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Local Antibodies to α-Casein and β-Lactoglobulin in the Saliva of Infants

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ABSTRACT. Salivary antibodies were studied in 112 infants between 1 day and 8 yr of life. SIgA anticasein was present from birth in breast-fed and bottle-fed infants. Bottle-feeding resulted in significantly higher concentrations of SIgA anticasein at 3 wk to 3 months of life as compared to breast-feeding. Salivary anticasein declined toward the end of the 1st yr and was present in less than half of the children older than 1 yr. Salivary anti-β-lactoglobulin was also present at birth in some infants. Levels increased slightly over the following 3 months but remained low. Only a minority of older children had this antibody in their saliva. (*Pediatr Res* 22: 399–401, 1987)

Abbreviations

SC, secretory component
BSA, bovine serum albumin

Transplacentally acquired IgG protects the newborn infant against most systemic infections. During the immediate postnatal period some of this IgG can be recovered from mucosal membranes and may contribute to mucosal defense mechanisms. SIgA, the major secretory immunoglobulin, has been detected in saliva as early as 7 days of life and adult levels were present at 4 wk in 92% of infants (1, 2). Secretory antibodies have been shown to represent an immune response against replicating and nonreplicating antigens on mucosal surfaces and act to exclude such antigens from penetrating mucosal membranes.

Cow's milk proteins are among the first antigens encountered by many human neonates. The systemic immune response to these proteins has been characterized in healthy term infants, in premature infants as well as in children and adults during health and disease (3–8).

Since milk proteins are continuously presented to the local immune system of the gut and since these proteins have repeatedly been implicated in the pathogenesis of atopic disease it was of interest to study local antibody formation to the two major cow's milk proteins, α-casein and β-lactoglobulin during the 1st yr of life.

MATERIALS AND METHODS

One hundred twelve infants and children between 1 day and 8 yr of life were studied. Neonates and infants were all born at term and were without clinical problems. Beyond the immediate postnatal period until 3 yr of age infants were seen during well baby visits. The older children were outpatients who had illnesses not relating to the immune system. In infants, unstimulated saliva was obtained by gentle suctioning with a soft rubber tube over 10–15 min. Saliva was obtained at least 1 h after the last meal to avoid contamination of the samples with milk. Saliva was heated for 20 min at 51° C, centrifuged at 5000 × *g* for 15 min and kept at −20° C until analyzed. Each individual was studied on a single occasion. Among the ten 1- to 5-day-old neonates, four were fully breast-fed, three were bottle-fed, and three received mixed feedings. In the 3-wk to 4-month-old group 10 infants were fully breast-fed; among the remaining 21 bottle-fed infants, three were still receiving some additional breast milk and 11 had received breast milk for an average of 4 wk. After 4 months of age all except two infants were exclusively bottle-fed.

Antibody determinations. Antibodies against α-casein and β-lactoglobulin of the IgA-isotype were determined by a direct ELISA as published previously (5, 8). Microtiter plates (Nunc Laboratories, Roskilde, Denmark) were coated with α-casein or β-lactoglobulin at concentrations of 20 μg/ml. Rabbit IgG-antibody against human SC was purchased from Nordic Immunological Laboratories, Tilburg, Netherlands. Alkaline phosphatase conjugated goat antihuman α-chain, alkaline phosphatase conjugated goat antihuman μ-chain, and goat antirabbit IgG were obtained from Medac Lab., Hamburg, FRG.

To determine the specificity of the ELISA for salivary antibody the following additional controls were carried out. Serial dilutions of saliva were incubated in microtiter plates coated with a 2% human serum albumin solution. After addition of alkaline phosphatase conjugated second antibody to α-chain, further incubation and subsequent addition of substrate no extinction was found.

After addition of rabbit antihuman SC to microtiter plates coated with human serum albumin, addition of alkaline phosphatase conjugated antibody to rabbit IgG, further incubation and subsequent addition of substrate no extinction was found.

After incubation of salivary anti-α-casein or anti-β-lactoglobulin with the respective antigens in a concentration of 1 mg/ml all antibody activity was abolished. Anticasein activity in saliva was not affected by prior incubation with β-lactoglobulin; anti-β-lactoglobulin activity was not affected by prior incubation with α-casein. Results were expressed as reciprocal value of the saliva dilution which gave an OD of 1.0. Antibody determinations for each antigen were performed in a single run to ensure optimal comparability of all samples.

Statistical methods. Comparison between breast-fed and bottle-fed infants (Fig. 3) was performed by the Student's *t* test, as values showed a normal distribution.

RESULTS

The determinations of salivary IgA were performed using a second antibody against α-chain and in addition a second antibody against secretory component. This was done to demonstrate the secretory nature of the antibody and, at the same time, to

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assess for the possible presence of secretory IgM. Whenever IgA-anticasein or anti-β-lactoglobulin was found, the presence of SC-containing antibody was consistantly demonstrable, whereas in the absence of SC-containing antibody no IgA antibody was found.

In Figure 1, salivary antibodies to casein are shown from all infants in whom enough saliva was present to determine both IgA- and total SC-containing antibody. Anticasein was present in the saliva of eight of nine neonates who were 1–5 days old. The median of SC containing-antibody was several fold higher than the median concentration of total IgA-associated antibody against casein suggesting the presence of secretory IgM at this time. A higher concentration of IgA-anticasein as compared to total SC-anticasein was found only in one infant of this group. This 4-day-old infant had the highest concentrations of both IgA and total SC containing antibody of the whole group. The median of IgA-anticasein increased during the first 3–7 wk whereas total SC-containing antibody decreased.

The median of IgA antibody declined over the following 9 months at which time IgA-anticasein was detectable in only six of 18 infants. Of the 12 children more than 1 yr old, seven had measurable IgA-anticasein in their saliva.

In Figure 2, salivary antibodies to β-lactoglobulin are shown from all infants in whom enough saliva was present to determine both IgA- and total SC-containing antibody. During the immediate postnatal period IgA-anti-lactoglobulin was detected in the saliva of only two of five infants and SC-containing antibody was found in three. Between 3 wk and 3 months of age six of 20 infants had IgA-antilactoglobulin and 16 of 20 had SC-containing antibody. After 10 months of age no IgA-antilactoglobulin was detected in any of 10 individuals tested. Total SC-containing antibody was present in only half of the individuals tested and at very low concentrations.

Both anticasein and antilactoglobulin were detected during the first 5 days of life, anticasein in some infants at the age of 1 day. In this group of neonates, four infants were fully breast-fed and prior exposure to cow's milk protein could be excluded. Concentrations of salivary antibodies to casein in these breast-fed infants were comparable to the rest of the group. In contrast, antibody concentrations in infants of 3 wk to 4 months of age showed a relationship to antigen exposure. The mean of SIgA-anticasein concentrations in the bottle-fed infants was significantly higher than in the breastfed group ($p < 0.05$) even though some exposure to cow protein had occurred in all of these latter infants. Salivary antilactoglobulin levels in breast-fed infants were also lower compared to bottle-fed infants but this difference did not reach statistical significance (Fig. 3). After 4 months of age the incidence of breast-feeding was low and all infants received cow's milk products as part of their regular diet.

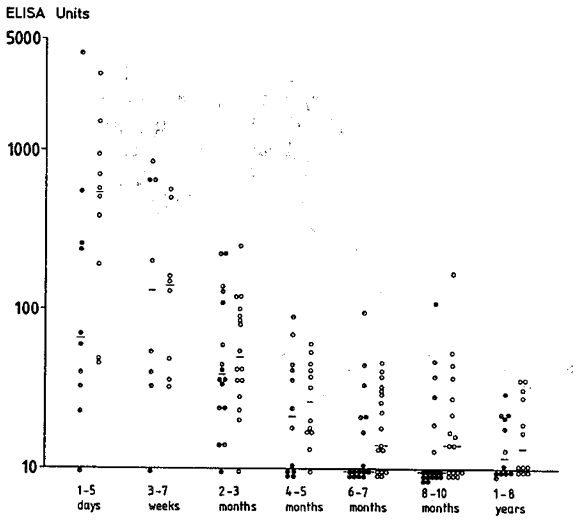

Fig. 1. Concentrations of anticasein in saliva of infants and children at various ages. The *horizontal bars* represent the median values of each group. ●, IgA-associated antibody; O, total SC-containing antibody.

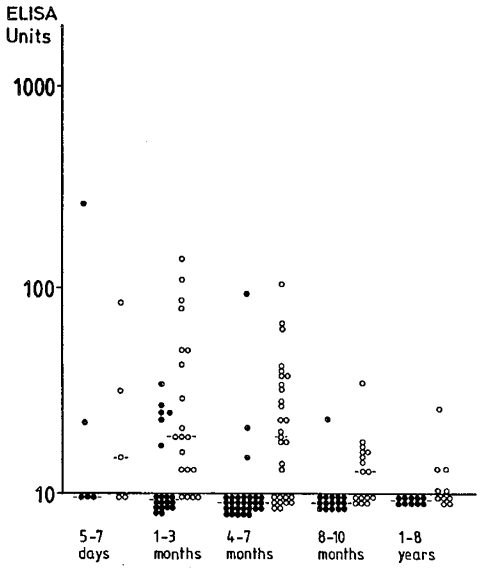

Fig. 2. Concentrations of anti-β-lactoglobulin in saliva of infants and children at various ages. The *horizontal bars* represent the median values of each group. ●, IgA-associated antibody; O, total SC-containing antibody.

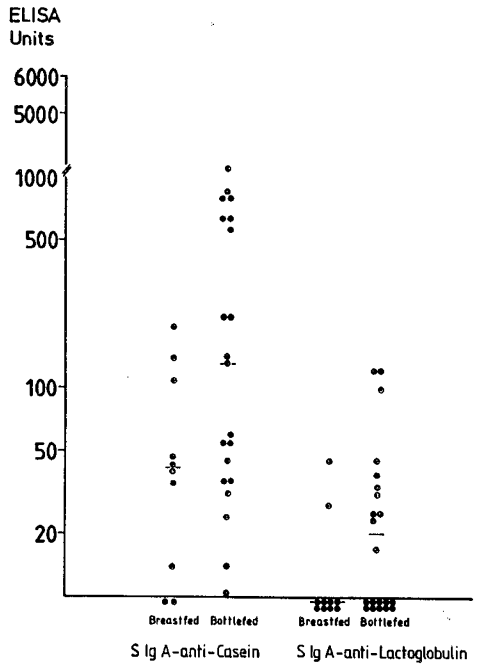

Fig. 3. Comparison between salivary IgA-associated anticasein and anti-β-lactoglobulin in breast-fed and bottle-fed infants of 3 wk to 3 months of age.

In most infants, particularly in the youngest group, insufficient amounts of saliva were available to test for the presence of IgM directly. However, in 14 infants of 3 wk to 5 months of age, a comparison between IgA, total SC-containing antibody and IgM-associated antibody to casein and β-lactoglobulin was possible. Although 13 of 14 infants of this group had no detectable IgA-anti-β-lactoglobulin, SC-containing anti-β-lactoglobulin was found in 13 (19–110 EU, median 40 EU) and IgM anti-β-lactoglobulin in 8 (13–160 EU, median 26 EU). In the same group SC-containing anticasein was found in 14 (13–160 EU, median 36 EU), IgA anticasein in nine (10–90 EU, median 40

EU), and IgM anticasein in only two infants (27 EU, 26 EU). These results agree with the assumption that, in the absence of IgA-antibody, SC-containing antibody reflects specific IgM.

DISCUSSION

The formation of serum antibodies to cow's milk protein is clearly related to antigenic exposure and occurs about 2 wk after initiation of feeding (9). In contrast, secretory antibodies were detected in this study after the 1st day of life even in infants who had been exclusively breast-fed.

The findings of anticasein and antilactoglobulin in the saliva of the study infants cannot be explained by contamination with breast milk since comparable concentrations of secretory antibodies were found in breast-fed infants and in infants who had not received mother's milk. Also, the high amounts of total secretory piece containing antibody as compared to IgA-associated antibody suggest the presence of secretory IgM which is not present in breast milk (10). The occurrence of antibodies to cow's milk proteins in newborn infants is new, albeit not unexpected (11), and is similar to results of Swedish authors. Mellander *et al.* (12) found SIgA-antibodies to *Escherichia coli* O antigens in saliva, amniotic fluid, and meconium of neonates. A comparison of secretory antibodies against *E. coli* between Swedish and Pakistani infants showed continuously low levels of salivary SIgA antibodies and a high ratio between total SC antibody and IgA in Swedish infants. In contrast, the more heavily exposed Pakistani group showed an increase of antibody levels to *E. coli* after 2–3 wk and a decrease of the SC/IgA ratio to 1.0 (13). In the present study a similar development was seen: concentrations of salivary antibodies to casein, the major antigenic protein of cow's milk, were significantly higher during the first 4 months in bottle-fed than in breast-fed infants.

The concentration of antigenic lactoglobulin in commercial formulas is about half of the concentration of antigenic casein (14). The lower levels of salivary antibodies to β-lactoglobulin and the observed persistence of a higher concentration of total SC-containing antibody compared to IgA-associated antibody might therefore be explained by less antigenic stimulation by β-lactoglobulin.

At present there appear to be two possible explanations for the occurrence of secretory antibodies in neonates. The fetus could have been exposed to cow's milk protein *in utero*. Protein antigens were, indeed, detected in amniotic fluid (Chandra R, personal communication). The detection of SIgA-antipolio in Swedish neonates, however, argues against the possibility of intrauterine exposure. Also, secretory antibodies to milk were a constant finding in the present study whereas milk proteins would be expected to contaminate amniotic fluid to varying degrees and only in certain cases.

An alternate explanation would be the induction of secretory antibody body by maternal antiidiotypic antibody. This possibility is suggested by the observation that administration of antiidiotypic antibody to the K 13 polysaccharides at birth primed mice for protection against challenge with *E. coli* K 13 (15).

Both secretory anticasein and anti-β-lactoglobulin declined toward the end of the 1st yr of life. In older children anticasein was present in half of the individuals tested and only at low levels. IgA anti-β-lactoglobulin was not found after 1 yr of age. This result is in agreement with findings of Kletter *et al.* (16) who detected coproantibodies to milk in most infants less than 1 yr, but only in one of 10 healthy children more than 1 yr old. This could be due to the fact that milk protein makes up a lower proportion of food protein with increasing age. Also, older individuals develop an immunologic tolerance against milk protein. Antibodies to BSA, *e.g.* are found only in about 30% of young adults (17).

The significance of local antibody against food protein is unclear, but may be related to exclusion of antigenic peptides from the gut wall and prevention of undue sensitization. Prospective evaluation of infants at risk may be able to answer this question.

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REFERENCES

1. Selner JC, Merill DA, Claman HN 1968 Salivary immunoglobulin and albumin: development during the newborn period. J Pediatr 72:685–689
2. Gleeson M, Dripps AW, Clancy RL, Hensley MJ, Dobson AJ, Firman DW Breast feeding conditions a differential developmental pattern of mucosal immunity. Clin Exp Immunol (in press)
3. Rothberg RM, Farr RS 1965 Anti-bovine serum albumin and antialphalactalbumin in serum of children and adults. Pediatrics 35:571–588
4. Rieger CHL, Rothberg RM 1975 Development of the capacity to produce specific antibody to an ingested food antigen in the premature infants. J Pediatr 87:515–518
5. Müller W, Lippmann A, Rieger CHL 1983 Oral immunization to milk protein in human infants in the presence of passive antibody. Pediatr Res 17:724–728
6. Kletter B, Gery I, Freier S, Davies AM 1971 Immune response of normal infants to cow milk. Int Arch Allergy 40:656–666
7. Müller W, Rieger CHL, von der Hardt H 1985 Increased concentrations of milk antibodies in recurrent pulmonary aspiration in infants and young children. Acta Paediatr Scand 74:660–663
8. Müller G, Bernsau I, Müller W, Weissbarth-Riedel E, Natzschka J, Rieger CHL 1986 Cow milk antigens and antibodies in the serum of premature infants. J Pediatr 109:869–873
9. Rothberg RM 1969 Immunoglobulin and specific antibody synthesis during the first weeks of life of premature infants. J Pediatr 75:391–399
10. Mellander L, Carlsson B, Fehmida Jalil, Söderström T, Hanson LÅ 1985 Secretory IgA antibody response against Escherichia coli antigens in infants in relation to exposure. J Pediatr 107:430–432
11. Hanson LÅ, Ahlstedt S, Andersson B, Carlsson B, Fallström SP, Mellander L, Porras O, Söderström T, Edén CS 1985 Protective factors in milk and the development of the immune system. Pediatrics 75(suppl):172–176
12. Mellander L, Carlsson B, Hanson LÅ 1986 Secretory IgA and IgM antibodies to E. coli O and Poliovirus type 1 antigens occur in amniotic fluid and saliva from newborns. Clin Exp Immunol 63:555–561
13. Mellander L, Carlsson B, Hanson LÅ 1984 Appearance of secretory IgM and IgA antibodies to Escherichia coli in saliva during early infancy and childhood. J Pediatr 104:564–568
14. Helms I, Rieger CHL 1987 Decreased production of specific antibodies to cow milk proteins in premature infants during the first six months of life. Eur J Pediatr 146:131–134
15. Stein KE, Söderström T 1984 Neonatal administration of idiotype or antiidiotype primes for protection against Escherichia coli K 13 infection in mice. J Exp Med 160:1001–1011
16. Kletter B, Freier S, Michael Davies A, Gary I 1981 The significance of coproantibodies to cow's milk proteins. Acta Paediatr Scand 60:173–180
17. Korenblat PE, Rothberg RM, Minden P, Farr RS 1968 Immune response of human adults after oral and parenteral exposure to bovine serum albumin. J Allergy 41:226–235