Protection Against Rotavirus-Induced Gastroenteritis in a Murine Model by Passively Acquired Gastrointestinal But Not Circulating Antibodies

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Newborn mice suckled on dams immunized either orally or parenterally with primate rotavirus SA-11 were protected against diarrhea induced by SA-11 virus challenge. Experimental oral administration of milk from orally immunized dams protected suckling mice against challenge; protective activity was detected both in the anti-rotavirus immunoglobulin A (IgA) and IgG fractions, but IgA was more potent in vivo than IgG. Oral administration of milk from parentally immunized dams also protected suckling mice against challenge; in this case, protective activity was detected in the anti-rotavirus IgG fraction. In newborn mice foster-nursed by seronegative dams, circulating rotavirus-specific antibodies in high titer did not protect mice against oral SA-11 virus challenge. It appears that the most effective rotavirus vaccine will be that which induces an efficient production of antibodies active at the intestinal cell surface.

Rotaviruses are one of the most important causes of acute gastroenteritis both in the United States and in developing countries (4, 24). The worldwide impact of these viruses has excited interest in disease prevention via the development of a vaccine (25, 45, 46). The development of a successful vaccine will be facilitated by defining the immune response necessary for protection against challenge.

Rotaviruses have been identified as a cause of infection and gastroenteritis in the young of most species of domestic and laboratory animals (22, 23, 27, 31, 37, 39, 48, 49). However, rotaviruses of common laboratory animals are either poorly characterized or are not adapted to growth in tissue culture. Previous investigations into rotavirus pathogenesis and immunogenesis in mice have been of limited value because the poor growth of murine rotaviruses in tissue culture has prevented accurate quantitation of infectivity, plaque purification, and efficient production of purified viruses. Studies of murine rotaviruses have not led to the clear differentiation of the immunological correlates of protection against challenge (9, 29, 38). The only animal models currently used for the study of rotavirus infection with tissue culture-adapted strains are gnotobiotic calves, lambs, and pigs (28, 36, 40). However, the limited availability of gnotobiotic large animals has hampered animal model studies. We have recently developed a murine model for oral infection with a tissue culture-adapted primate rotavirus (simian strain SA-11), using conventionally bred mice (33). Studies of the relative roles of passively acquired circulating and intestinal rotavirus-specific antibodies in protecting suckling mice against diarrhea induced by SA-11 virus challenge are described in this report.

MATERIALS AND METHODS

Animals. Conventionally bred pregnant CD-1 mice obtained from Charles River Breeding Laboratories (Wilmington, Mass.) were housed in separate isolation units. Dams were bled on arrival by retroorbital capillary plexus punc-

ture, and sera were tested by radioimmunoassay (RIA) as previously described (34); litters whose dams were seronegative were used in these studies.

Cells and virus. MA-104 cells were grown in antibiotic-free BHK cell medium (30) supplemented with 10% newborn bovine serum.

A seed stock of simian rotavirus SA-11 was obtained from H. H. Malherbe (University of Texas, San Antonio, Tex.). Plaque-purified stocks of SA-11 virus for use in these studies were prepared in MA-104 cells. Viral growth, purification, quantitation, and viral infectivity plaque assays were performed as previously described (35).

Cross-fostering studies. (i) **Immunization and handling of dams.** Eight-week-old female CD-1 mice were immunized with SA-11 virus under one of the following protocols: (i) mice were orally inoculated with 3×10^6 PFU of SA-11 virus and inoculated orally 3 and 6 weeks later with the same viral dose, or (ii) mice were inoculated intraperitoneally with 3×10^6 PFU of SA-11 virus emulsified in a 50% suspension of complete Freund adjuvant and inoculated intraperitoneally 6 weeks later with the same viral dose in incomplete Freund adjuvant. On the day of the final booster inoculation, mice were bred in a female-to-male ratio of 1:1; the animals were left together for 2.5 weeks. Pregnant immunized mothers were then placed in cages with slotted bottoms, which allowed their progeny to fall to a lower level, inaccessible to the mother upon delivery.

(ii) **Infection of infant mice.** Five-day-old mice were orally inoculated with 6×10^6 PFU of SA-11 virus, a dose shown previously to cause diarrhea in ca. 90% of inoculated animals (33). Infants were inspected daily for diarrhea after gentle palpation of their abdomens.

(iii) **Specimen collection.** On the day of inoculation of suckling mice, sera were obtained from the foster and birth mothers by retroorbital capillary plexus puncture and from two neonates in each litter by decapitation.

In vivo neutralization studies in infant mice. (i) **Collection of serum and milk.** Sera and milks for use in these studies were collected from immunized dams 14 days after delivery (ca. 5

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weeks after the final booster inoculation). Milk was collected by a modification of the technique described by Feller and Boretos (11). Briefly, litters were removed ca. 16 h before milking. To secure adequate yields of milk, it was necessary to inoculate the lactating mice intraperitoneally with 0.1 U of oxytocin (Parke, Davis & Co., Detroit, Mich.). Mice were then milked with the suction apparatus previously described (11).

(ii) Serum and milk immunoglobulin adsorption protocol. Milk and serum preparations were used at 1:25 and 1:50 dilutions, respectively, in phosphate-buffered saline (PBS).

To selectively adsorb immunoglobulin A (IgA) out of the serum and milk preparations, 50 µg of rabbit antiserum to mouse IgA (courtesy of Walter Gerhard, The Wistar Institute) was added per ml of a 10% (wt/vol) suspension of *Staphylococcus aureus* Cowan I and adsorbed for 1 h at 0°C. The bacteria were pelleted at $12,800 \times g$ for 2 min and washed once with PBS. To block the protein A sites not bound to immunoglobulin, 2 mg of human IgG (courtesy of Walter Gerhard) per ml of 10% *S. aureus* Cowan I was then added and adsorbed for 1 h at 0°C. The bacteria were pelleted and washed four times with PBS. Serum or milk was adsorbed with the bacteria containing bound rabbit anti-mouse IgA, using a concentration of 10 µg of bacteria per ml. After 18 h of incubation at 4°C, bacteria were removed by centrifugation and supernatant fluids were used for further testing.

To selectively adsorb IgG, serum or milk preparations were adsorbed four times with 5 mg of *S. aureus* Cowan I per ml. Each adsorption consisted of incubation at 0°C for 30 min and removal of the bacteria by centrifugation. Supernatant fluids were used for further testing.

(iii) Inoculation of animals and specimen collection. Five-day-old CD-1 mice nursed by unimmunized dams were orally inoculated with 100 µl of the unadsorbed or immunoglobulin-adsorbed serum or milk preparations at various dilutions as described below. After 5 to 10 min, mice were orally challenged with 6×10^6 PFU of SA-11 virus. Mice were checked daily for diarrhea as described above.

RIA. Purified SA-11 virus (100 ng) in sodium carbonate buffer (15 mM Na_2CO_3 , 35 mM NaHCO_3) was added to individual wells of round-bottomed, 96-well polyvinyl plates and kept at room temperature overnight. Each well was then treated with 1% bovine serum albumin in PBS. Immunoglobulin-adsorbed and unadsorbed serum and milk samples were diluted in 1% bovine serum albumin in PBS and incubated with viral immunoabsorbent for 90 min at room temperature. Serum and milk obtained from unimmunized animals served as controls. The wells were then washed with PBS and incubated for 90 min with ^{125}I -labeled, affinity-purified goat antimouse IgA, IgG, or IgM (Cappel Laboratories, Malvern, Pa.). Isotyping reagents were labeled with ^{125}I by the chloramine T method (16). After a final washing procedure, the wells were separated from the plate and assayed for radioactivity in a gamma radiation counter. Serum or milk titer endpoints were calculated as the reciprocal of the highest dilution giving a value equal to twice the control value at the same dilution.

The specificity and sensitivity of the isotyping reagents were determined by using murine myeloma immunoglobulins (IgA, IgM, and IgG1; Miles Scientific, Naperville, Ill.). Myeloma immunoglobulins diluted in sodium carbonate buffer were adsorbed to individual wells of 96-well polyvinyl plates at room temperature. Each well was treated with 1% bovine serum albumin in PBS. The wells were then washed with PBS and incubated for 90 min with the ^{125}I -labeled

isotyping reagents. The wells were washed and assayed for radioactivity in a gamma radiation counter. Final values greater than twice the control values obtained from wells without immunoabsorbent were considered positive. Although the goat antimouse IgG and IgM were isotype specific, the goat antimouse IgA detected murine IgG1 and IgA at concentrations of 100 and 120 ng/ml, respectively. This cross-reactivity was ablated when 10 µg of purified murine IgG (courtesy of Walter Gerhard) was added to 8 µg of ^{125}I -labeled goat antimouse IgA.

By the use of ^{125}I -labeled goat antimouse IgA (blocked with purified murine IgG), IgG, and IgM, the thresholds for detection of myeloma immunoglobulins were 70, 120, and 120 ng/ml, respectively, for IgA, IgG, and IgM.

Plaque reduction neutralization assay. The plaque reduction neutralization (PRN) assay was a modification of the technique described by Matsuno et al. (32) and was performed as previously described, using SA-11 virus (35).

RESULTS

For a determination of the relative contributions of transplacentally acquired and colostrum-milk antibodies in protecting infant mice against rotavirus challenge, a series of cross-fostering experiments was performed with unimmunized, orally immunized, and parenterally immunized dams.

Neonates from two dams orally immunized with SA-11 virus were foster nursed by unimmunized dams (Table 1). Nine of 10 neonates developed diarrhea 2 days after challenge with SA-11 virus. The duration (3 to 4 days) and severity of the diarrhea were equivalent to those observed in neonates from unimmunized dams (33). Anti-rotavirus IgG concentrations representing ca. 5% of maternal levels were detected in neonatal sera collected on the day of inoculation. The neutralization titers by PRN of neonatal sera ranged from less than 1:100 to 1:365.

Neonates from three dams parenterally immunized with SA-11 virus were foster nursed by unimmunized dams (Table 1). Infant mice were orally challenged with SA-11 virus as described above. Eleven of 15 neonates developed diarrhea. Anti-rotavirus IgG concentrations representing ca. 1% of maternal levels were detected in neonatal sera collected on the day of inoculation. Anti-rotavirus IgA and IgM were never detected in neonatal sera despite their presence in certain of the maternal sera. These findings are consistent with those of previous investigations of the passive transfer of immunoglobulins into the murine neonatal circulation, which indicated that neither IgA nor IgM was transported either transplacentally or by absorption of ingested colostrum-milk antibodies into the circulation (1, 10). Circulating PRN antibodies in titers ranging from 1:3,550 to 1:6,000 were clearly shown to be nonprotective.

Neonates from three unimmunized dams were foster nursed by dams orally immunized with SA-11 virus (Table 2). None of 19 neonates developed diarrhea after SA-11 virus inoculation. Anti-rotavirus IgG concentrations representing from 12 to 100% of foster nurse levels were detected in neonatal sera collected on the day of inoculation. Anti-rotavirus PRN activity in neonatal sera ranged from undetectable levels to 35% of foster nurse levels. However, regardless of the serum antibody levels detected, all neonates were protected against rotavirus challenge.

Neonates from two unimmunized dams were foster nursed by dams parenterally immunized with SA-11 virus (Table 2). Of 11 neonates, none developed diarrhea after virus chal-

TABLE 1. Cross-fostering studies of suckling mice challenged orally with SA-11 virus: role of passively acquired, circulating rotavirus-specific antibodies in protection against challenge^a

Birth dam no.	Serum antibody profile (μg/ml) by RIA			PRN titer ^b	Neonate no.	Serum antibody concn (μg/ml) by RIA			PRN titer	Susceptibility to challenge ^c
	IgM	IgA	IgG			IgM	IgA	IgG		
3B ₂	<0.12	<0.07	650	900	1	<0.12	<0.07	30	<100	3/4
					2	<0.12	<0.07	30	<100	
1B ₂	<0.12	<0.07	200	1,880	1	<0.12	<0.07	7.8	365	6/6
					2	<0.12	<0.07	7.8	180	
4-1	30	<0.07	12,000	225,500	1	<0.12	<0.07	120	5,200	5/7
					2	<0.12	<0.07	96	3,550	
4-4	<0.12	<0.07	42,000	325,000	1	<0.12	<0.07	150	5,190	3/4
					2	<0.12	<0.07	156	6,000	
4-6	750	87.5	19,200	246,500	1	<0.12	<0.07	600	4,740	3/4
					2	<0.12	<0.07	648	5,290	

^a Suckling mice from dams immunized with SA-11 virus orally (3B₂ and 1B₂) or parenterally (4-1, 4-4, and 4-6) were foster nursed by unimmunized dams. Five days after parturition, sera were obtained from two neonates (1 and 2) in each litter by decapitation and from the birth dam by retroorbital capillary plexus puncture. Sera were tested by RIA and PRN. The remaining neonates in each litter were orally challenged with 6×10^6 PFU of SA-11 virus.

^b Expressed as reciprocal of serum dilution.

^c Number of mice with diarrhea/number of mice inoculated with SA-11 virus.

lenge. All neonates exhibited substantial levels of IgG antibodies detected by RIA and PRN activity.

These studies demonstrate that protection against rotavirus-induced gastroenteritis was passively transferred to suckling mice via rotavirus-specific antibodies in colostrum-milk and not via circulating antibodies acquired transplacentally.

For a determination of the relative contributions of passively acquired gastrointestinal anti-rotavirus IgG and IgA in protecting infant mice against SA-11 virus challenge, suckling mice were orally inoculated with unadsorbed or immunoglobulin-adsorbed milk (Table 3) or serum (Table 4) preparations. Milk and serum were obtained from dams inoculated either orally or parentally with SA-11 virus.

Milk from orally infected animals contained anti-rotavirus IgG and IgA antibodies as determined by RIA and PRN (Table 3). There was a ca. 7-fold greater concentration of anti-rotavirus IgG than IgA in this preparation. Although in vivo protective activity was detected in both the anti-rotavirus IgG and IgA fractions, the quantity of IgG required to protect 50% of the mice was 0.021 μg, compared with a quantity of only 0.002 μg required for IgA. Therefore, milk

IgA (presumably secretory IgA) had a greater protective efficiency in this system than IgG. In vivo protective activity was not detected in an IgG-IgA-adsorbed preparation which nevertheless retained ca. 2% of its original PRN activity.

Milk from a parenterally immunized dam contained a ca. 200-fold greater concentration of rotavirus-specific IgG than IgA (Table 3). Although the IgA concentration of this preparation by RIA was similar to that observed in milk from an orally immunized dam, there was no detectable rotavirus-specific neutralizing activity in vitro (PRN) or in vivo. Both the protective and the PRN activity of this milk were associated only with the anti-rotavirus IgG fraction; the protective efficacy of this preparation was ca. 30-fold less than that observed for anti-rotavirus IgG in milk from an orally immunized dam.

Milk from an unimmunized dam contained no detectable anti-rotavirus antibodies by PRN or RIA. Oral inoculation of this preparation, at a dilution of 1:50, did not protect against diarrhea induced by SA-11 virus.

Sera from orally and parentally immunized dams contained a ca. 180-fold greater concentration of rotavirus-specific IgG than IgA (Table 4). Both the protective and the

TABLE 2. Cross-fostering studies of suckling mice challenged orally with SA-11 virus: role of passively acquired colostrum-milk rotavirus-specific antibodies in protection against challenge^a

Foster dam no.	Serum antibody profile (μg/ml) by RIA			PRN titer ^b	Neonate no.	Serum antibody concn (μg/ml) by RIA			PRN titer	Susceptibility to challenge ^c
	IgM	IgA	IgG			IgM	IgA	IgG		
3B ₁	<0.12	6.3	516	4,500	1	<0.12	<0.07	216	1,290	0/6
					2	<0.12	<0.07	132	1,140	
3B ₂	<0.12	<0.07	650	900	1	<0.12	<0.07	78	<100	0/6
					2	<0.12	<0.07	120	<100	
1B ₂	<0.12	<0.07	200	1,800	1	<0.12	<0.07	200	260	0/7
					2	<0.12	<0.07	114	355	
4A ₁	<0.12	17.5	3,750	181,000	1	<0.12	<0.07	2,040	11,000	0/5
					2	<0.12	<0.07	1,800	>12,500	
4A ₂	<0.12	42	7,200	234,000	1	<0.12	<0.07	744	2,500	0/6
					2	<0.12	<0.07	1,200	>12,500	

^a Suckling mice from unimmunized dams were foster nursed by dams immunized either orally (3B₁, 3B₂, and 1B₂) or parenterally (4A₁ and 4A₂) with SA-11 virus. Five days after parturition, sera were obtained from two neonates (1 and 2) in each litter by decapitation and from the foster dam by retroorbital capillary plexus puncture. Sera were tested by RIA and PRN. The remaining neonates in each litter were orally challenged with 6×10^6 PFU of SA-11 virus.

^b Expressed as reciprocal of serum dilution.

^c Number of mice with diarrhea/number of mice inoculated with SA-11 virus.

TABLE 3. Passive protection of suckling mice by oral inoculation of milk from SA-11 virus-immunized dams: analysis of rotavirus-specific IgG and IgA activity in vivo^a

Immunization status of milk donor	Milk antibody profile				In vivo protection test		In vivo protective titer ^d	Protective potency ^e
	Isotype analysis (μg/ml) by RIA			PRN ^b	Milk dilution (reciprocal)	Infection rate ^c		
	IgM	IgA	IgG					
Unimmunized	<0.12	<0.07	<0.12	<100	50	7/9	<50	
SA-11 oral								
Unadsorbed	<0.12	42	276	26,700	250 1,250 6,250	0/9 2/8 9/9	2,200	
IgA-adsorbed	<0.12	<0.07	180	20,500	500 2,500 12,500	3/10 8/9 10/10	850	0.021
IgG-adsorbed	<0.12	15.4	<0.12	4,230	100 500 2,500	1/7 4/9 9/11	660	0.002
IgG/A-adsorbed	<0.12	<0.07	<0.12	580	50	7/9	<50	
SA-11 parenteral								
Unadsorbed	<0.12	17.5	3,600	9,260	500 2,500 12,500	1/8 6/7 6/6	1,200	
IgA-adsorbed	<0.12	<0.07	1,400	7,300	100 500 2,500	0/9 8/8 9/9	230	0.64
IgG-adsorbed	<0.12	14	<0.12	<100	100 500	8/8 9/9	<100	

^a Milk was obtained from dams immunized either orally or parenterally with SA-11 virus. Unadsorbed and immunoglobulin-adsorbed milk preparations were tested for rotavirus-specific activity by RIA and PRN. Five-day-old mice nursed by unimmunized dams were orally inoculated with various dilutions of these milk preparations and then orally challenged with 6×10^6 PFU of SA-11 virus.

^b Titers are expressed as reciprocal of milk dilution.

^c Number of mice with diarrhea/number of mice inoculated with SA-11 virus.

^d Reciprocal of dilution of milk at which 50% of suckling mice were protected.

^e Quantity (in micrograms) of immunoglobulin isotype at which 50% of suckling mice were protected.

PRN activities of these preparations were associated with the anti-rotavirus IgG fraction. The IgG-IgA-adsorbed preparations lacking rotavirus-specific antibodies by RIA retained traces of in vitro PRN activity but no in vivo neutralizing activity.

Serum from an unimmunized dam contained no anti-rotavirus antibodies detectable by RIA or PRN. This preparation at a dilution of 1:50 did not protect mice against challenge virus (Table 4).

DISCUSSION

The suckling mouse model has allowed us to demonstrate in vivo protection against rotavirus challenge by rotavirus-specific antibodies in postcolostral milk. We found that protection against SA-11 virus challenge in suckling mice correlated with the level of rotavirus-neutralizing activity in milk measured by PRN assay. Several investigators have shown that neonatal gnotobiotic calves, lambs, and pigs were protected against rotavirus challenge when fed colostrum from orally or parenterally immunized animals (28, 36, 40, 41). However, studies of calves (36) and suckling mice (29) nursed by naturally infected animals have revealed that newborns are not protected against gastroenteritis induced by homologous rotavirus challenge. Although numerous studies have documented the presence of rotavirus-specific antibodies in human colostrum and milk (6, 7, 43, 50), epidemiological investigations of human neonates have revealed no evidence that breastfeeding protects against symptomatic rotavirus infection (17, 18). Indeed, we found that suckling mice were not protected against SA-11 virus challenge when nursed by dams inoculated once orally with SA-11 virus; these dams had rotavirus-specific antibodies

detected by RIA at dilutions similar to those found in naturally rotavirus-seropositive adult mice presumably infected with murine rotavirus (data not shown). Only neonates nursed by dams "hyperimmunized" orally or parenterally with SA-11 virus were protected against SA-11 virus challenge (Table 2). The lack of protection afforded by breastfeeding in animals and humans may relate to the level of rotavirus-neutralizing antibodies evoked in milk under natural as compared with experimental conditions of infection.

The presence of high titers of passively acquired rotavirus-neutralizing antibodies in the serum of suckling mice was not alone capable of protecting neonates against rotavirus-induced gastroenteritis (Table 1). Homologous virus-neutralizing antibodies in the serum have been shown to be highly protective against infection with viruses known to be disseminated by the bloodstream, such as poliovirus (19), measles (2), and rubella (8). However, the protective effect of serum antibodies appears to be less complete for pathogens producing disease in the gastrointestinal tract mucosa (12, 13, 20, 21, 42). Our data are consistent with those of Snodgrass and co-workers (39), who found that protection against rotavirus challenge in lambs did not correlate with the level of serum rotavirus-specific antibodies. Epidemiological studies have found no correlation between serum antibodies directed against rotaviruses and a reduced risk of rotavirus-induced gastroenteritis in human neonates (18) and adults (47). However, Kapikian et al. (25) found that in adult volunteers challenged with human rotavirus, the prechallenge serum rotavirus-specific antibody titers correlated with resistance to diarrhea and a decreased shedding of rotavirus; the relationship between preexisting local neutralizing activ-

TABLE 4. Passive protection of suckling mice by oral inoculation of serum from SA-11 virus-immunized dams: analysis of rotavirus-specific IgG and IgA activity *in vivo*^a

Immunization status of serum donor	Serum antibody profile				In vivo protection test		In vivo protective titer ^d	Protective potency ^e
	Isotype analysis (µg/ml) by RIA			PRN ^b	Milk dilution (reciprocal)	Infection rate ^c		
	IgM	IgA	IgG					
Unimmunized	<0.12	<0.07	<0.12	<100	50	7/9	<50	
SA-11 oral								
Unadsorbed	<0.12	38	7,200	312,500	500 2,500 12,500	0/8 2/9 7/8	5,000	
IgA-adsorbed	<0.12	<0.07	2,400	211,000	500 2,500 12,500	0/9 7/9 9/11	1,500	0.063
IgG-adsorbed	<0.12	12.6	<0.12	2,000	200 1,000	6/9 7/8	<200	
IgG/A-adsorbed	<0.12	<0.07	<0.12	200	<100	9/10	<100	
SA-11 parenteral								
Unadsorbed	<0.12	42	7,200	234,000	2,500 12,500 62,500	0/10 2/8 8/9	23,000	
IgA-adsorbed	<0.12	<0.07	3,000	129,000	2,500 12,500 62,500	0/9 4/9 7/9	17,000	0.018
IgG-adsorbed	<0.12	14	<0.12	459	200 1,000	9/9 7/7	<200	

^a Serum was obtained from dams immunized either orally or parenterally with SA-11 virus. Unadsorbed and immunoglobulin-adsorbed serum preparations were tested for rotavirus-specific activity by RIA and PRN. Five-day-old mice nursed by unimmunized dams were orally inoculated with various dilutions of these serum preparations and then orally challenged with 6×10^6 PFU of SA-11 virus.

^b Titers are expressed as reciprocal of serum dilution.

^c Number of mice with diarrhea/number of mice inoculated with SA-11 virus.

^d Reciprocal of dilution of serum at which 50% of suckling mice were protected.

^e Quantity (in micrograms) of immunoglobulin isotype at which 50% of suckling mice were protected.

ity in intestinal fluid and protection against challenge was less clear-cut. The cross-fostering design in our studies clearly facilitates a definition of the distinction between circulating and gut-associated antibodies in protecting suckling mice against rotavirus challenge. The degree to which our suckling mouse model is predictive of the important determinants of protection against human rotavirus infection awaits further investigation.

We found that suckling mice inoculated orally with SA-11 virus-specific milk antibodies were protected against SA-11 virus challenge; protection against challenge was detected in both the anti-rotavirus IgG and IgA fractions of milk from an orally immunized dam but only in the anti-rotavirus IgG fraction of milk from a parenterally immunized dam. In our studies, anti-rotavirus IgA derived from milk (presumably secretory IgA) was more potent than either milk- or serum-derived IgG in protecting mice against SA-11 virus-induced diarrhea. Previous studies of pigs infected with transmissible gastroenteritis virus (5) and mice infected with cholera (42) have indicated that the presence of gut-associated IgA is correlated more closely with protection against challenge than is intestinal IgG. These findings may be explained by the unique adaptability of secretory IgA to the milieu of intestinal tract secretions; secretory IgA is more resistant to degradation by trypsin, chymotrypsin, and pepsin than is IgG or serum IgA (26, 44).

We found by PRN that the anti-rotavirus IgA and IgG fractions in milk from an orally infected dam each contained rotavirus-neutralizing activity greater than that found in the corresponding isotype fractions of milk from a parenterally immunized dam (Table 3). Goldblum and co-workers (15) have found that lymphocytes sensitized in the gastrointesti-

nal tract are selectively transported to the mammary gland. SA-11 virus probably replicates in the gastrointestinal tract to a greater extent than in the peritoneal cavity. Perhaps viral replication in the gut results in a more efficient stimulation of lymphocytes producing neutralizing antibodies.

We found that the ratios of the concentrations of rotavirus-specific IgG to IgA in milks 200:1 and 7:1 from parenterally and orally immunized dams, were 200:1 and 7:1, respectively. These data are consistent with those of Fuhrman and Cebra (14), who found that mucosal priming with cholera toxin generated a greater percentage of secondary B cells in the lamina propria whose progeny expressed exclusively IgA; intraperitoneal priming resulted in clones expressing the IgG isotype.

Serum from SA-11 virus-immunized dams inoculated orally into suckling mice protected against SA-11 virus challenge; protection resided in the anti-rotavirus IgG fraction. Previous investigators have found that serum containing anti-rotavirus antibodies fed to lambs (41) and pigs (28) protected against homologous rotavirus challenge. Gamma globulin fed to naturally infected human neonates during the first week of life resulted in delayed excretion of rotavirus and amelioration of symptoms of infection as compared with placebo-fed controls (3). The suitability of feeding milk or serum containing rotavirus-specific antibodies to humans for the purpose of either ameliorating disease or decreasing the shedding of infectious virus requires further study.

We have not yet examined the effect of passively transferred rotavirus-specific antibodies on viral shedding, gastrointestinal cellular histopathological changes, or the development of an active immune response in suckling mice orally infected with SA-11 virus. These parameters of infection are

currently being investigated in our laboratory. It appears to be clear, however, that the most effective rotavirus vaccine will be that which induces an efficient production of antibodies active at the intestinal cell surface.

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