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Transfer of Antirotaviral Antibodies from Mothers to Their Infants

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Levels of rotavirus-specific immunoglobulin G (IgG), IgA, IgM, and secretory immunoglobulin in maternal and cord serum, colostrum and milk, and infants' stools were measured by enzyme-linked immunosorbent assay in 92 mothers and their infants. Although antirotaviral IgG, IgA, and secretory immunoglobulin were present in most maternal sera, only IgG crossed the placenta. All samples of colostrum and milk tested contained antirotaviral secretory immunoglobulin and IgA except those of two women in whom IgA deficiency was subsequently described. Specific IgM and IgG were also detected in many colostral samples. Antirotaviral IgA was detected in many colostral samples. Antirotaviral IgA was detected in stools of breast-fed but not bottle-fed neonates. Apparently the human infant receives rotaviral antibodies both transplacentally and via maternal colostrum and milk.

Previous investigations have demonstrated the presence of antibodies directed against a number of enteropathogenic agents in human colostrum and milk, including poliovirus (2, 10) and *Escherichia coli* (7, 11). Most of these lacteal immunoglobulins are recoverable from the feces of breast-fed infants (11, 16). Recently there have been a number of reports of the detection of rotaviral antibodies in human colostrum and milk by various techniques (4, 5, 8, 18). Using an enzyme-linked immunosorbent assay developed previously (6, 14), we have studied the classes of rotavirus-specific immunoglobulins present in maternal colostrum, milk, and serum and followed their transfer to the infant during the first 5 to 8 days of life. At this time, passively acquired immunity in the gut is likely to be of greatest importance to the infant (13).

MATERIALS AND METHODS

Collection of specimens. Samples were accumulated from 92 mothers and their infants in four postnatal wards at the Royal Women's Hospital, Melbourne, from October 1977 to April 1978. All stools passed by each infant were collected into disposable diapers from the first day after birth until they were discharged (5 to 8 days). Samples were initially refrigerated (8 to 48 h), and all feces collected on one day from each baby were pooled and frozen at −70°C. One gram of pooled feces was separated for processing. Those mothers who were breast-feeding their infants (total, 49) expressed samples of 1 to 10 ml of colostrum and milk aseptically each day during the period of stool collection. Specimens were refrigerated immediately on collection and frozen at −70°C within 48 h. Babies designated "bottle-fed" received only artificial diets. Samples of maternal and cord sera taken routinely at delivery for blood grouping were obtained from 79 of the 92 mother-infant pairs and were frozen within 48 h.

Processing of specimens. Approximately 1 g of pooled feces was extracted for electron microscopy as described by Schnagl et al. (17). The supernatant fluid obtained after ultracentrifugation was frozen to await antibody testing. Samples of 1 to 2 ml of thawed colostrum or milk were clarified at 10,000 × *g* for 60 min at 4°C. The supernatant fluid below the lipid phase was frozen for antibody testing.

All specimens were tested under code.

Class-specific micro-enzyme-linked immunosorbent assay. Details of the method have been previously described (6, 14).

Horseradish peroxidase conjugated to anti-human immunoglobulins was used. Goat antisera to human immunoglobulins (immunoglobulins G [IgG], γ-chain specific; IgM, μ-chain specific) were purchased from Hyland Division, Travenol Laboratories, Costa Mesa, Calif.; chromatographically purified goat anti-human IgA (α-chain specific) was purchased from Antibodies Inc., Davis, Calif.; and rabbit anti-human secretory piece was obtained from Dako Immunoglobulins, Copenhagen, Denmark.

The controls included were of the same type as the samples tested (e.g., milk control when testing milks). A conjugate control and three positive controls of known titer were routinely included. Results were acceptable if these positive controls showed no more than twofold variation from the expected titers. The rejection rate was 5%. The highest concentration of each sample was reacted against control antigen (uninfected primary cynomologus monkey kidney [PMK] cells), and reactors were titrated as indicated previously (6).

Simian rotavirus (SA 11) grown in PMK cells and partially purified was applied to wells of polyvinyl microtiter trays. Trays were washed, twofold dilutions of test samples were applied, and the trays were incubated and then rewashed. The appropriate enzyme-

immunoglobulin conjugate was added, and after incubation and washing, the enzyme substrate was applied. The last serum dilution showing a darker color than that of the conjugate control was regarded as the endpoint.

The working dilution of each conjugate was reacted with dilutions of purified human IgG, IgM (Cappel Laboratories, Cochranville, Pa.), and human colostral IgA (gift of H. Watanabe, Queen Elizabeth II Medical Centre, Perth, Western Australia) adsorbed to wells of polyvinyl microtiter trays. At the dilutions used, immunoglobulin adsorbtion was virtually complete (14). Each endpoint represented the minimum amount of the immunoglobulin class (in micrograms per milliliter) detectable by the conjugate.

These endpoints demonstrated the specificity of each conjugate for one immunoglobulin class relative to the other classes and provided factors for the conversion of titers to weight-per-volume units (14).

Total serum immunoglobulin levels. Immunodiffusion was carried out in Tri-Partigen and S-Partigen plates (Behring) as directed by the manufacturer.

Examination of milk and colostrum for rotavirus particles. A 0.1-ml portion of each of 50 randomly selected clarified samples was pooled and centrifuged at 80,000 × *g* for 60 min at 4°C. The pellet was suspended in 2 to 3 drops of distilled water, negatively stained with 1:10 saturated solution of ammonium molybdate, and examined by electron microscopy. No virus particles were seen.

Statistical methods. Linear regression analysis was carried out using the method of least squares. The correlation coefficient, *r*, and the probability of the results arising by chance, *P*, are quoted in the text.

RESULTS

Antibody classes in sera. Rotavirus-specific IgG was detected in all maternal and cord sera tested. Figure 1 shows the relationship between maternal and cord serum-specific IgG levels.

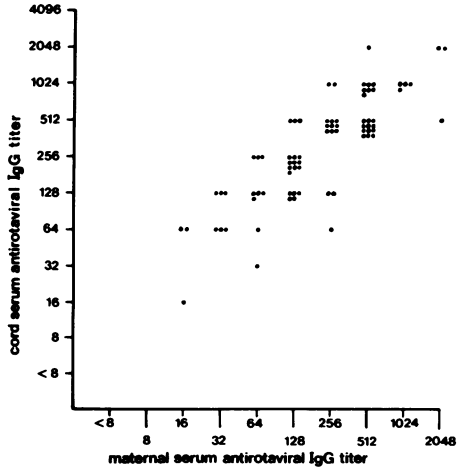

FIG. 1. *Transfer of rotavirus-specifc IgG from mother to infant across the placenta. A titer of 1:8 corresponds to 2.5 μg of antirotaviral IgG per ml.*

These levels show strong positive correlation ($r = 0.84$; $P < 0.001$), with cord sera showing slightly higher levels than maternal sera in most cases. Antirotaviral IgA was also present in all maternal sera tested, with two exceptions which are described below. However, rotavirus-specific IgA was not detected in any cord serum sample tested. The geometric mean titers of antirotaviral IgG and IgA in maternal and cord sera, along with the corresponding estimates of immunoglobulin concentrations, are presented in Table 1. Generally, rotavirus-specific IgA levels were lower than specific IgG levels in maternal sera. Although high levels of these two classes of antirotaviral immunoglobulins appeared to occur together (Fig. 2), their overall correlation was much less striking but still significant ($r = 0.43$; $P < 0.001$). Antirotaviral IgM was not found in any serum sample tested. Rotavirus-specific secretory immunoglobulin (ScIg) was detected in 53% of maternal serum samples, but was not found in cord sera. Figure 3 presents the relationship between specific ScIg and IgA in maternal sera ($r = 0.73$; $P < 0.001$). ScIg levels were

TABLE 1. *Geometric mean levels of rotavirus-specific IgG and IgA in maternal and cord sera*

Antibody	Geometric mean titer	Geometric mean immunoglobulin concn (μg/ml)
Maternal IgG	212	66
Maternal IgA	91	10
Cord IgG	313	97

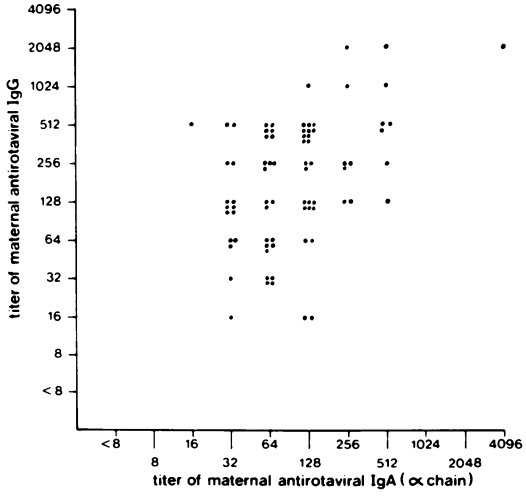

FIG. 2. *Relationship between rotavirus-specific IgA and IgG levels in maternal sera. A titer of 1:8 corresponds to 2.5 μg of IgG per ml and to 0.9 μg of IgA per ml.*

approximately threefold lower than the corresponding total specific IgA level, and ScIg was not detectable for IgA levels below 3.5 μg/ml with the available conjugates. No relationship was evident between maternal serum antirotaviral IgG and ScIg levels. The relationship between maternal age and parity and serum antirotaviral antibody levels is illustrated in Fig. 4. Increasing age and parity was associated with increasing levels of serum rotavirus-specific IgG and IgA. This is a further indication (6) of the likelihood of frequent rotavirus infections in adults.

Antibody classes in colostrum, milk, and fecal supernatants. Figure 5 shows the percentage of colostrum and milk samples containing detectable antirotaviral immunoglobulins. Specific IgA and ScIg were found in all but two samples tested (see below), whereas the frequency of detection of IgM and IgG fell from 59% and 28%, respectively, on day 1 postpartum to less than 10% on day 5. Geometric mean levels of rotavirus-specific immunoglobulin classes for each day postpartum showed a similar decline, reaching a steady low level by day 3 or day 4 (Fig. 6). IgA/ScIg was the most commonly found

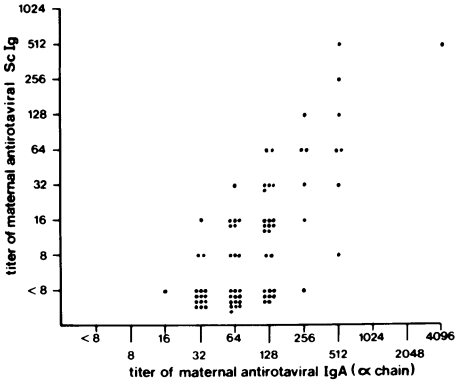

FIG. 3. *Relationship between antirotaviral ScIg and IgA levels in maternal sera. A titer of 1:8 corresponds to 1.1 μg of ScIg per ml and to 0.9 μg of IgA per ml.*

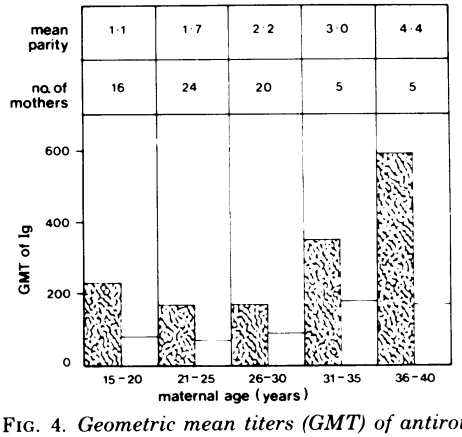

FIG. 4. *Geometric mean titers (GMT) of antirotaviral IgG and IgA in maternal serum with increasing age and parity. Stippled bars, antirotaviral IgG; open bars, antirotaviral IgA.*

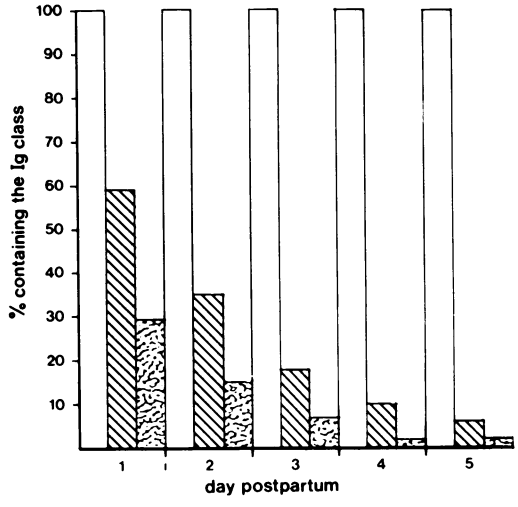

FIG. 5. *Percentage of colostrum and milk samples in which the various antirotaviral immunoglobulin classes were detected. Open bars, IgA, ScIg; hatched bars, IgM; stippled bars, IgG.*

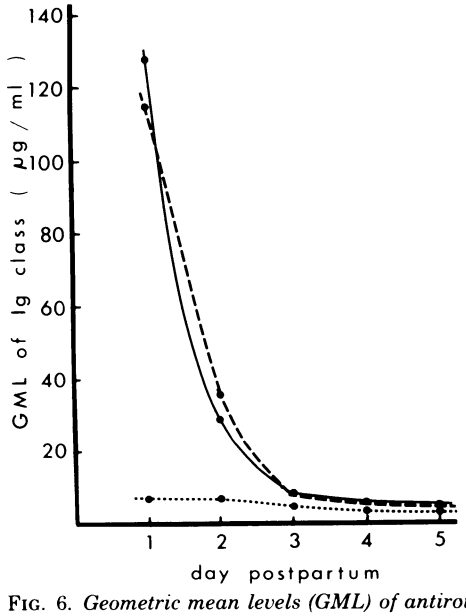

FIG. 6. *Geometric mean levels (GML) of antirotaviral immunoglobulin classes in lacteal secretions. (——) IgA; (— — —) ScIg; (·····) IgG, IgM.*

class of immunoglobulin; it was also present in the greatest quantity on any one day, along with smaller amounts of IgM and IgG. The strong positive correlation ($r = 0.89$; $P < 0.001$) between rotavirus-specific IgA and ScIg levels in lacteal secretions is shown in Fig. 7. It is thus likely that a large part of the ScIg detected was IgA. Levels of antirotaviral IgA and ScIg also showed some correlation with levels of rotavirus-specific IgM in colostrum ($r = 0.62$; 0.59; $P < 0.001$). However, levels of antirotaviral immunoglobulins in sera, when compared with levels of specific immunoglobulins in lacteal secretions, showed poor correlation ($r < 0.4$; $0.10 > P > 0.01$). Two typical examples of transfer of antirotaviral IgA from mothers via colostrum and milk to their infants are shown in Fig. 8. Clearly, specific IgA was detectable in fecal supernatants, even when maternal milk specific IgA concentrations were low. No antirotaviral IgA was detected in the fecal supernatants of any bottle-fed infant.

Detection of selective IgA deficiency. Only two mothers surveyed showed no detectable antirotaviral IgA (or ScIg) in their serum or lacteal secretions. Both mothers exhibited normal specific IgG levels in serum (Table 2), with IgG also predominating in their colostrum and milk. No antirotaviral IgA was detected in the stool supernatants of their infants. Table 3 presents the total serum immunoglobulin levels of these mothers, with the normal ranges for comparison. No IgA was demonstrated on immunodiffusion plates capable of detecting 3 mg of IgA per 100 ml of serum. In addition, mother 2 showed an elevated total IgM level. These findings are consistent with a diagnosis of selective IgA deficiency in both cases (3).

DISCUSSION

Quantitation of the antirotaviral immunoglobulin classes present in maternal and cord sera, maternal colostrum and milk, and neonatal fecal extracts has provided evidence of two routes of passive transfer of rotavirus-specific antibodies from mother to infant. All mothers showed serological evidence of previous rotavirus infection (IgG, IgA, secretory IgA [ScIgA]), and all their infants showed corresponding IgG levels in cord serum. Although the impermeability of the human placenta to IgA (1) precludes the serological transfer of IgA, all mothers also possessed antirotaviral antibodies in their lacteal secretions (mostly ScIgA), which were transferred by

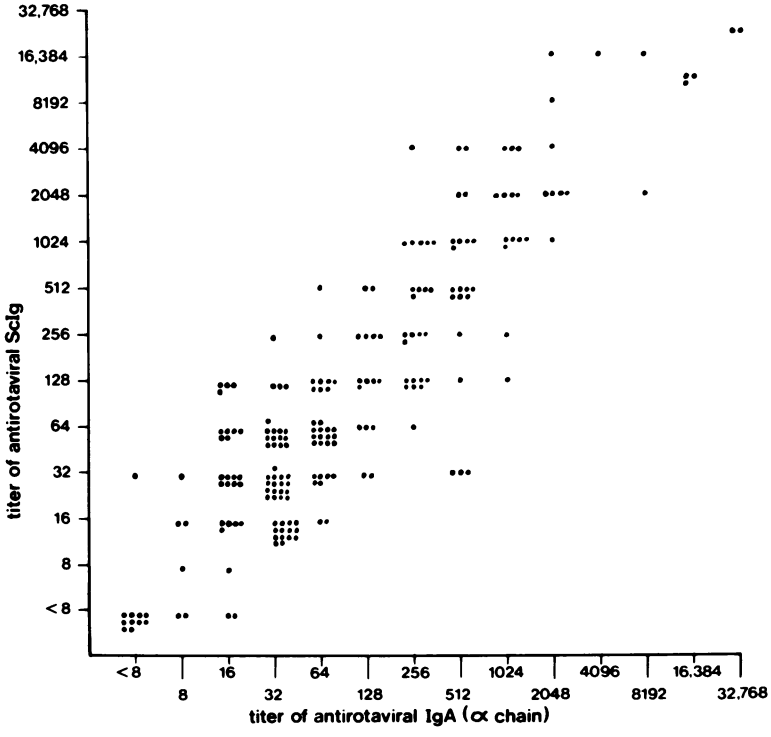

FIG. 7. *Relationship between lacteal rotavirus-specific ScIg and IgA levels. A titer of 1:8 corresponds to 1.1 μg of ScIg per ml and to 0.9 μg of IgA per ml.*

breast feeding and could be detected in the stools of their infants in almost all cases.

Previous studies have shown serum antibodies to have a doubtful protective capacity against rotavirus infections in humans (9). In lambs (19), calves (15), and piglets (12), colostrum is effective in the prevention of rotaviral diarrhea. Although the acquisition of antibodies in the neonatal period by these animals shows variations from the human system, it is likely that antibodies in colostrum and milk can provide passive protection against rotavirus infection in human infants (B. McLean and I. H. Holmes, submitted for publication).

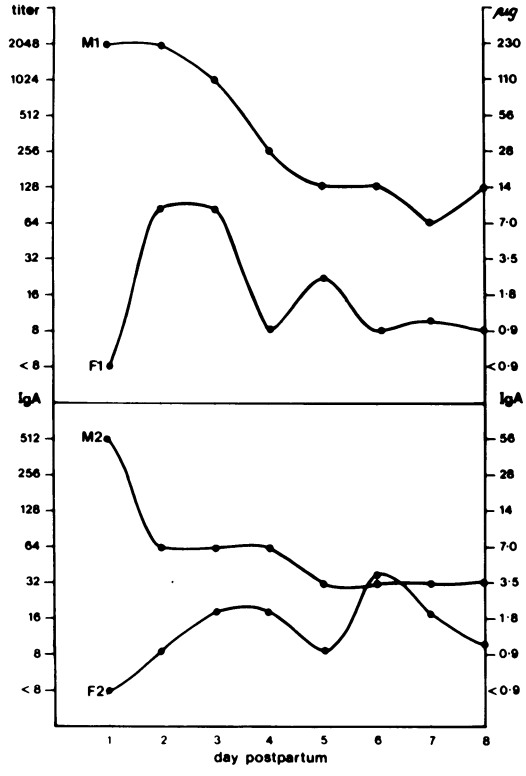

FIG. 8. *Typical patterns of transfer of antirotaviral IgA via colostrum and milk from mother to baby. The μg axis refers to micrograms per milliliter in milk or micrograms per gram in feces. (M) Maternal colostrum and milk; (F) corresponding infant's stools.*

ScIgA has been demonstrated at levels averaging 30 μg/ml in the serum of most normal individuals. Levels in lactating women averaged five times that amount (22). Approximately 2% of the total serum IgA of women in our study was directed against rotavirus, and a proportion of this rotavirus-specific IgA was ScIgA in 53% of sera. Serum antirotaviral ScIgA levels in the remainder of women surveyed are likely to have been below the limits of detection of this enzyme-linked immunosorbent assay (1.1 μg/ml). Information on the origin of serum ScIgA is lacking. It has been suggested that elevated serum levels of ScIgA result from "spill-over" of ScIgA from certain of its synthesis sites, such as the liver in obstructive liver disease (21, 22). Excess serum ScIgA in lactating women is thus likely to originate from the mammary gland.

In colostrum and milk, unlike serum, levels of antirotaviral IgA and ScIg corresponded closely so that titration with either anti-α-chain or anti-

TABLE 3. *Total serum immunoglobulin levels of mothers with selective IgA deficiency*

Serum	Immunoglobulin level (mg/dl)		
	IgA	IgG	IgM
Mother 1	<3	1,591	>400
Mother 2	<3	1,778	83
Normal (20)	100–400	750–1,800	45–150

TABLE 2. *ELISA titers of antirotaviral immunoglobulins present in specimens from mothers exhibiting selective IgA deficiency, and from their infants*

Specimen	Day postpartum	Mother-infant pair 1			Mother-infant pair 2		
		IgA, ScIg	IgG	IgM	IgA, ScIg	IgG	IgM
Colostrum and milk	1	<8	256	<8	<8	256	16
	2	<8	16	<8	<8	32	<8
	3	<8	16	<8	<8	<8	<8
	4	<8	16	<8	<8	<8	<8
	5	<8	32	<8	<8	<8	<8
	6	<8	32	<8	<8	<8	<8
	7	<8	32	<8	<8	<8	<8
Serum							
Maternal		<8	1,024		<8	512	
Cord		<8	1,024		<8	512	

secretory component enzyme conjugates would measure a major part of the lacteal complement of rotaviral antibodies.

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