

Local production of rotavirus specific IgA in breast tissue and transfer to neonates

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SUMMARY Rotavirus specific IgA, secretory component, and IgG were measured by enzyme linked immunosorbent assay in 20 pairs of mothers and babies to estimate antibody transfer from the mother, particularly from breast milk to neonatal faeces. Colostrum contained high titres of specific IgA and secretory component, which decreased gradually. Faeces after breast feeding for three days showed detectable titres of IgA and secretory component, with further increases by seven days. There was a positive correlation between titres of secretory component in breast milk and in faeces. To clarify the mechanism of high anti-rotavirus activity in breast milk, ratios of rotavirus specific IgA in maternal serum samples to breast milk were calculated and compared with those that were herpes simplex virus specific. Significantly higher concentrations were obtained for the herpes simplex virus specific samples, indicating that anti-rotavirus IgA is selectively produced in breast tissue.

During the past decade rotaviruses have been shown to be a major cause of neonatal and infantile gastroenteritis throughout the world. They still constitute a great threat to young children, causing morbidity as well as mortality, particularly in those in developing countries. Breast feeding has been shown to protect neonates and infants from various infections.¹ This protection extends to rotaviral gastroenteritis, at least to some extent, as lower incidences of viral diarrhoea have been found in breast fed infants.² Newborns and infants in general under 6 months of age have been shown to have mild or asymptomatic rotaviral infection.³

Gut associated lymphoid tissue (as the local defence mechanism against this virus) consists of Peyer's patches and other lymphoid tissues in the intestine, including mesenteric lymph nodes. After antigenic stimulation in the gut primed B cells, particularly cells that produce IgA, can migrate from there to various sites of external secretions, such as breast tissue, to produce local antibody there.⁴ This study was undertaken to determine the reason for relative resistance to rotaviral gastroenteritis in the newborn period by estimating the antibody transfer from the mother and also to explore the role of maternal gut associated lymphoid tissue against neonatal rotavirus infection.

Patients and methods

Preparation of antigen. For rotavirus antigen, human rotavirus strain Wa was grown and propagated in MA104 cells, then concentrated by precipitation with polyethylene glycol, and finally purified by sucrose gradient centrifugation.⁵ Protein concentration of the extract was 800 µg/ml as estimated by the Lowry method. For herpes simplex virus antigen, the HF strain of the virus type I was propagated in Vero cells and purified by sucrose gradient centrifugation. Protein concentration was estimated and diluted with carbonate buffer (pH 9.5) to obtain a final concentration of 2.5 µg/ml during use.

Samples. Breast milk, serum, and faecal samples were collected from 20 pairs of mothers and neonates. The mothers and babies were enrolled in the study at random according to delivery serials in the hospital, but very low birthweight babies and their mothers were excluded.

Samples of breast milk were collected three times: before beginning lactation and three days and seven days after lactation. These samples were first centrifuged at 2000 g for 10 minutes, and then upper lipid and lower cellular portions were discarded and recentrifuged at 4500 g for 30 minutes so that the middle portion could be collected.⁶ Each mother had a serum sample taken before lactation, and each

neonate had serum samples taken before breast feeding began and seven days after breast feeding had started. Three faecal samples were taken from each neonate, one before breast feeding and one three days and one seven days after breast feeding had begun. The faecal samples were mixed with phosphate buffered saline, homogenised, and centrifuged at 10 000 revolutions per minute for 30 minutes at 4°C to collect the supernatant fluid. Latex agglutination tests were performed on the faecal samples by using Rotalex kit (Orion Diagnostica, Denmark) to exclude asymptomatic rotaviral infection during the study. All the faecal samples yielded negative results, indicating the absence of rotavirus.

Enzyme linked immunosorbent assay. Immunolon microelisa plates (Dynatech, Virginia, United States) were used for measuring antibodies. IgA, secretory component, and IgG titres against rotavirus and herpes simplex virus were determined using the procedure we have reported previously.⁷ The rotavirus antigen was thawed and treated with sodium thiocyanate to increase its ability to attach to the solid phase.⁸ To exclude the possibility of non-specific reactions, antigen free plates were used as controls every time. Peroxidase conjugated rabbit immunoglobulins to human secretory component (Dako, Copenhagen, Denmark) were used at dilutions of 1:200. Peroxidase conjugated, affinity purified, heavy chain specific goat antibodies to human IgA and IgG (Tago, California) were used at dilutions of 1:500 and 1:2000, respectively.

Results of the assay were expressed with reference to standard curves. The standard curves were prepared separately for secretory component, IgA, and IgG from several estimations of specific antibodies to twofold serial dilutions of different serum and colostrum samples. Samples that had higher titres of antibodies were chosen as standards. The antibody titres of samples were decided in terms of the dilutions (2^n) of standard samples, the optical density of which corresponded to that of the desired sample.

Results

Transfer of antibody from mother to neonate. Figure 1 shows the transplacental transfer and kinetics of rotavirus specific antibodies in the serum of the neonates. All the maternal serum samples contained high titres of IgA (2^{8-64}) as well as of IgG (2^{6-2}). The IgG transfer (2^{6-25}) was almost in the same titre, whereas IgA transfer (2^{1-57}) was low. Secretory component was not detectable in either maternal or neonatal serum samples.

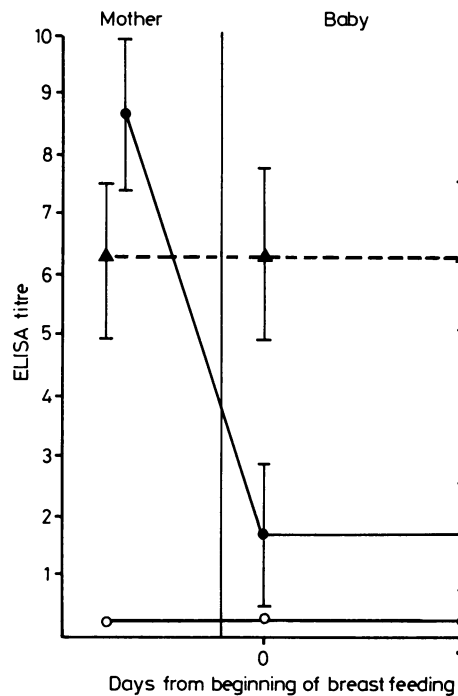


Fig. 1 Transplacental transfer and kinetics in the baby's serum of rotavirus specific antibodies. Enzyme linked immunosorbent assay titres of antibodies IgA (●—●), IgG (250×2^n) (▲—▲), and secretory component (100×2^n) (○—○) expressed in comparison with the standard curves. Vertical lines indicate standard deviations.

The titres of rotavirus specific antibody in breast milk are shown in Figure 2(a). Titres of IgA (2^{9-15}) and secretory component (2^{8-75}) were present in high concentrations in all colostrum samples, but the IgG titre (2^{1-67}) was low. All the antibody titres showed a rapid decrease in the samples taken three days after lactation, with a gradual decrease afterwards. On the other hand, antibody titres in neonatal faeces showed reverse trends. While we could not detect any antibody in the meconium (Fig. 2(b)), titres of rotavirus specific IgA (2^{4-95}) and secretory component (2^{3-25}) were detected in significant amounts in the samples taken three days after breast feeding, which showed a further increase (2^{6-25} and 2^{4-35} , respectively) by day 7. IgG remained undetectable even after seven days of breast feeding. A definite correlation between rotavirus specific secretory component in breast milk and neonatal faeces was found ($r=0.763$) (Fig. 3).

Ratios of serum IgA to breast milk IgA against rotavirus and herpes simplex virus. To clarify the

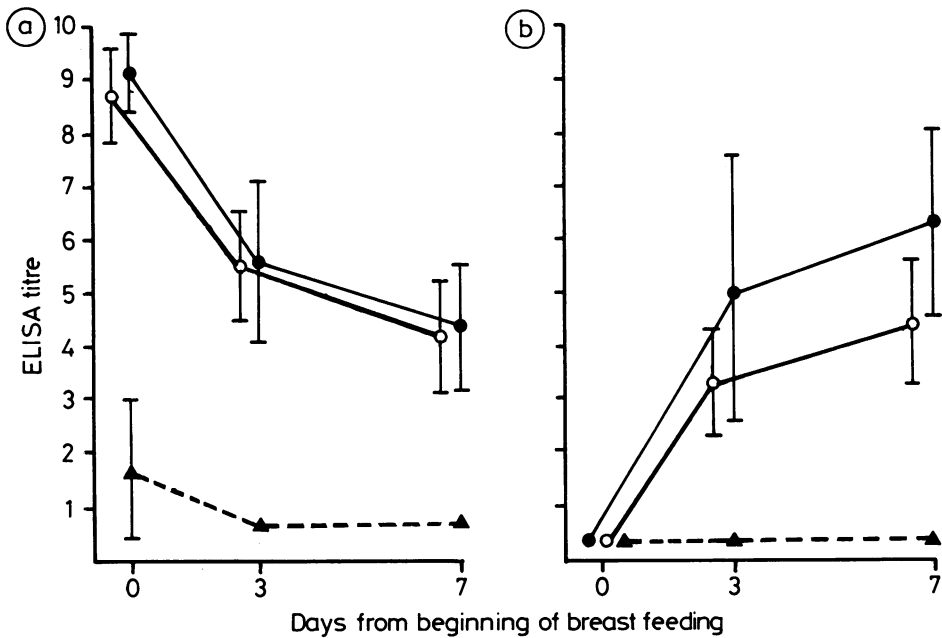


Fig. 2 Rotavirus specific antibodies IgA (●—●), IgG (▲---▲), and secretory component (○—○) in breast milk and baby's faeces. (a) Enzyme linked immunosorbent assay titres of antibodies IgA (1000×2^n), IgG (40×2^n), and secretory component (200×2^n) in breast milk; (b) titres of antibodies IgA, IgG, and secretory component (100×2^n) in baby's faeces. Titres expressed in comparison with the standard curves. Vertical lines indicate standard deviations.

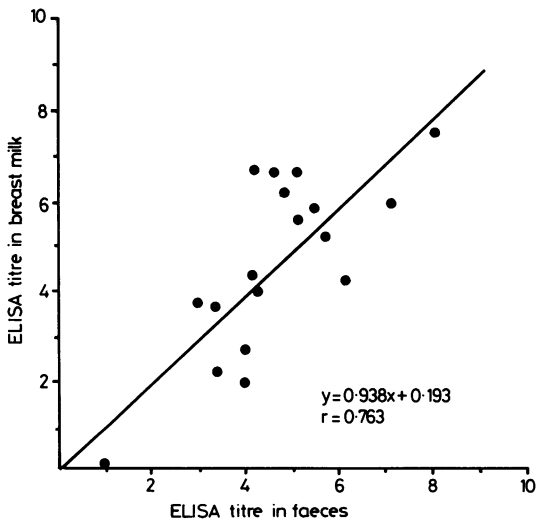


Fig. 3 Correlation between rotavirus specific secretory component in breast milk and baby's faeces. Enzyme linked immunosorbent assay titres in breast milk (200×2^n) and faeces (100×2^n) expressed in comparison with the standard curves.

reason for high titres of rotavirus specific IgA in breast milk, we compared the ratios of serum to milk titres (Table). For comparison, the ratios were estimated in the same manner for herpes simplex virus, which cannot replicate in the gut. There was a significant difference (Student's *t* test for paired groups) between the ratios of rotavirus specific IgA in maternal serum to breast milk and the herpes simplex virus specific ratios, the latter showing a higher value ($p < 0.05$). The mean (SD) serum: breast milk ratio was 2.8 (2.1) for rotavirus and 4.5

Table Mean (SD) ratios of serum to breast milk IgA titres against rotavirus and herpes simplex virus type 1

Virus	ELISA titres of antiviral IgA (2^n)		Ratio of ELISA titres (serum: breast milk)
	Serum†	Breast milk‡	
Rotavirus	8.7 (1.9)	5.9 (1.9)	2.8 (2.1)
Herpes simplex virus type 1	7.0 (0.7)	2.5 (1.6)	4.5 (1.7)

ELISA=Enzyme linked immunosorbent assay.

* $p < 0.05$.

†Maternal serum at 250 times' dilution.

‡At 1000 times' dilution.

(1·7) for herpes simplex virus. Thus rotavirus specific IgA in breast milk was significantly higher, indicating that the antibody is locally synthesised in breast tissue.

Discussion

Our results show that transplacental transfer of rotavirus specific IgA and IgG corresponds to the general antibody transfer as shown by other studies.⁹ Iyenger *et al* have shown that there is preferential transfer of IgG in comparison with IgA and IgM.¹⁰ We have observed that rotavirus specific IgG transfer is also preferential.

As far as the fate of rotavirus specific antibodies in breast milk fed to neonates is concerned, most of the IgA and secretory component were excreted in the faeces of the neonates. Before or during the process of excretion, these antibodies probably form a covering over the microvilli of the upper intestinal tract, giving passive protection, as shown by Nagura *et al* in the gastrointestinal tract of rats.¹¹ Rotavirus specific IgA and secretory component detected in neonatal faeces after breast feeding are thought to be derived entirely from milk as no rotaviral outbreak occurred during the study and asymptomatic infections were also ruled out by negative latex agglutination tests in the faecal samples. Although the breast milk samples showed more diminished titres of antibodies than colostrum samples, faecal samples showed increased titres of IgA and secretory component. This is possibly due to the fact that the amount of milk taken by the neonate increased day by day, thus increasing the total antibody intake.

From our study, we have seen that all the mothers had high titres of rotavirus specific antibodies in breast milk and serum. This indicates that all of them had previous rotavirus infection, which corresponds to other studies, indicating the ubiquitous nature of the rotavirus and rotavirus specific antibodies.¹² Titres of rotavirus specific IgA and secretory component in the colostrum samples were much higher than in the serum samples. The IgA in breast milk is believed to be mainly secretory IgA in nature. This points us to the assumption that anti-rotavirus antibody is produced locally in the breast tissue by stimulation of IgA producing cells that have migrated from the gut associated lymphoid tissue, because direct infection of the mammary tissue with rotavirus does not occur. Antigens in the gut lumen are thought to stimulate the precommitted (IgA producing) lymphoid cells there, and some of these cells migrate to external secretory tissues—for example, breast tissue—for production of

antibody.⁴ Comparatively lower titres of breast milk antibody against herpes simplex virus, which cannot multiply in the gut, also support our hypothesis. Our previous study showed that there is anamnestic response for IgA production in the gut after reinfection with rotavirus.⁷ By some mechanisms, the reinfection in the gut may also cause production of specific secretory IgA in breast tissue.

Our study has the limitation that we have observed antibody titres for only seven days after lactation and after the start of breast feeding. But it suggests that if the initial rotavirus specific antibodies are high then the neonate may get passive protection in the intestinal tract for prolonged periods of breast feeding. Local antibodies to rotaviruses in the gut rather than systemic antibody are thought to be of primary importance in protection from infection. Animal studies have indicated that rotavirus infection occurs when the viral antigen titre exceeds the colostral neutralising antibody titre.¹³ The role of maternal breast milk IgA, in supplying local IgA to the gut in the early neonatal period, is immense and hence the role of maternal gut associated lymphoid tissue is indirectly also important.

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Fifty years ago

Plasma phosphatase in the diagnosis of rickets

Noah Morris, Mary M Stevenson, Olive D Peden, and Jean M D Small (Glasgow)—*Arch Dis Child* 1937;**12**:45-58

Plasma phosphatase was measured in 506 children in the first two years of life, all of whom had manifest rickets or might have been deficient in vitamin D. Raised values were found in 84% of cases with clinical or x ray evidence of rickets, and a high plasma phosphatase value was more often associated with rickets than a reduced serum phosphorus concentration. In general the phosphatase activity ran parallel with the degree of rickets, but the correlation was not a close one. While healing of the rickets was associated with a fall in the phosphatase activity, in no case did this precede radiological evidence of healing. In fact, the initiation of antirachitic therapy was occasionally followed by a transient rise in the plasma phosphatase. The authors postulated that the rare occurrence of a normal phosphatase activity with signs of active rickets might be explained on the assumption that growth had temporarily stopped, while the presence of a high plasma phosphatase activity in the absence of evidence of rickets could be attributed to an insufficient supply of calcium to meet the demands of active bone cells. Rise in the plasma phosphatase activity was considered to be an earlier manifestation of rickets than any provided by clinical or radiological examination and also a more sensitive test than the fall in serum phosphorus or the calcium-phosphorus product.

Comment. This was one of the earliest reports on the value of the plasma phosphatase activity in the diagnosis of early rickets. The conclusion that the alkaline phosphatase activity rises before there are radiological changes may be erroneous, and the earliest sign of rickets is probably loss of radiological definition of the metaphyseal ends of long bones such as the radius and ulna. Interpretation of minor x ray changes is open to the criticism, however, that it is highly subjective. The much more recent ability to measure the plasma 25-hydroxycholecalciferol concentration probably supplies us with a more reliable index of the vitamin D state of a community.

(Noah Morris was biochemist to the Royal Hospital for Sick Children in Glasgow. He was responsible for introducing a series of young graduates to the excitements of research in paediatrics; two of these later occupied chairs in paediatrics and one a chair in pathology. Shortly after publication of this paper Morris was appointed to the Chair of Materia Medica in Glasgow and he devoted the rest of his too short life to clinical practice and research in adult medicine. His book, 'Acidosis and alkalosis', written in conjunction with Professor Stanley Graham, was a minor classic in the 1930s when few British paediatricians appreciated the value of biochemistry to the clinician.)

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