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Breast feeding increases concentrations of IgA in infants' urine

A PRENTICE

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SUMMARY To investigate the influence of breast feeding on mucosal immunity the concentrations and daily outputs of IgA and lactoferrin in urine were measured in 10 breast fed and 12 infants fed on formula milk at 6 and 12 weeks of age. The concentrations and outputs of secretory IgA in urine were significantly higher in the breast fed group by a factor of three. The secretion of IgA in urine by the breast fed infants was characteristic of the baby and was not related to the intake of IgA from breast milk. Lactoferrin concentrations were similar in the two groups at both ages. In addition to secretory IgA, two thirds of all samples contained proteins with α chain but no secretory component antigenic determinants. Breast feeding seems to increase the local production of secretory IgA into the urinary tract during early childhood, thus providing enhanced protection from infection.

Epidemiological studies have shown that breast feeding confers a measure of protection against infection on an infant. The mechanisms entailed, however, are still not fully understood. There is some evidence that breast feeding enhances the mucosal defence system,[1 2] although the data are conflicting.[3 4] The purpose of this study was to quantitate two immune proteins IgA and lactoferrin in the urine of healthy infants fed exclusively on breast milk, or on formula milk with a cow's milk base, to assess mucosal immunity at a site remote from the gastrointestinal tract.

Patients and methods

Ten breast fed and 12 formula fed infants in the Cambridge area were studied. They were all born at term with a mean birth weight of 3400 g, and were all healthy with no history of urinary tract infections. Total collections of urine and faeces were made over seven day periods when they were 6 and 12 weeks old. The collections were made in the subject's home using preweighed disposable nappies which were frozen immediately. The study, which had the approval of the ethical committees of the Dunn Nutrition Unit and the Cambridge Area Health Authority, formed part of an investigation into the use of breast milk immune protein. Milk intakes were measured using the deuterium oxide dilution method, and samples of breast milk and formula milk were obtained daily for analysis. Nappies were also collected from five each of the breast fed and formula fed subjects at 26 weeks of age, once they had been introduced to solid foods. Full details of the subjects and methods, together with the results of the faecal and milk analyses, have been published.[5]

Unsoiled nappies were gently squeezed and the urine collected. Any nappy with traces of faecal material was regarded as soiled and rejected from the urine analysis. The urine specimens were pooled in proportion to the weight of urine contained in each nappy.

Samples were analysed for human total IgA, secretory IgA, and lactoferrin, using enzyme linked immunoabsorbent assays.[5] Standards used were purified human secretory IgA (Cappell) and human lactoferrin (Calbiochem-Behring). The total IgA assay detects any molecules containing α chain determinants—for example, secretory IgA, serum IgA, IgA fragments—while the secretory IgA assay is specific for molecules with determinants for both α chains and secretory component (secretory IgA). The two IgA assays give identical results when secretory IgA is the only IgA species present.[5] Other species which contain α chains but no secretory component, such as serum IgA and certain IgA degradation products, are detected only by the total IgA assay. Fragments of IgA that do not contain α chain determinants are not detected by

these assay systems. No degradation of secretory IgA or lactoferrin has been shown during the analytical procedures.[5] The thresholds of detection are 30 μg/l for both the IgA assays and 2 μg/l for lactoferrin. Concentration of urine samples before analysis was unnecessary due to the high sensitivity of the assays.

Immune protein concentrations in urine were transformed to logarithms because of the skewed distribution of the data. Concentrations below the detection limit were entered into calculations at the threshold value. Calculation of urinary outputs was based on the weight of urine collected, on the assumption that 1 ml of urine weighed 1 g. The weight of urine present in each soiled nappy was estimated as the average weight of urine in unsoiled nappies over the collection period. Mean (+1 geometric standard error) values are given. Statistical methods used were Student's *t* test (for independent and dependent values as appropriate), linear regression, and analysis of variance.

Results

The Table shows the concentrations of secretory IgA in urine which were significantly higher in the breast fed infants than in the formula fed group. The magnitude of the difference was threefold at both 6 and 12 weeks of age. The mean values (μg/l) were: at 6 weeks, breast fed=148, formula fed=44, $p<0.01$; at 12 weeks, breast fed=237, formula fed=71, $p<0.001$. Secretory IgA concentrations rose significantly between the time points in the formula fed group ($p<0.05$), but not in the breast fed infants. At 6 weeks, two breast fed and seven formula fed infants had IgA concentrations below the detection limit. By 12 weeks all subjects had measurable IgA.

Measurements obtained using the total IgA assay exceeded secretory IgA values in 66% of samples. This showed the presence, in addition to secretory IgA, of molecular species containing α chain but no secretory component determinants. In several samples the concentration of total IgA (using secretory IgA as standard) was more than double that of secretory IgA. The nature of the molecular species entailed has yet to be elucidated. Total IgA concentrations in urine were significantly higher in those infants breast fed than in those formula fed ($p<0.01$). This difference was due to the increased secretory IgA concentration, as the amounts of non-secretory IgA species were similar in the two groups. The ratio of total IgA to secretory IgA in samples of individual children rose between 6 and 12 weeks, showing an increased abundance of non-secretory IgA species; in the breast fed babies at 6 weeks it was 1·1 (0·1), and at 12 weeks 1·4 (0·1) $p<0.02$; in the formula fed babies at 6 weeks it was 1·2 (0·1) and at 12 weeks it was 1·6 (0·2), $p<0.05$. Consequently, the total IgA concentration also increased between the two ages ($p<0.05$).

Lactoferrin concentrations in urine were not significantly different between the breast fed and formula fed infants and showed no change between the two ages. A wide range of concentrations was observed, the mean value recorded being 22 μg/l (12 μg/day). At 6 weeks, one breast fed and three formula fed infants had concentrations of lactoferrin

Table *Immune proteins in urine of infants either breast fed or formula fed*

	Breast fed at		*Formula fed at*	
	6 weeks (n=10)	*12 weeks (n=12)*	*6 weeks (n=10)*	*12 weeks (n=12)*
Secretory IgA:				
Concentration μg/l, mean (geometric SE)	148 (66)†	237 (69)‡	44 (7)	71 (15)¶
range	(30–760)	(80–790)	(30–110)	(30–180)
Output μg/day, mean (geometric SE)	70 (22)*	126 (36)†	22 (4)	39 (8)¶
range	(15–440)	(42–417)	(15–77)	(15–104)
Total IgA:				
Concentration μg/l, mean (geometric SE)	159 (76)†	322 (102)†	49 (9)	112 (35)¶
range	(30–1070)	(105–1390)	(30–129)	(33–609)
Output μg/day, mean (geometric SE)	84 (18)†	172 (56)¶	25 (5)	63 (18)§
range	(15–625)	(56–811)	(15–94)	(21–371)
Lactoferrin:				
Concentration μg/l, mean (geometric SE)	30 (22)	37 (20)	12 (7)	21 (13)
range	(2–542)	(6–266)	(2–130)	(2–849)
Output μg/day, mean (geometric SE)	18 (12)	19 (10)	6 (3)	12 (32)
range	(1–258)	(3–132)	(1–89)	(1–446)
Urine volume:				
L/day, mean (geometric SE)	0·53 (0·02)	0·53 (0·03)	0·55 (0·04)	0·58 (0·04)
range	(0·45–0·61)	(0·42–0·70)	(0·37–0·83)	(0·38–0·81)

Significant differences for breast *v* formula fed infants at each age=*$p<0.02$; †$p<0.01$; ‡$p<0.001$.
Significant differences for breast *v* 12 week values in each group=¶$p<0.05$; §$p<0.01$.

below the threshold of measurement. By 12 weeks all breast fed and 11 formula fed infants had detectable amounts.

The concentrations of secretory IgA and lactoferrin in urine samples were positively correlated within each group of children (breast fed $p<0{\cdot}01$, formula fed $p<0{\cdot}05$). No associations could be found in the breast fed infants between the concentrations of these urinary proteins and daily intakes of secretory IgA and lactoferrin from breast milk at either age.

Estimated daily volumes of urine were similar in breast fed and formula fed children at both ages, with an average of 0·55 l/day. No association was found between urine volume and concentrations of immune protein in either group of infants. Consequently, the variations in daily output of urinary IgA and lactoferrin paralleled the concentrations.

The breast fed infants had characteristic concentrations of IgA and lactoferrin in urine at the two ages as judged by analysis of variance ($p<0{\cdot}01$ for both proteins). No correlations existed between the total:secretory IgA ratio nor the IgA:lactoferrin ratio in urine and in faeces of any child. These findings showed that the accidental contamination of urine with faecal material, which contains comparatively high amounts of these two proteins, was minimal.[5]

Between 12 and 26 weeks of age the concentration and daily output of secretory IgA increased in the small group of formula fed subjects on whom repeat studies were made ($p<0{\cdot}05$). reaching mean (SEM) values of 134 (85) µg/l and 74 (47) µg/day, respectively, whereas the concentrations noted for the five breast fed infants were unchanged. The ranking of individual breast fed infants according to the urinary secretory IgA concentrations was maintained until 26 weeks ($p<0{\cdot}02$). Significant differences in secretory IgA concentrations could not be detected between the breast fed and formula fed infants at 26 weeks. Urinary volumes and lactoferrin concentrations remained unchanged and were similar in the two groups.

Discussion

Secretory IgA and lactoferrin are antimicrobial proteins that form an important part of the mucosal defence system and are present in all human external secretions.[6] The results of this study show that breast feeding increases the concentration and daily production of secretory IgA in urine during the first three months of life.

The daily intake of IgA from breast milk is about 1 g/day.[5] The increased concentrations of IgA in urine may represent the transfer of small quantities of maternal antibody from the infant's gut via the blood. This seems unlikely, however, because concentrations of lactoferrin in urine were not increased in the breast fed group despite the high concentrations of lactoferrin in breast milk. In addition, IgA concentrations in urine were not related to daily intake, and secretory IgA is a very large protein (molecular weight 375 000) that is unlikely to be filtered through the kidneys. A more likely explanation is that there are factors in breast milk which promote the local production of secretory IgA, possibly by the stimulation of IgA producing lymphocytes in the child's gastrointestinal tract which migrate to other mucosal sites. A similar mechanism has been proposed for the appearance in breast milk of IgA antibodies specific for antigens in the mother's gut.[7] In addition, breast milk has been shown to contain a factor that stimulates the differentiation of cord blood lymphocytes into IgA producing cells in vitro.[8]

Raised IgA concentrations in the saliva and nasopharyngeal secretions of breast fed infants have been noted during the first days of life,[1 2] although other authors have failed to detect them.[3] These studies have shown that the differences either become negligible[2] or reverse[4] as the child becomes older. The reason may be that local antibody production in formula fed infants is stimulated by exposure to ingested material. The study presented here showed long term differences between healthy breast fed and formula fed infants in the concentrations of secretory IgA measured in a fluid distant from the gastrointestinal tract. The observations that the concentration of secretory IgA remained consistently raised in the urine of individual breast fed infants between 6 and 12 weeks of age at a time when the concentration was increasing in formula fed infants, and that by 26 weeks of age IgA concentrations in the formula fed group had approached those in the breast fed group, suggest that breast feeding promotes the maturation of local antibody production in the first weeks of life.

The mean output of lactoferrin recorded in the present study for healthy infants born at term was 12 µg/day. No differences were detected between the breast fed and formula fed groups. A recent study has shown that very low birthweight babies fed on a formula based on human milk excreted grossly increased amounts of lactoferrin compared with formula fed babies (1140 µg/day *v* 15 µg/day).[9] It seems likely that these very high values were a reflection of the prematurity of the subjects.

The production of IgA in urine is low at birth and increases with age.[10] The daily outputs of IgA from urine recorded in the Cambridge infants were within normal limits reported for children[10] but were about

one tenth of adult values.[11] Several studies have shown that low concentrations of IgA in secretions from the urinary tract,[12 13] vagina,[14 15] nose,[16] and breast[17] are associated with an increased susceptibility to mucosal infections. It would seem likely, therefore, that the threefold increase of IgA in the urine of breast fed infants at 6 and 12 weeks of age enhances mucosal immunity during the vulnerable first months of life.

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References

[1] Roberts SA, Freed DLJ. Neonatal IgA secretion enhanced by breast feeding. *Lancet* 1977;ii:1131.
[2] Taylor CE, Toms GL. Immunoglobulin concentrations in nasopharyngeal secretions. *Arch Dis Child* 1984;**59:**48–53.
[3] Gross SJ, Buckley RH. IgA in saliva of breast-fed and bottle-fed infants. *Lancet* 1980;ii:543.
[4] Østergaard PAA. Serum and saliva Ig-levels in infants of non-atopic mothers fed breast milk or cow's milk-based formulas. *Acta Paediatr Scand* 1985;**74:**555–9.
[5] Prentice A, Ewing G, Roberts SB, Lucas A, MacCarthy A, Jarjou LMA, Whitehead RG. The nutritional role of breast-milk IgA and lactoferrin. *Acta Paediatr Scand* (in press).
[6] Hanson LA, Brandtzaeg P. The mucosal defence system. In: Stiehm ER, Fulginit VA, ed. *Immunologic disorders in infants and children*. 2nd ed. Philadelphia: WB Saunders, 1980:137–64.
[7] Hanson LA, Ahlstedt S, Carlsson B, *et al*. New knowledge in human milk immunoglobulin. *Acta Paediatr Scand* 1978;**67:** 577–82.
[8] Pittard WB, Bill K. Differentiation of cord blood lymphocytes into IgA-producing cells in response to breast-milk stimulatory factor. *Clin Immunol Immunopathol* 1979;**13:**430–4.
[9] Goldblum RM, Garza C, Schanler RJ, Goldman AS. Immunologic outcomes of feeding human milk to very low birth weight infants. In: Schaub J, ed. *Composition and physiological properties of human milk*. Amsterdam: Elsevier, 1986:323–8.
[10] Uehling DT, Steihm ER. Elevated urinary secretory-IgA in children with urinary tract infection. *Pediatrics* 1971;**47:**40–6.
[11] Bienenstock J, Tomasi TB. Secretory gamma-A in normal urine. *J Clin Invest* 1968;**47:**1162–71.
[12] Fleidner M, Mehls O, Rauterberg EW, Ritz E. Urinary sIgA in children with urinary tract infection. *J Pediatr* 1986;**109:**416–21.
[13] Riedasch G, Heck P, Rauterberg E, Ritz E. Does low urinary SIgA predispose to urinary tract infection? *Kidney Int* 1983;**23:**759–63.
[14] Stamey TA, Wehner N, Mihara G, Condy M. The immunological basis of recurrent bacteriuria. Role of cervicovaginal antibody in enterobacterial colonization of the introital mucosa. *Medicine* 1978;**57:**47–56.
[15] Tuttle JP, Sarvas H, Koistinen J. The role of vaginal immunoglobulin A in girls with recurrent urinary tract infections. *J Urol* 1978;**120:**742–4.
[16] Rossen RD, Butler WT, Waldman RH, *et al*. The proteins of nasal secretions. II. A longitudinal study of IgA and neutralising antibody levels in nasal washings from men infected with influenza virus. *JAMA* 1970;**211:**1157–61.
[17] Prentice A, Prentice AM, Lamb WH. Mastitis in rural Gambian mothers and the protection of the breast by milk antimicrobial factors. *Trans R Soc Trop Med Hyg* 1985;**79:**90–5.

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