

Mucosal IgA, mucosal cow's milk antibodies, serum cow's milk antibodies and gastrointestinal permeability in infants

Kuitunen M, Savilahti E. Mucosal IgA, mucosal cow's milk antibodies, serum cow's milk antibodies and gastrointestinal permeability in infants. *Pediatr Allergy Immunol* 1995; 6: 30–35. © Munksgaard 1995.

We have studied development of the levels of IgA cow's milk (CM) antibodies in the saliva, faeces and serum of 20 term and 20 preterm infants from birth to 8 months. All infants already had IgA in their saliva during the first week of life. The levels peaked at the age of one month, thereafter decreasing in both groups; from the age of three months levels remained stable. Term infants had higher levels than preterm infants, but no differences were found between breastfed and CM-fed infants. Breast-fed infants had higher levels of IgA in their faeces than did CM-fed infants; the IgA levels were similar in breast-fed term and preterm infants, being highest at birth, and decreasing thereafter. We also showed rising titers of serum IgA CM antibodies, with higher levels in infants regularly exposed to CM than in breast-fed infants. We sought associations between the magnitude of intestinal permeability to human α -lactalbumin (ALA) measured at the ages of 4–7 days and one month and the levels of IgG antibodies to CM, but no such relation was found.

M. Kuitunen, E. Savilahti

Children's Hospital, University of Helsinki, Finland

Mikael Kuitunen, Children's Hospital, University of Helsinki, Stenbäckinkatu 11, SF-00290 Helsinki, Finland

Key words: human α -lactalbumin; macromolecular absorption; milk; cow's milk antibody

Received April 19, 1994

Accepted September 23, 1994

The mucosal immune system is vital for humans in coping with infectious agents and soluble antigens. Exposure to cow's milk (CM) in the early months of life, the timing and length of the weaning period, and exposure before weaning through the mother's milk or as repeated small doses of CM, all may affect the development of mucosal immune responses and may relate to the appearance of atopy. The normal effect of feeding protein antigens during early infancy is the induction of specific nonreactivity to the same antigen, termed oral tolerance (1, 2). This involves local activation of specific secretory IgA and suppression of systemic immune responses, mainly via T-suppressor cells (3, 4). In early infancy, however, the permeability of the gut to protein antigens is increased, which leads to the formation of circulating antibodies and possibly to infants' sensitization (5).

The aim of this study was to characterize the development of the mucosal immune system by measuring the levels of total IgA and IgA CM antibodies in saliva, faeces and serum during the infants' first 8 months and in their mothers' milk during the same period. We also investigated the effect of IgA in breast milk (BM) on the levels of IgA in infants'

saliva and faeces and the effect of gut permeability on serum CM antibody-levels.

Material and methods

Subjects and sampling – With the informed, written consent of the mothers, 20 term and 23 preterm infants (median gestational age 34 weeks, range 32.6–36.6) were recruited into the study. All infants were healthy and free from perinatal infections, and enteral feeding was started on the first postnatal day. The infants were examined by MK during the first week of life (mean age 4 days, range 2–5, for term; 7 days, range 2–19, for preterm infants), at 1, 2, 3, 5, and 8 months of age, immediately before the planned weaning, and one and two weeks after it. Four of the preterm infants were withdrawn after the initial visit, because of inconvenience to the family.

At each visit before weaning, the mothers were advised to adhere strictly to a CM-free diet. Their compliance with this diet was tested by means of a questionnaire on which the mother noted every new food started at home. The mother was specifically asked at each visit about possible inadvertent CM-exposure.

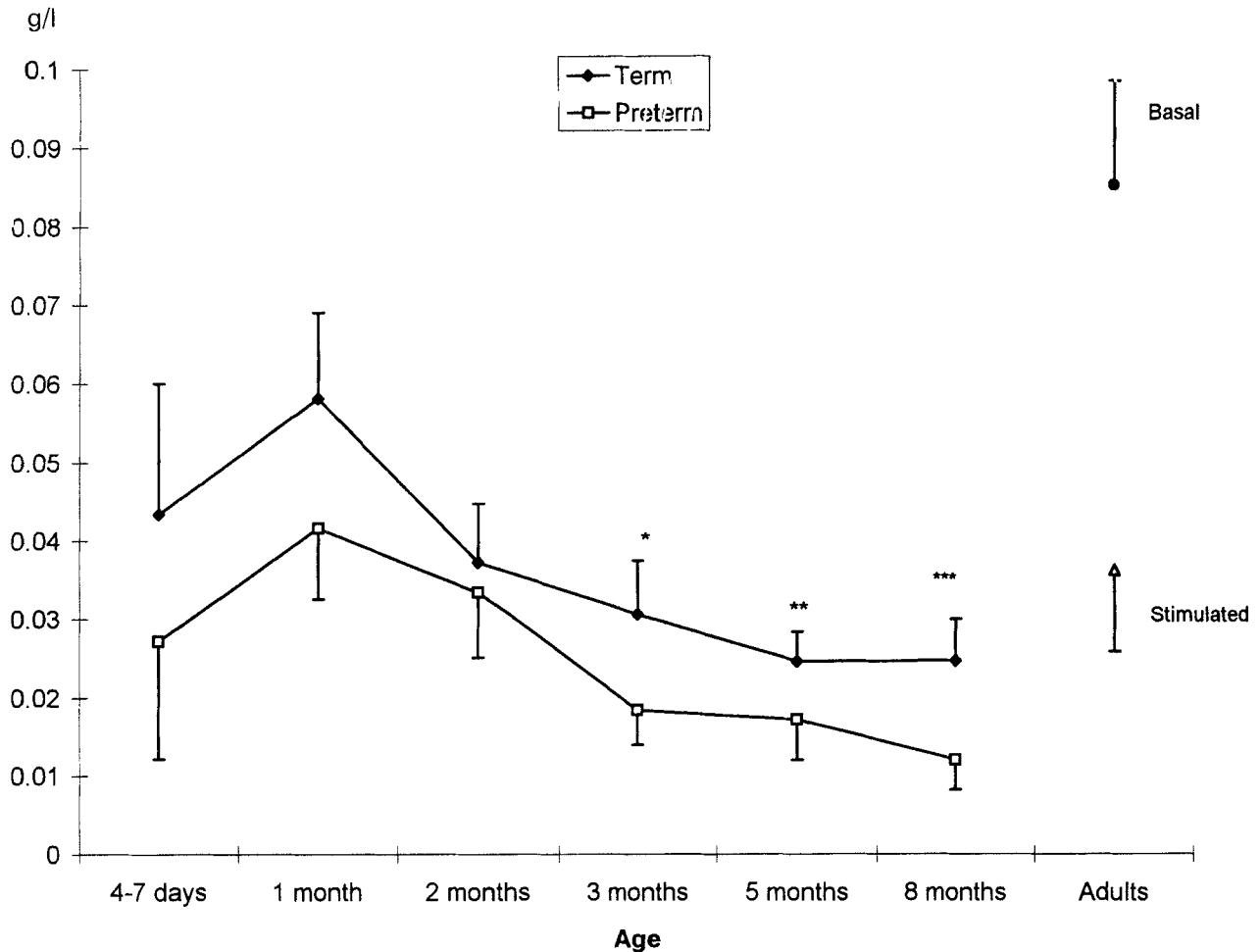


Fig. 1. IgA levels in saliva (mean, 95% confidence intervals) in term and preterm infants followed longitudinally during the first 8 months of life and in healthy adults. * $p=0.02$, ** $p=0.03$ and *** $p=0.001$ (Mann-Whitney U test).

Furthermore, we measured β -lactoglobulin (BLG) from the sera of some infants who were solely breast-fed, but detected no BLG. Fruit, berries, and vegetables were introduced at three months, and meat, fish, eggs, and cereals at 5 months. Infants attended well-baby clinics, but any medical care needed during the study was provided by MK. Not all the mothers and infants were able to make every planned visit, but on each visit the mother furnished a faecal sample from the child and a sample of mixed breast milk consisting of equal amounts of fore and hind milk. At each visit the infants consumed a measured amount of breast milk and/or CM. After this milk feed, the infant was given water. One hour later a blood sample was drawn, the infant's mouth was inspected and rinsed if necessary with water, 3–4 small citric acid crystals were placed in its mouth, and a salivary sample was collected with a plastic Pasteur-pipette. The infants had no symptoms nor signs of infections during any visit.

We also collected unstimulated and similarly stimulated salivary samples from 16 healthy adults who had fasted for 2–4 h.

Serum was stored at -20°C . Breast milk samples were stored at -20°C . Before analysis, these were defatted by centrifugation at $10\,000 \times g$. Faecal samples were lyophilized, eluted with PBS, and centrifuged at $10\,000 \times g$; the supernatants were stored at -70°C . Saliva was mixed with BSA (Smith Ltd., UK) in PBS to give a final BSA concentration of 0.3%, and centrifuged at $17\,300 \times g$; supernatants were stored at -70°C .

Laboratory measurements – Total levels of IgA were measured by immunoturbidometry using a serum standard (6). The detection limit was 0.0057 g/l. CM antibodies (CMAb) were measured with an ELISA (7). Results were compared with a positive reference serum and expressed as percentages. A sensitive solid-phase double-sandwich time-resolved fluoroimmunoassay was used to measure serum human α -lactalbumin (ALA) (8). Serum levels are expressed in relation to the amount of milk protein per meal as $\mu\text{g ALA/l serum/g ALA given/kg body weight}$. The sensitivity of the assay was 0.25 $\mu\text{g/l}$. The inter-assay coefficients of variance

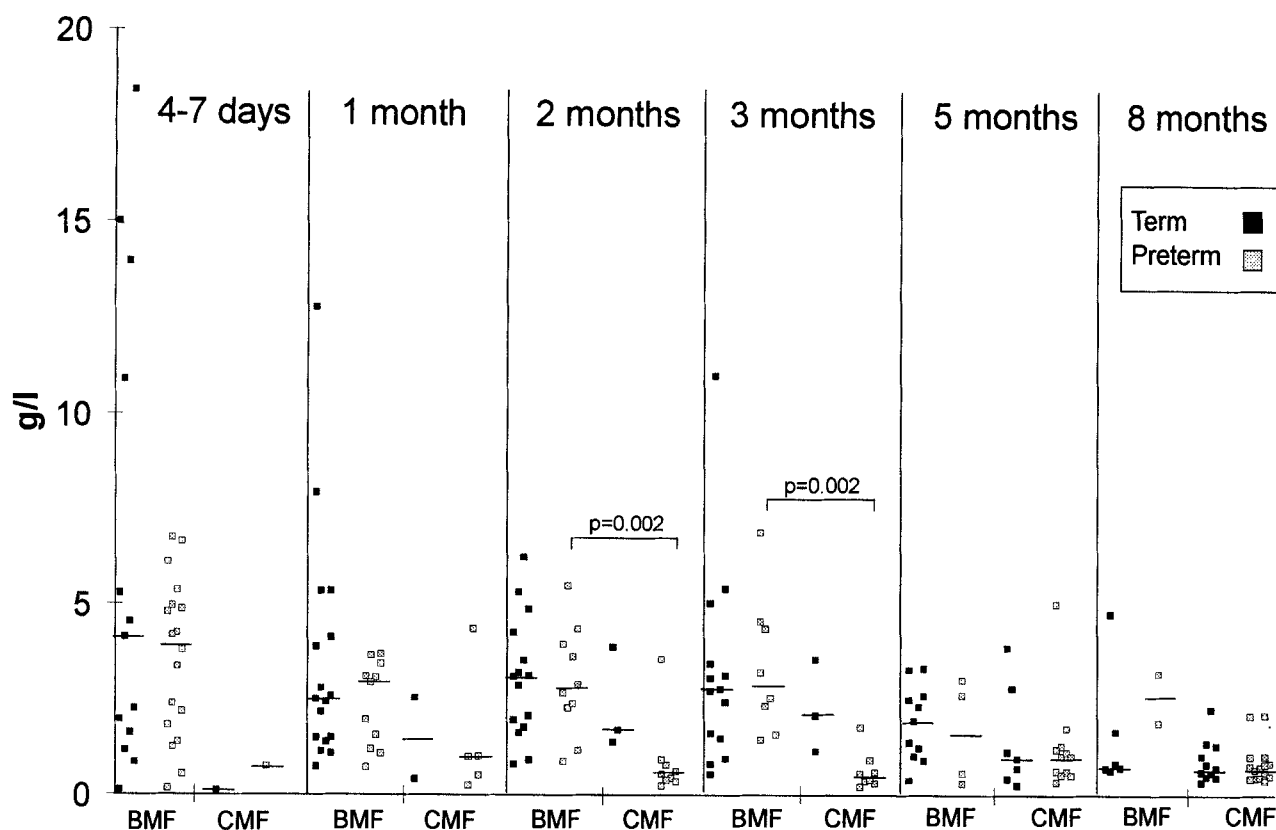


Fig. 2. Levels of faecal IgA in breast fed (BMF) and formula fed (CMF) term and preterm infants followed longitudinally during the first 8 months of life. — = median values. Significant differences between two sets of data are indicated by a p-value (Mann-Whitney U test).

for the methods used were: IgA 11%, IgG CMAb 5.0%, IgA CMAb 13%, ALA 3.0–24 %.

Statistical methods – We compared differences between groups with the Mann-Whitney U test because of the skewed distribution of the data. Results are given as two-tailed p-values. We sought associations between data with Spearman's rank correlation test; results are given as correlation coefficient Rho and two-sided p-value. $p < 0.05$ was considered statistically significant.

Ethics – The study was approved by the Ethics Committee of the Children's Hospital, University of Helsinki, Finland.

Results

Feeding patterns – The mean duration of exclusive breast feeding (no CM-formula, no solid foods) was 3.1 months (range 0–5.1) in term infants, and 2.1 months (0–6.4) in preterm infants. CM-formula was introduced at a mean age of 5.5 months (0–11) in term and 2.9 months (0–8.1) in preterm infants. Solid foods (CM-free) were introduced at a mean age of 3.5 months (2.6–5.1) in term and 3.7 months (2.0–6.4) in preterm infants. The mean age for partial breast feeding was 6.9 months (2.3–12) in term and 4.2 months (0–9) in preterm infants.

Salivary IgA (Fig. 1) – IgA was detected in saliva of all infants during the first week of life. Levels were higher in term than in preterm infants; at 3, 5, and 8 months the difference was significant. No statistically significant differences were found between BM-fed (BMF) and CM-fed (CMF) infants. Stimulated salivary IgA levels in healthy adults did not differ from levels in term infants except for levels at the age of one month ($p = 0.007$). Preterm infants at the age of 3, 5, and 8 months had lower levels than adults, $p = 0.005$, 0.003 and 0.0001 , respectively. In adults, levels of IgA in unstimulated saliva were higher than in stimulated saliva, $p < 0.0001$. IgA in BM and in infants' saliva showed no statistically significant correlation.

Faecal IgA (Fig. 2) – IgA was detected in faeces of all infants from birth onwards. Levels in term and preterm infants were similar. BMF infants showed a higher concentration of IgA in faeces throughout the study, but the difference between the feeding groups was significant only in preterm infants at two and three months ($p = 0.002$). Faecal IgA levels in term infants throughout the study showed a positive correlation with the levels of IgA in BM (Rho 0.30, $n = 121$, $p = 0.001$); however, no significant correlation was found for preterm infants.

Table 1. Percent of positive values for salivary and faecal IgA cow's milk antibodies (SaCMAB and FCMAb) in all infants and in breast-fed infants alone.

	4-7 days	1 month	2 months	3 months	5 months	8 months
All infants						
SaCMAB (term)	5.6	5.0	11	5.3	11	35
SaCMAB (preterm)	23	22	26	6	12	24
FCMAb (term)	28	45	32	41	22	0
FCMAb (preterm)	53	26	21	6	19	18
Breast fed infants						
SaCMAB (term)	5.9	0	6.3	6.3	7.7	33
SaCMAB (preterm)	24	7.7	10	0	20	0
FCMAb (term)	29	44	25	43	25	0
FCMAb (preterm)	56	40	20	13	0	0

IgA in saliva vs. faeces – No statistically significant correlation was found in term or preterm infants at any age.

Breast milk IgA – IgA levels were highest during the first week after delivery in mothers of term (median 1.3 g/l) and preterm (median 0.7 g/l) infants, then fell to 0.4 g/l at one month and 0.3 g/l at two months in both groups. At 3, 5 and 8 months the levels in term and preterm infants were similar, i.e. 0.2–0.5 g/l.

Salivary, faecal, and breast milk IgA CMAB – Few infants had detectable levels of CMAB's in their saliva. Preterm infants tended to show antibodies more often and earlier than did term infants, but the differences were not significant ($p > 0.05$, when tested with the Chi-squared test). CMAB's were detected in up to 50% of the infants' faeces during the first month, but thereafter were detected less often (Table 1). Levels of IgA CMAB's in saliva and faeces were similar in BMF and CMF infants (data not shown). All mothers had detectable levels of CMAB's in BM. Median levels at the different times in mothers of term and preterm infants varied between 0.3–1.5% of positive reference.

Table 2. Median levels for serum IgG cow's milk antibodies (% of positive reference) at the ages of 3, 5, and 8 months when infant-groups were divided into upper and lower halves, based on a permeability test using human α -lactalbumin (ALA) as a marker, at the ages of 4–7 days and 1 month in term and preterm infants; differences non-significant (Mann-Whitney U test).

Age	ALA half	3 months	5 months	8 months
Term infants				
4-7 days	Upper	0.4	0.3	2.7
	Lower	0.3	0.4	1.4
1 month	Upper	0.2	0.2	1.4
	Lower	0.3	0.4	2.4
Preterm infants				
4-7 days	Upper	0.6	0.3	2.5
	Lower	0.7	1.0	3.4
1 month	Upper	0.4	0.3	9.6
	Lower	0.6	0.6	2.5

Serum CMAB's – Levels of IgG CMAB's in BMF infants were highest at birth and then gradually fell; median levels at 4–7 days, and 1, 2, 3, 5 and 8 months in term and preterm infants were 1.1 and 0.4, 0.8 and 0.3, 0.4 and 0.5, 0.2 and 0.4, 0.2 and 0.1, and 0.1 and 0.1% of pos. ref., respectively. CMF infants had higher levels of IgG CMAB's than BMF infants (p-value in parenthesis if significant); median levels in term and preterm infants were 1.1 and 0.1 at 4–7 days, 2.0 and 1.4 ($p = 0.04$) at 1, 3.8 and 2.6 at 2, 4.4 and 8.0 ($p = 0.003$) at 3, 1.5 ($p = 0.01$) and 1.3 ($p = 0.02$) at 5, and 1.8 and 6.6 at 8 months. IgA CMAB's were detected in 0/18 and 1/17, 1/18 and 0/13, 0/16 and 0/10, 0/16 and 1/8, 5/12 and 0/4, and 4/6 and 0/2 of term and preterm BMF infants tested at 4–7 days, and 1, 2, 3, 5 and 8 months, respectively. Levels were low. CMF infants had higher levels of IgA CMAB's than BMF infants (p-value in parenthesis if significant); median levels at 4–7 days, and 1, 2, 3, 5 and 8 months in term and preterm infants were 1.1 and 0.1, 1.8 and 0.5 ($p = 0.02$), 7.5 and 0.6 ($p = 0.001$), 7.7 and 1.5 ($p = 0.0006$), 2.2 and 0.3 ($p = 0.01$), and 0.7 and 0.8, respectively.

Gut permeability vs. serum IgG CMAB's – We compared the gut permeability to ALA, expressed as serum levels of ALA after a milk feed at 4–7 days and one month of age, with the levels of serum IgG CM-antibodies. No relation was observed (Table 2).

Discussion

We found IgA in the saliva of all infants as early as 2–7 days after birth. Some studies (9, 10) have detected IgA in saliva in the first week of life, but other studies have failed to do so (11, 12). The levels peaked at the age of one month, followed by a decrease at two and three months, after which age the levels remained stable, which agrees with earlier reports (13). Term infants had higher levels than preterm infants, but the mode of feeding did not influence the levels of IgA, nor were IgA levels in saliva related to the levels of IgA in breast milk. Hence we believe salivary IgA reflects the proper

secretion of infants, and that this secretion is relatively unaffected by feeding. Most studies demonstrate lower levels of IgA in saliva during the first month of life in breast-fed infants (12, 14, 15), and the authors speculate this is due to low antigenic stimuli. However, one study (9) showed higher levels in breast-fed infants and interpreted this as a result of the stimulatory effect of colostrum. Most studies agree that adult salivary IgA levels are reached later in childhood (11, 15, 16), although some studies have shown adult levels being reached even during the first month of life (12, 17, 18), a finding which agrees with ours. Our results indicate that the salivary IgA system is already well developed during the first week of life, as judged by levels in infants' stimulated saliva. We are confident that we have measured salivary IgA and not BM IgA, since the salivary samples were taken one hour after the milk meal; moreover if visible milk was present the mouth was rinsed with water.

Faecal IgA was detected in all infants from birth, with levels comparable in term and preterm infants. Levels in BMF infants were highest at birth, then decreased. BMF infants had higher levels than CMF infants throughout the study. Only small changes were observed in CMF infants. We thus infer that faecal IgA levels reflect breast-milk IgA content, because along with the fall in breast-milk IgA levels, the level of IgA in faeces fell. Since levels of IgA in faeces were 3–9 times higher than levels in BM, either BM may have a stimulatory effect, or BM IgA may be concentrated in the colon, or both. Jatsyk et al. (19) found IgA in faeces by the second day only in breast-fed infants and by one month of age in 30% of CMF infants. Koutras et al. (20), finding no IgA in faeces at birth and thereafter rising levels up to 8 weeks in BMF infants, argued that breast milk had a stimulatory effect on gastrointestinal humoral immunologic development. Rognum et al. (21) and Perkkio et al. (22), using immunohistochemistry, found IgA-producing immunocytes neither in the duodenal nor in the rectal epithelium during the first week.

Preterm BMF infants showed an earlier salivary CMAb-response than did term infants. It is thus possible that CM proteins in BM serve as an antigenic stimulus for these antibodies, because β -lactoglobulin was found more often in milk from mothers of preterm infants (37%) than in milk from mothers of term infants (13%) (8, 23). Specific IgA CM antibodies have been shown (24, 25) in newborns before the first meal and in BMF newborns, making the authors of those studies suspect antigenic contamination via amniotic fluid and breast milk.

Serum CM antibody levels are related to the intake of antigens in infants' food (26). We found rising levels of IgG, and IgA CMAb with age in all

infants, with higher levels in the CMF infants. Several studies have failed to demonstrate such differences in IgA CMAb's between fully breast-fed infants and infants regularly exposed to CM (14, 27, 28). The present study also revealed the appearance of IgA CM antibodies after the age of three months in BMF infants. The stimulus for these antibodies remains obscure.

The levels of IgG CMAb at the ages of 3 to 8 months were unrelated to the gut permeability to human ALA measured at the ages of 4–7 days and one month. The reason for our use of the results of the permeability study at 4–7 days and at one month, was that most infants at those ages had demonstrable levels of the marker, but thereafter gut-permeability was rapidly reduced. Furthermore, the permeability for individual infants was consistent, i.e. infants with high absorption a few days after birth also had high absorption at one and two months (8, 23). Our results suggest that the amount of antigen that passes through the gut epithelia to come into contact with immunocompetent cells does not affect the magnitude of the serum antibody-response. Although the antibody responses to the dietary protein fraction show the penetration of these fractions in immunogenic form, there may be no generalized association between these phenomena—though the reverse has indeed been suggested (5, 7). We have shown earlier a similar permeability to ALA in allergic and in non-allergic infants (8). Thus, neither high gut permeability nor a high serum food antibody level (8) can predict the development of allergy in an infant.

Acknowledgements

We thank Annikki Sarnesto, Tuula Ahokas, Terttu Louhio and Marjatta Viikari for their assistance. Support was by Finska Läkaresällskapet, the Foundation for Paediatric Research, the Sigrid Jusélius Foundation and the University of Helsinki, Finland.

References

1. HANSON DG. Ontogeny of orally induced tolerance to soluble proteins in mice. I. Priming and tolerance in newborns. *J Immunol* 1981; 127: 1518–24.
2. KAGNOFF MF. Oral tolerance. *Monogr Allergy* 1988; 24: 222–6.
3. CHALLACOMBE SJ, TOMASI TB. Systemic tolerance and secretory immunity after oral immunization. *J Exp Med* 1980; 152: 1459–72.
4. SWARBRICK ET, STOKES CR, SOOTHILL JF. Absorption of antigens after oral immunization and simultaneous induction of specific systemic tolerance. *Gut* 1979; 20: 121–5.
5. WALKER WA. Absorption of protein and protein fragments in the developing intestine: Role in immunologic/allergic reactions. *Pediatrics* 1985; 75 suppl(1): 167–71.
6. TEPPA A-M. Immunoturbidometry of albumin and immunoglobulin G in urine. *Clin Chem* 1982; 28(6): 1359–61.
7. TAINIO V-M, SAVILAHTI E, ARJOMAA P, SALMENPERÄ L, PERHEENTUPA J, SIIMES MA. Plasma antibodies to cow's milk are increased by early weaning and consumption of unmodified milk, but production of plasma IgA and IgM

- cow's milk antibodies is stimulated even during exclusive breast-feeding. *Acta Pædiatr Scand* 1988; 77: 807-11.
8. KUITUNEN M, SAVILAHTI E, SARNESTO A. Human α -lactalbumin and bovine β -lactoglobulin absorption in infants. *Allergy* 1994; 49: 354-60.
 9. ROBERTS SA, FREED DLJ. Neonatal IgA secretion enhanced by breast feeding. *Lancet* 1977; ii: 1131.
 10. MELLANDER L, CARLSSON B, HANSON LA, ET AL. Appearance of secretory IgM and IgA antibodies to *Escherichia coli* in saliva during early infancy and childhood. *J Pediatr* 1984; 104: 564-8.
 11. BURGIO GR, LANZAVECCHIA A, PLEBANI A, JAYAKAR S, UGAZIO AG. Ontogeny of secretory immunity: Levels of secretory IgA and natural antibodies in saliva. *Pediatr Res* 1980; 14: 1111-4.
 12. GLEESON M, CRIPPS AW, CLANCY RL, HENSLEY MJ, DOBSON AJ, FIRMAN DW. Breast feeding conditions a differential developmental pattern of mucosal immunity. *Clin Exp Immunol* 1986; 66: 216-22.
 13. BRANDTZAEG P, NILSSEN DE, ROGNUM TO, THRANE PS. Ontogeny of the mucosal immune system and IgA deficiency. *Gastroenterol Clin North Am* 1991; 20: 397-439.
 14. ØSTERGAARD PA. Serum and saliva Ig-levels in infants of non-atopic mothers fed breast milk or cow's milk-based formulas. *Acta Pædiatr Scand* 1985; 74: 555-9.
 15. AVANZINI MA, PLEBANI A, MONAFO V, ET AL. A comparison of secretory antibodies in breast-fed and formula-fed infants over the first six months of life. *Acta Pædiatr* 1992; 81: 296-301.
 16. THRANE PS, ROGNUM TO, BRANDTZAEG P. Ontogenesis of the secretory immune system and innate defence factors in human parotid glands. *Clin Exp Immunol* 1991; 86: 342-8.
 17. HAWARTH JC, DILLING L. Concentration of γ A-globulin in serum, saliva, and nasopharyngeal secretions of infants and children. *J Lab Clin Med* 1966; 67: 922-33.
 18. SELNER JC, MERRILL DA, CLAMAN HN. Salivary immunoglobulin and albumin: Development during the newborn period. *J Pediatr* 1968; 72: 685-9.
 19. JATSYK GV, KUVAEVA IB, GRIBAKIN SG. Immunological protection of the neonatal gastrointestinal tract: the importance of breast feeding. *Acta Pædiatr Scand* 1985; 74: 246-9.
 20. KOUTRAS AK, VIGORITA VJ. Fecal secretory immunoglobulin A in breast milk versus formula feeding in early infancy. *J Pediatr Gastroenterol Nutr* 1989; 9: 58-61.
 21. ROGNUM TO, THRANE PS, STOLTENBERG L, VEGE Å, BRANDTZAEG P. Development of intestinal mucosal immunity in fetal life and the first postnatal months. *Pediatr Res* 1992; 32: 145-9.
 22. PERKKIÖ M, SAVILAHTI E. Time of appearance of immunoglobulin-containing cells in the mucosa of the neonatal intestine. *Pediatr Res* 1980; 14: 953-5.
 23. KUITUNEN M, SAVILAHTI E, SARNESTO A. Human α -lactalbumin and bovine β -lactoglobulin absorption in premature infants. *Pediatr Res* 1994; 35: 344-7.
 24. FRICK M, RIEGER HL. Local antibodies to α -casein and β -lactoglobulin in the saliva of infants. *Pediatr Res* 1987; 22: 399-401.
 25. RENZ H, VESTNER R, PETZOLDT S, BREHLER C, PRINZ H, RIEGER CHL. Elevated concentrations of salivary secretory immunoglobulin A anti-cow's milk protein in newborns at risk of allergy. *Int Arch Allergy Appl Immunol* 1990; 92: 247-53.
 26. FÄLLSTRÖM SP, AHLSTEDT S, CARLSSON B, WETTERGREN B, HANSSON LÅ. Influence of breast feeding on the development of cow's milk protein antibodies and the IgE level. *Int Archs Allergy Appl Immunol* 1984; 75: 87-91.
 27. WEAVER LT, WADD N, TAYLOR CE, GREENWELL J, TOMS GL. The ontogeny of serum IgA in the newborn. *Pediatr Allergy Immunol* 1991; 2: 72-5.
 28. FÄLTH-MAGNUSSON K, KJELLMAN N-IM, MAGNUSSON K-E. Antibodies IgG, IgA and IgM to food antigens during the first 18 months of life in relation to feeding and development of atopic disease. *J Allergy Clin Immunol* 1988; 81: 743-9.