milk

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Human milk obtained from 21 American nursing mothers was studied for the presence of secretory immunoglobulin A antibody to rotavirus, the most common etiological agent of infantile gastroenteritis. Antibody was quantitated by adaptation of a recently described solid-phase radioimmunoassay technique that employs simian rotavirus as a convenient substitute antigen for human rotavirus. Of the mothers tested, 80% (12 of 15) possessed milk antibody within a week of parturition, whereas 56% of those tested (5 of 9) secreted milk antibody as late as 6 or 9 months postpartum. Specificity of the radioimmunoassay was demonstrated by absorption of antibody with purified rotavirus. Our detection by radioimmunoassay of antibody to rotavirus in milk samples collected past the colostrum stage is in contrast to other studies that have failed to detect antibody in human milk by immunofluorescence or neutralization tests. The present study also suggested that the appearance of secretory immunoglobulin A antibody in the milk of mothers previously lacking milk antibody may be correlated with subclinical infection of the mother with rotavirus.

Rotavirus is an important etiological agent of infantile gastroenteritis in all parts of the world (1, 3, 6, 12, 16). It is a major cause of infant morbidity and mortality in regions with poor sanitation, nutrition, and access to medical care. Rotavirus infection is also a common reason for the hospitalization of infants in developed areas. Epidemiological studies, in emerging as well as modern societies, support the widely held clinical impression that breast feeding plays a major role in protection against diarrheal disease (8, 10). To study the nature of this protective effect, we adapted a highly sensitive solid-phase radioimmunoassay (RIA) technique (G. Cukor, M. K. Berry, and N. R. Blacklow, J. Infect. Dis., in press) to the detection of secretory immunoglobulin A (IgA) antibody to rotavirus in human milk. We now report that this type of antibody is secreted for at least 6 to 9 months postpartum by a high percentage of women in a small group of middle class American nursing mothers.

Solid-phase RIA and the analogous enzyme-linked immunosorbent assay have recently been employed as highly efficient methods for the detection of serological responses to a variety of infectious agents, including rotavirus (20). A hyperimmune animal serum that reacts with rotavirus is a critical reagent in these assays. Human rotavirus does not replicate completely in cell culture and therefore can be used as an immunogen only when collected from human stool. Rather than immunize animals with a stool-derived virus preparation which could contain extraneous antigens causing nonspecific reactivity, we chose to prepare antibody against a simian rotavirus (SA-11) which is immunologically closely related to the human agent but in addition is capable of efficient growth in monkey kidney cell culture. We have reported elsewhere details of our preparation of this antiserum and its use in an accurate and sensitive RIA for the detection of rotavirus in stools of human infants with diarrhea (G. Cukor et al., in press). We have now adapted this RIA to the measurement of secretory IgA antibody to rotavirus in human milk specimens.

MATERIALS AND METHODS

RIA for detection of secretory IgA antibody in milk. Milk was collected around the time of parturition and at various times for up to 9 months afterwards from 21 healthy mothers who gave informed consent per approval of the Memorial Hospital Human Experimentation Committee. Milk samples were centrifuged at 12,000 × *g* for 1 h. Starting at a 1:20 concentration, fourfold dilutions of the aqueous phase were prepared in phosphate-buffered saline (PBS) for subsequent testing by RIA. SA-11 virus, a simian rotavirus, was grown to high titer in MA-104, a continuous line of rhesus monkey kidney cells. The virus was purified on sucrose gradients and used to immunize guinea pigs as previously described (G. Cukor et al., in press). The RIA procedure was as follows. A 100-μl amount of a 1:

1,000 dilution of SA-11 hyperimmune guinea pig serum was used to coat the wells of a microtiter plate. Plates were incubated for 4 h at 4°C, washed twice, and saturated with 1% bovine serum albumin. After further washing, 10^7 50% tissue culture infective doses of sucrose gradient-purified SA-11 virus were added to each well for the test proper. Control wells received only PBS. Plates were incubated overnight at 4°C and, after washing five times, 25 μl of each milk dilution was added to two experimental and two control wells. After overnight incubation at 4°C and five more washes, 200,000 cpm of ^{125}I-labeled anti-human secretory component rabbit globulin was added. After 4 h of incubation at 37°C, the plates were again washed five times, and the bound radioactivity was determined. The source of the rabbit globulin was Behring Diagnostics, Sommerville, N.J., and it was iodinated by the method of Hunter and Greenwood (4). Virus-specific counts were calculated by subtraction of the counts obtained in the control well without virus from the counts obtained by the same milk sample (at the same dilution) in the experimental well containing virus. A positive-to-negative (P/N) ratio was obtained by dividing the virus-specific counts of a milk sample by the virus-specific counts obtained with a buffer control. The reciprocal of the highest dilution of a milk sample yielding a P/N ratio of at least 2 was considered to be its titer. P/N values for positive samples ranged from 2.00 to 5.30, with a mean of 2.70. For negative samples, the range of P/N values was from 0.14 to 1.90, with a mean of 1.16.

Milk antibody absorption test. Portions (0.5 ml each) of the aqueous phase of a milk sample were incubated for 1 h at 37°C and overnight at 4°C with either 10^8 50% tissue culture infective doses of gradient-purified SA-11 virus or an equivalent volume of PBS. All samples were then centrifuged at 100,000 × *g* for 1 h, and the supernatant was then diluted 1:20 for use in the RIA.

RESULTS

A total of 21 nursing mothers were studied for the presence of secretory IgA antibody to rotavirus in their milk. Within less than a week of parturition, 80% of women (12 of 15) from whom milk specimens were available had milk titers of antirotavirus secretory IgA of 1:20 or greater (Table 1). Seven to 14 days postpartum, 13 of 26 available milk specimens possessed titers ≥1:20, whereas after that period for up to 9 months, about one-third of available samples were antibody positive. Of the mothers tested, 56% (5 of 9) secreted milk antibody as late as 6 or 9 months postpartum.

Six of the 21 mothers initially tested negative for milk antibody (titers <1:20) (Table 1, patients A-F). Three of these women continued to test negative throughout the duration of their study (group I). Six women who initially tested positive for milk antibody tested negative within a month and remained so for the duration of their study (group III). Six of the mothers who initially tested positive maintained for the most part positive milk antibody titers, and milk antibody positivity persisted for as long as 6 to 9 months (group IV). Group V contained three women from whom only initial milk specimens were available.

The specificity of the RIA for antibody to rotavirus was demonstrated by the elimination of milk antibody positivity by absorption of samples with purified SA-11 virus (Table 2). Milk samples which were positive (P/N > 2) all had their P/N ratios reduced to less than 2 by the

TABLE 1. *Rotavirus antibody titers in milk at various times following parturition as determined by RIA*

Group	Patient	No. of weeks postpartum[a]								
		<1	1	2	4	6	8	12	24	36
I	A	—			—				—	—
	B		—		—	—	—	—		
	C		—	—	—		—			
II	D	—	—	—	—	—	80	20		
	E		—	—	—	20	20	—		
	F	—		—	—	—	—	—		80
III	G	20			—	—	—	—		
	H	20	—	—	—	—	—	—		
	I		20	20	—	—	—	—		
	J	20	—							
	K	80		80	80	—	—	—	—	—
	L	20		20	—	—	—	—	—	—
IV	M		20	80	80	80	20	—	320	20
	N	20	80	320	80	80	20	20	20	—
	O	80	—	—	—	—	—	—	—	80
	P	320	80	80	—	80	80	—		
	Q			20	—	80	20	—	—	—
	R	20	20	20	320	80	20	—	80	
V	S	20								
	T	80								
	U	20								

[a] —, RIA antibody titer <20.

TABLE 2. *Absorption of milk antibody by gradient-purified SA-11 rotavirus*

Milk sample	Absorbed with	P/N at 1:20 dilution
1	PBS	2.21
	Virus	0.86
2	PBS	2.13
	Virus	0.73
3	PBS	3.20
	Virus	1.06
4	PBS	3.56
	Virus	1.82
5	PBS	1.83
	Virus	1.53

absorption process. Negative milk was unaffected by absorption.

DISCUSSION

Secretory IgA antibody to rotavirus was observed in human milk specimens for up to 9 months postpartum. This finding is in contrast to recent reports that have failed to find antibody to rotavirus in human milk past the colostrum stage by fluorescent-antibody or neutralization tests (15, 18). Our experience is that the fluorescent-antibody test for antibody in milk is not sufficiently specific or sensitive (unpublished data). The efficiency of replication of some rotaviruses in culture can be enhanced by trypsin, and the presence of milk in a plaque reduction or neutralization test could conceivably lead to nonspecific results due to inactivation of the enzyme by the milk. In addition, milk has been reported to contain antiviral neutralizing activity, detectable in tissue culture systems, that is unrelated to antibodies (11). Therefore it seems that an in vitro immunological test such as RIA would be a preferred method for measuring antibody to rotavirus in milk. A recent letter to the editor (17) indicates that enzyme-linked immunosorbent assay can be used to demonstrate the presence of antibody to rotavirus in colostrum. We have confirmed that observation by RIA and have extended it to show that antibody may be present in milk for at least 6 to 9 months postpartum.

It is known that IgA antibody levels are normally highest in colostrum and decline fairly rapidly with time (13, 14). The rising and falling pattern of rotavirus antibody titer that we observed in some women might be explainable by infection of the mother with rotavirus, although we have no direct virus detection data to confirm this hypothesis. It has been shown that subclinical infection with rotavirus commonly occurs in adult contacts of infected infants even in the presence of serum antibody (7). We have found, as has also been recently noted (5), that antibody levels in colostrum do not necessarily correlate with antirotavirus titers in maternal sera obtained at parturition. However, in the case of two mothers, we have had the opportunity to demonstrate that a rise in milk antibody titer was accompanied by a serum antibody rise. Patient M had a serum antibody titer to rotavirus of 1:32 as determined by immunofluorescence (fluorescent antibody) (2) 12 weeks postpartum. The next available serum specimen, obtained at 36 weeks, showed an antibody titer of 1:128. During the intervening period, a sharp rise in milk antibody titer occurred (Table 1). Similarly, patient D seroconverted after she began to secrete milk antibody to rotavirus. At 12 weeks postpartum, her serum titer was 1:512, whereas at parturition she had a serum titer of 1:128.

Animal studies indicate that ingestion of rotavirus antibody in milk is protective for the young (9, 19). An important area for further study is to see whether certain levels of rotavirus antibody in human milk may be effective in preventing diarrheal disease in the infant.

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