

Antibodies IgG, IgA, and IgM to food antigens during the first 18 months of life in relation to feeding and development of atopic disease

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Serum levels of IgG, IgA, and IgM antibodies against ovalbumin, β -lactoglobulin, and gliadin were measured in a series of 210 "allergy-risk" infants and their mothers. The antibody levels were determined with ELISA in sera obtained from mothers at delivery, and from their babies at birth, 6 weeks, 6 months, and 18 months. High levels of maternal IgG, IgA, or IgM antibodies to food at delivery did not appear to protect the baby against development of atopic disease. Maternal avoidance of cow's milk and egg during pregnancy had no significant influence on the level of food antibodies in cord blood, but the mother's intake of these foods during lactation affected the immunologic response of the baby, not only to these antigens but also to gliadin as well. Babies with minimal cow's milk exposure before 6 months had significantly lower levels of IgG to β -lactoglobulin than babies regularly exposed to cow's milk. We conclude that maternal elimination diet during lactation influences the immunologic response of the baby, but if this prevents the development of atopic disease or just delays the immunologic maturation remains to be evaluated. (J ALLERGY CLIN IMMUNOL 1988;81:743-9.)

Increased levels of serum IgE and IgG antibodies to food antigens are common in infants and children with food allergy.¹ High levels of IgG, IgA, and IgM antibodies to foods have also been found in food-allergic subjects.²⁻⁴ Such antibodies may, however, also be demonstrated in healthy children,⁵ and the kinetics of production of these antibodies are influenced by the type of feeding.⁶

General advice on the prevention of atopic disease in babies "at risk" often include maternal elimination diet during pregnancy and lactation,⁷ or at least a restricted intake of CM and egg during lactation.⁸ From animal studies it is determined, however, that the young animal must be exposed to sufficient quantities of antigen to develop a normal immune response, which probably includes a depression of the production of IgE antibodies.^{9, 10} In rat pups,

Abbreviations used

AU:	Arbitrary absorbance units
BLG:	β -Lactoglobulin
BM:	Breast milk
CM:	Cow's milk
GL:	Gliadin
OA:	Ovalbumin
PBS:	Phosphate-buffered saline
PBS-Tween:	PBS with 0.05% Tween 20
SPT:	Skin prick test
D:	Diet totally devoid of cow's milk plus an egg
ND:	Normal food containing about 0.5 L of milk per day and three to five eggs per week

the maternal IgG antibodies modulate the immune response.¹⁰

We have previously reported results from a prospective, randomized study concerning the effects of maternal elimination of CM and egg during late pregnancy in "allergy-risk" pregnancies.^{11, 12} We found no significant differences in the development of atopic diseases among babies of D mothers as compared to ND mothers. Abstention from CM and egg caused a significant fall of serum IgG antibody levels to these antigens in D mothers, but there was no significant difference between cord blood IgG in babies

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of D mothers compared to ND mothers. However, atopic mothers had significantly higher levels of IgG antibodies to food already before D, and their babies had significantly higher levels of cord blood IgG antibodies to food than babies of nonatopic mothers.

After delivery, the mothers chose their diet freely, but some mothers continued taking an exclusion/restriction diet. In this article, we present the results concerning the levels of food antibodies in mothers at delivery, relating them to maternal D or ND during pregnancy, and in the babies, relating the levels to (1) maternal D or ND during pregnancy and lactation, (2) the age of the baby, (3) the exposure to CM, and (4) the development of atopic disease in the baby.

MATERIAL AND METHODS

Study population

In order to study the effect of maternal elimination D during pregnancy, 212 pregnant women were engaged into a prospective, randomized study. There was a history of atopic disease (atopic eczema, allergic rhinoconjunctivitis, and/or bronchial asthma) in at least one family member (mother, father, or sibling) in all families enrolled in the study. Thus, the study comprised both mothers having atopic diseases themselves and nonatopic mothers (with a husband or previous child suffering from atopic disease). The pregnant women volunteered for the study in midpregnancy and were randomly allocated to two different groups from the twenty-eighth gestational week to delivery. D meant a diet totally devoid of CM (but substituted with extra calcium and a casein-hydrolysate, Nutramigen; Bristol-Myers, Stockholm). ND was normal food containing about 0.5 L of milk per day and three to five eggs per week. The maternal D, that is, strict elimination of all CM and egg during late pregnancy, was guided by the advices of a dietician.¹¹ After delivery, the diet was determined by the mother's own choice.

The development of atopic disease in the babies was monitored by SPT, questionnaires, and blinded physical examination by pediatric allergist when the babies were 18 months of age.¹²

Feeding of the babies

Breastfeeding was encouraged. All babies, who needed formula partially or fully, were offered a casein-hydrolysate without charge until the babies were 3 months of age. One baby, fed CM-based formula from birth because of maternal choice, was not included in this presentation.

Mothers were advised not to introduce solid food before the babies were 4 months of age.

At 6 months of age, 79 babies were entirely fed CM-based formula (and not BM) for 1 to 3 months. Twelve babies, however, were exclusively breast-fed, and their mothers had postponed the introduction of CM in baby food beyond 6 months of age.

At 6 months of age, GL was introduced for breast-fed babies as porridge, and for bottle-fed babies, as formulas containing wheat flour.

Maternal diet during lactation

By their own choice, 10 mothers in the D group continued a strictly CM- and egg-free diet after delivery. Fifty-eight mothers in the D group and 29 in the ND group chose a "low-allergenic diet" regimen, allowing up to 0.2 L of milk per day and two eggs per week. In the delivery ward, all babies in the study were fed only casein-hydrolysate as supplement and no banked BM (not to jeopardize the maternal choice of sparing the baby from CM and egg antigen in the breast milk).

During the first 6 weeks of lactation, the diets of the mothers could be described as (1) strictly CM- and egg-free diet ($N = 10$, referred to as "allergen-free"), (2) restricted, "low-allergenic regimen" diet ($N = 87$, referred to as "allergen-reduced"), and (3) normal diet ($N = 92$, referred to as "normal").

Detection of atopic signs in the babies

SPT with commercially available extracts and skimmed milk were performed when the babies were 6 months (to food) and 18 months of age (to food, pollen, mite, and molds). At 18 months of age, most children ($N = 120$) were examined by a pediatric allergist (N.-I. M. K.) to find out whether the child up to 18 months of age had demonstrated definite, probable, possible, or no signs of atopic disease.¹² The selection of babies for the physical examination was based on information from questionnaires and SPT. The examiner did not know whether the mother had been receiving the D during pregnancy and lactation or not. For this presentation, children with a diagnosis of "definite" or "probable" atopic disease will be referred to as "atopic," whereas children with a diagnosis of "possible" or "no" will be referred to as "nonatopic." Further details regarding the atopic diseases in the babies are available elsewhere.¹²

Blood sampling

Venous blood was drawn from the mothers at the delivery. Cord blood was obtained after the cord was cut and wiped dry after delivery. Venous blood was drawn from the babies at about 6 weeks, 6 months, and 18 months of age. All the blood samples were allowed to clot at room temperature and were then centrifuged. Aliquots of serum were frozen at -20°C until analysis. Serum samples were available from mothers at the delivery ($N = 191$), from babies' cord blood ($N = 177$), at 6 weeks of age ($N = 156$), at 6 months of age ($N = 192$), and at 18 months of age ($N = 186$).

Determination of antibodies to food antigens

Protein antigen preparations were obtained from Sigma Chemical Co. (St. Louis, Mo.), namely, OA (type A 5503), BLG (L 0130), and GL (G 3375).

As antisera, horseradish-peroxidase-conjugated rabbit antihuman immunoglobulins were used (Dakopatts, Stockholm), that is, anti-IgG (type P 214), anti-IgA (P 216), and anti-IgM (P 215).

ELISA¹³ was performed in microtiter plates with 96 wells from Haeggar Plastics (Oslo, Norway). Absorbance was read

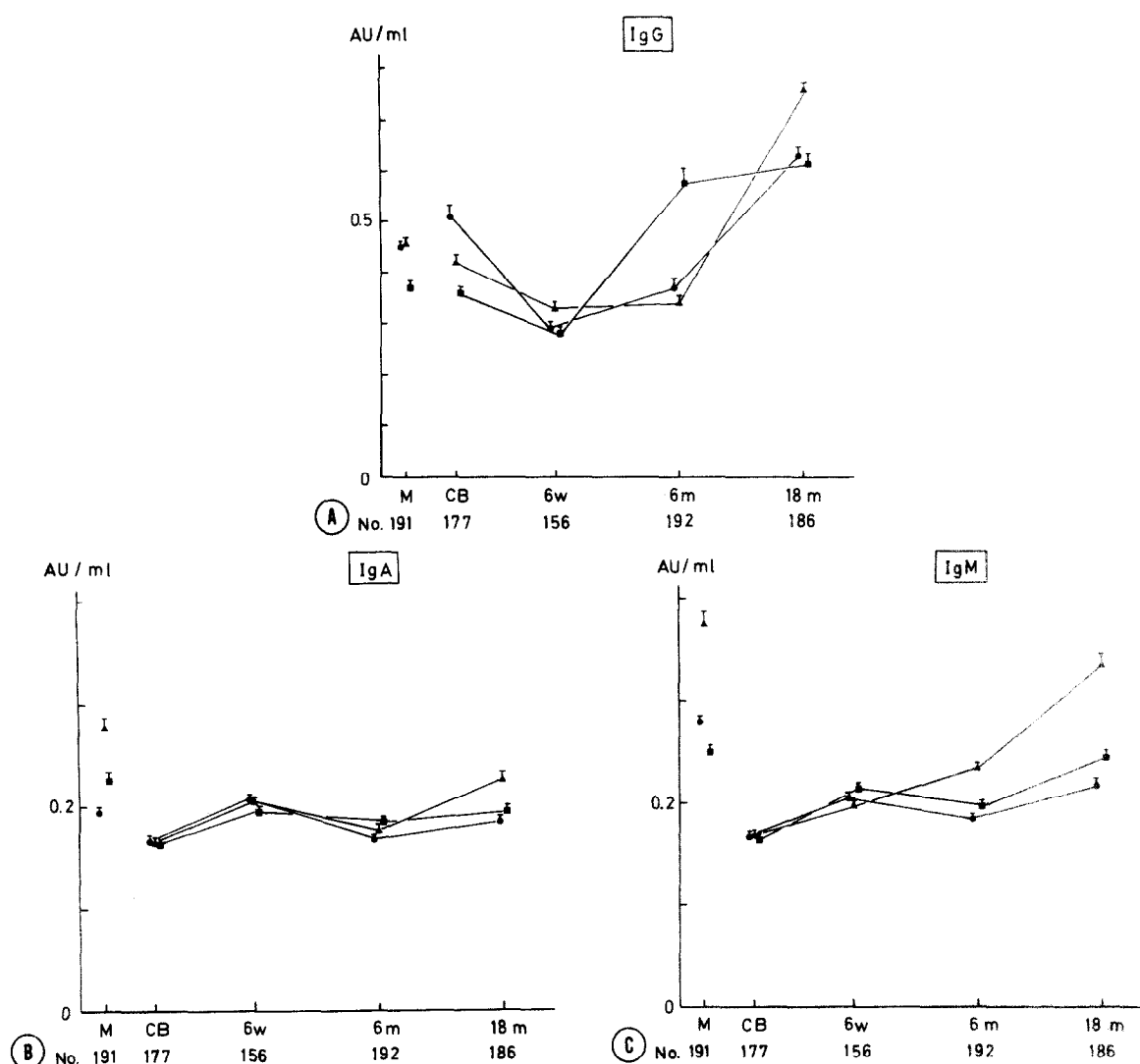


FIG. 1. Serum levels of immunoglobulins (mean $\pm$ SEM; arbitrary absorbance units per milliliter) against OA (●), BLG (■) and GL (▲). **A**, IgG. **B**, IgA. **C**, IgM. M, maternal serum obtained at delivery; CB, cord blood; 6 w, 6 m, and 18 m, samples from the babies at 6 weeks, 6 months, and 18 months of age, respectively. No., number of analyzed samples.

with a Titertec Multiscan reader (Ef-lab Oy, Helsinki, Finland) at 492 nm. To prepare antigen solutions, OA and BLG were diluted in PBS (pH 7.3) to 25 μ g/ml, whereas GL was dissolved in 70% ethanol and diluted with PBS to 100 μ g/ml; 200 μ l of antigen solution was added to each well, incubated for 2 hours at 37°C, and then incubated overnight in a moisture chamber. The microtiter plates were washed three times with 250 μ l of PBS-Tween. Patient sera were diluted 1:25 in PBS-Tween and were heat activated in a thermostated water bath for 30 minutes at 56°C. Then, 200 μ l of serum samples was added to three separate wells for each antigen and immunoglobulin for 1 hour at 37°C; thereafter, the wells were washed three times with PBS-Tween and incubated with 200 μ l of the horseradish-peroxidase-conjugates (diluted 1:200 with PBS-Tween), washed again with PBS-Tween, and followed by substrate solution. The plates were light protected until they were read, and the

reaction was stopped after 30 minutes by adding 50 μ l 12.5% sulfuric acid. The substrate contained 100 ml of PBS, 0.4 ml of hydrogen peroxide (2%), and 10 ml of 1,4-phenylenediamine dissolved in methanol (10 mg/ml) and was freshly prepared and kept dark before use. To obtain a positive control in each plate, serum from a volunteer with measurable levels of anti-OA, anti-BLG, and anti-GL of all immunoglobulin classes was used, and, as negative controls, sera with negligible specific antibody activity, as well as PBS-Tween. The reference serum for positive control was obtained during the period of the investigation, frozen at -20°C in aliquots suitable for one day of experiments. When a deviation >10% was noted between a control measurement and the accumulated median of the positive or negative control, the whole determination was repeated. To reduce further the influence of methodological errors, the median value of the three samples was used for calculations.

and all analyses were performed by the same technician with the same equipment throughout the study. The sera obtained from each age group were analyzed in the same run. The results were expressed in arbitrary absorbance units per milliliter without subtraction of background, which was about 0.08 to 0.09 AU/ml.

Statistics

Student's *t* test, two-tailed, was used to compare the levels of antibodies at different ages (Fig. 1, A through C), whereas all other comparisons between groups were performed by the nonparametric Mann-Whitney U test. Correlation analysis by paired coefficients of correlation was used to compare paired maternal and fetal antibody levels and antibodies of different isotypes in the same individual on one occasion.

Ethics

Informed consent was given by all the parents whose babies were participating, and the study was approved by the Human Research Ethics Committee of the Medical Faculty, University of Linköping.

RESULTS

Maternal food antibody levels at delivery

Mothers, who had been receiving the strictly CM- and egg-free diet during late pregnancy, had lower levels of IgG food antibodies than mothers who had been receiving normal food. The difference was statistically significant only for OA ($p < 0.05$), whereas there was a tendency to difference for GL ($p < 0.1$). For IgA and IgM antibodies, there were no significant differences between D and ND mothers. Maternal antibody levels at delivery did not differ significantly between the group of mothers whose babies later developed atopic disease and the group of mothers whose babies remained healthy.

Cord blood-antibody levels

There were no significant differences between cord blood levels of IgG, IgA, and IgM to OA, BLG, or GL between babies of D mothers as compared to babies of ND mothers. The factor of correlation between paired mother-child samples was for IgG: 0.77 (OA), 0.81 (BLG), and 0.75 (GL); for IgA: 0.03 (OA), -0.11 (BLG), and -0.14 (GL); for IgM: -0.02 (OA), 0.12 (BLG), and -0.10 (GL).

Antibody levels at 6 weeks in relation to maternal diet during lactation

The results are depicted in Fig. 2, A through C, in which the effects of the three maternal diets during lactation are compared. The babies from the "allergen-free" group had significantly higher levels of IgG, IgA, and IgM antibodies to OA and GL than the other groups, and the difference for BLG was nonsignificant.

Antibody levels at 6 weeks in relation to breast-feeding

At 6 weeks, 189 babies received BM (162 exclusively, and 27 with casein-hydrolysate as supplement), while 21 babies were exclusively fed casein-hydrolysate. The antibody levels of all three isotypes were similar in the two groups of babies.

Antibody levels at 6 months in relation to CM exposure

At 6 months, the antibody levels of exclusively breast-fed babies were compared with the levels in exclusively formula-fed babies regularly exposed to CM. The IgG levels against BLG were significantly higher in the CM-exposed group, whereas the other levels were not significantly different. There were no significant differences between these two groups at birth for any isotype.

Antibody levels in the babies at different ages

The antibody levels in the different age groups against the three tested food antigens are illustrated in Fig. 1. The IgG antibody levels to OA and BLG increased significantly from 6 weeks to 6 months of age ($p < 0.001$) but not for GL (not significant, Fig. 1, A). From 6 months to 18 months of age, there was a significant increase ($p < 0.001$) for OA and GL, but not for BLG (not significant). The IgA antibody levels decreased from 6 weeks to 6 months of age (Fig. 1, B; $p < 0.001$ for OA, $p < 0.05$ for BLG, and $p < 0.001$ for GL) and increased from 6 to 18 months of age ($p < 0.001$ for OA, $p < 0.05$ for BLG, and $p < 0.001$ for GL). The IgM antibodies against OA and BLG also decreased from 6 weeks to 6 months of age (Fig. 1, C; $p < 0.001$ for OA and $p < 0.01$ for BLG), whereas those against GL increased ($p < 0.001$). There was a significant increase in IgM antibody levels for all three antigens from 6 months to 18 months of age ($p < 0.001$ for all).

At 6 weeks, the coefficients of correlation between IgA antibodies against OA, BLG, and GL were between 0.65 and 0.77. The correlation between IgA and IgM antibodies to GL was 0.72, and all other coefficients of correlation were considerably lower. At 6 months of age, the IgG antibodies of the children had reached the maternal levels and exceeded them at 18 months. IgA and IgM antibodies at 18 months of age still fell below the maternal levels.

Antibody levels at the different ages in relation to development of atopic disease to 18 months of age

At 6 weeks of age, the IgM levels toward OA were significantly higher in babies later classified as being

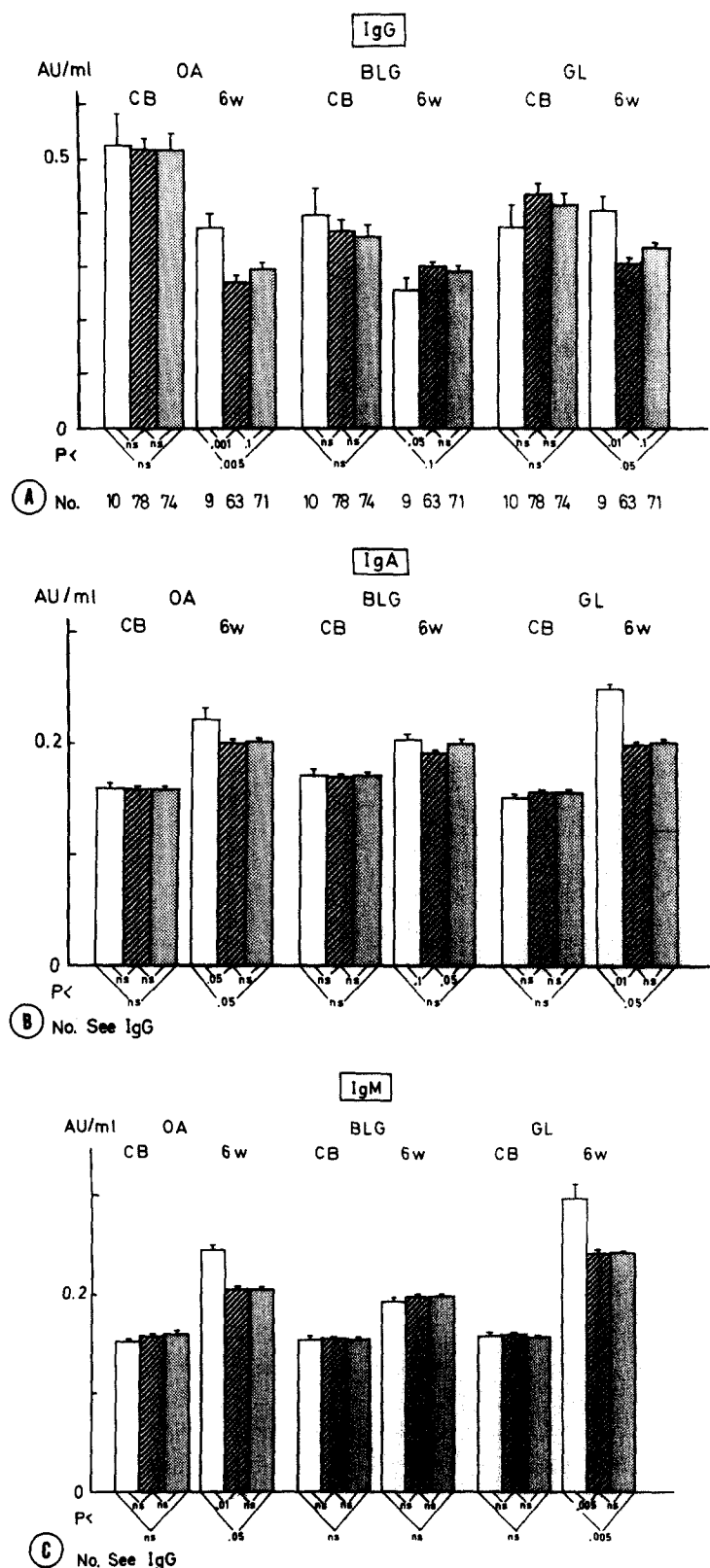


FIG. 2. Serum levels of immunoglobulins (mean $\pm$ SEM; arbitrary absorbance units per milliliter) against OA, BLG, and GL. A, IgG. B, IgA. C, IgM. CB, cord blood; 6 w, sample obtained from the baby at 6 weeks of age. *Open bars*: breast-feeding mothers receiving diet strictly free from CM and egg. *Hatched bars*: breast-feeding mothers taking reduced amounts of CM and egg. *Closed bars*: breast-feeding mothers taking normal food, including CM and egg. P, indicates possibly significant differences (tested with Mann-Whitney U test) between groups, connected with lines; ns, not significant differences; No., number of analyzed samples.

nonatopic than in babies classified as being atopic ($p < 0.05$). At 6 months of age, IgG levels to OA and GL were significantly higher in the atopic children ($p < 0.005$ for both). At 18 months of age, the atopic babies had higher levels of most antibodies, but significantly so only for IgG against OA ($p < 0.05$).

DISCUSSION

In this study, we monitored the development of serum antibodies to food in babies with a genetic propensity for atopic disease. Our results may not be valid for all infants, since the whole group had a family history of atopic disease. We were able to confirm previous observations^{4-6, 14} that exposure to CM influences the immunologic response of the baby.

We also studied the influence of maternal antibodies at delivery on the development of atopic disease in the babies. We were unable to confirm previous observations indicating a protective effect of high levels of maternal IgG antibodies to food against development of atopic disease in the baby.^{1, 15} Neither did high food-antibody levels in cord blood protect the baby against development of atopy. Varying maternal intake of CM and egg during lactation affected the food-antibody level in the baby.

It was previously demonstrated that IgG antibodies, but not IgA and IgM, cross the placental membrane.¹⁶ In this study, there were fairly good correlations between the maternal and fetal levels of IgG, but no corresponding correlations for maternal and fetal IgA and IgM antibodies, confirming that only IgG is transferred through placenta.

The levels of IgG antibodies to food antigens in the mothers were higher in the atopic subjects, confirming previous observations.^{1, 11, 12} In the mothers, the levels of IgG antibodies at delivery were lower in the D group than in the ND group, indicating that antigen exposure influences the antibody levels in the adult. The cord blood IgG levels did *not* differ between babies of D and ND mothers. Theoretically, the transport through placenta may be regulated by the best interest of the fetus in a not yet elucidated way.

In accordance with previous articles,^{1, 17} we found no convincing predictive capacity of IgG, IgA, and IgM antibody determinations with respect to development of atopic disease. At 18 months of age, none of the babies in our study had isolated, late allergic symptoms, which possibly might have provided a better correlation, since children with such symptoms have been described to have higher IgG levels.³

Studies on human B-lymphocyte function¹⁸ by the use of the lymphocyte activator, Epstein-Barr virus, demonstrated that adult levels of IgG production were reached at 24 months of age, whereas the capacity to

produce IgA was low for the first 2 years of life. IgM was already secreted at adult levels by lymphocytes from cord blood, stimulated with Epstein-Barr virus, but pokeweed mitogen (a T-lymphocyte-dependent activator) did not induce IgM secretion at adult levels before 6 months of age. The food antibodies of these three isotypes, measured in the infants in this study, demonstrated a similar pattern of development, as was demonstrated in these lymphocyte studies. A tendency of higher levels of IgG antibodies in children, who were considered to suffer from atopic disease, was noted both at 6 months and at 18 months of age.

The occurrence of dietary antigens, consumed by the mother in BM, has been well documented.¹⁹⁻²⁴ After ingestion of one raw egg, most mothers had detectable levels of OA in BM samples, but the concentration of OA in BM did *not* differ between mothers of eczematous or healthy babies.²² This finding might indicate that the individual propensity of the child is of greater importance for the development of atopic disease than the amount of antigen delivered into the BM.

During the first 6 weeks after delivery, the lactating mothers in this study had three different types of diet with varying content of CM and egg. It is hard to assess the amount of antigen reaching the babies whose mothers were receiving "restricted" or "normal" diet during lactation, since the range of consumption varied considerably within the reported limits, and the individual mammary permeability might have influenced the amount of antigen delivered into the BM. The gastrointestinal permeability may also have differed between the babies, which could ultimately have determined the uptake of antigen. By contrast, the BM from the 10 mothers receiving a strictly CM- and egg-free diet should evidently be free from these food antigens. All 10 mothers had been receiving the diet for about 3 months before delivery, making them familiar with the diet and thereby decreasing the risk of inadvertent intake of CM and egg. There was no restriction in our study concerning the intake of bread, and the brands of bread used by the D mothers contained as much gluten as those containing CM and egg.

Fully breast-fed babies, supposedly unexposed to CM and egg antigens by BM, had higher levels of IgA and IgG antibodies to OA and GL at 6 weeks of age (Fig. 2, A and B). Restriction of the intake of CM and egg during early lactation thus appears to influence the antibody response, not only to these antigens but also to the unrestricted antigen GL. These findings are interesting in relation to animal studies demonstrating the importance of early antigen exposure for the induction of tolerance.¹⁰

At 6 months of age, babies exposed to CM by BM only had significantly lower levels of IgG to BLG than formula-fed babies. This is in accordance with our findings in the mothers when they were receiving exclusion diet during pregnancy. The difference in the pattern of reaction in the babies, when babies were compared at 6 weeks and 6 months of age, may reflect a more mature immune system. The discrepancy observed at 6 weeks and 6 months of age could not be explained by differences in antibody levels at birth, since the cord blood levels were similar in the groups.

The effects of dietary manipulation, for example, maternal abstention from CM and egg or delayed CM introduction, affected the levels of IgG more than those of IgA and IgM. Possibly, since IgA and IgM have their main importance in secretions (Brandtzaeg²⁵), serum samples may not have truly reflected alterations in the production of these isotypes.

In conclusion, we found that manipulation of maternal diet during lactation influenced the immunologic response of the baby, which contrasted with our previous results concerning the effects of diet during pregnancy.^{11, 12} Maternal avoidance of CM and egg during lactation influenced not only the antibody response to these antigens but also to the unrestricted antigen GL. Whether antigen avoidance during lactation may prevent atopic disease in the babies or, on the contrary, increase the risk of atopy, must be studied prospectively in a study, exclusively designed for this purpose. Such studies are presently being performed by our group.

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REFERENCES

1. Dannaeus A, Johansson SGO, Foucard T. Clinical and immunological aspects of food allergy in childhood. I. Estimation of IgG, IgA, and IgE antibodies to food antigens in children with food allergy and atopic dermatitis. *Acta Paediatr Scand* 1977;66:31-7.
2. May CD, Remigio L, Feldman J, Bock SA, Carr RI. A study of serum antibodies to isolated milk proteins and ovalbumin in infants and children. *Clin Allergy* 1977;7:583-95.
3. Fällström SP, Ahlstedt S, Hanson LA. Specific antibodies in infants with gastrointestinal intolerance to cow's milk protein. *Int Arch Allergy Appl Immunol* 1978;56:97-105.
4. Fällström SP, Ahlstedt S, Carlsson B, Lönnerdal B, Hanson LÅ. Serum antibodies against native, processed, and digested cow's milk proteins in children with cow's milk protein intolerance. *Clin Allergy* 1986;16:417-23.
5. Kletter B, Gery I, Freier S, Davies AM. Immune responses of normal infants to cow milk. I. Antibody type and kinetics of production. *Int Arch Allergy Appl Immunol* 1971;40:656-66.
6. Kletter B, Gery I, Freier S, Davies AM. Immune responses of normal infants to cow milk. II. Decreased immune reactions in initially breast-fed infants. *Int Arch Allergy Appl Immunol* 1971;40:667-74.
7. Chandra RK, Puri S, Suraiya C, Cheema PS. Influence of maternal food-antigen avoidance during pregnancy and lactation on incidence of atopic eczema in infants. *Clin Allergy* 1986;16:563-71.
8. Businco L, Marchetti F, Pellegrini G, Perlini R. Predictive value of cord blood IgE levels in "at risk" newborn babies and influence of type of feeding. *Clin Allergy* 1983;13:503-8.
9. Jarrett EEE. Activation of IgE regulatory mechanisms by trans-mucosal absorption of antigen. *Lancet* 1977;2:223-6.
10. Jarrett EEE, Hall E. The development of IgE-suppressive immunocompetence in young animals: influence of exposure to antigen in the presence and absence of maternal immunity. *Immunology* 1984;53:365-73.
11. Fälth-Magnusson K, Öman H, Kjellman NIM. Maternal abstention from cow milk and egg in allergy-risk pregnancies: effect on antibody production in the mother and the newborn. *Allergy* 1987;42:64-73.
12. Fälth-Magnusson K, Kjellman NIM. Development of atopic disease in babies whose mothers were on exclusion diet during pregnancy—a randomized study. *J ALLERGY CLIN IMMUNOL* 1987;80:868-75.
13. Engvall E, Perlmann P. Enzyme-linked immunosorbent assay, ELISA. III. Quantification of specific antibodies by enzyme-labelled anti-immunoglobulin in antigen-coated tubes. *J Immunol* 1972;109:129-35.
14. Firer MA, Hosking CS, Hill DJ. Cow's milk allergy and eczema: patterns of the antibody response to cow's milk in allergic skin disease. *Clin Allergy* 1982;12:385-90.
15. Casimir G, Gossart B, Vis HL, Duchateau J. Antibody against betalactoglobulin (IgG) and cow's milk allergy [Abstract]. *J ALLERGY CLIN IMMUNOL* 1985;75:206.
16. Brambell FWR. The transmission of antibodies. In: Brambell FWR, ed. *The transmission of immunity from mother to young*. Amsterdam-London: North-Holland Publishing Co. 1970: 242-50.
17. Kjellman NIM, Johansson SGO. Soy versus cow's milk in infants with a biparental history of atopic disease: development of atopic disease and immunoglobulins from birth to four years of age. *Clin Allergy* 1979;9:347-58.
18. Andersson U, Bird G, Britton S. A sequential study of human B lymphocyte function from birth to two years of age. *Acta Paediatr Scand* 1981;70:837-42.
19. Shannon WR. Demonstration of food proteins in human breast milk by anaphylactic experiments on guinea pigs. *Am J Dis Child* 1921;22:223-31.
20. Kulangara AC. The demonstration of ingested wheat antigens in human breast milk. *IRCS Med Sci* 1980;8:19.
21. Kilshaw PJ, Cant AJ. The passage of maternal dietary proteins into human breast milk. *Int Arch Allergy Appl Immunol* 1984;75:8-15.
22. Cant A, Marsden RA, Kilshaw PJ. Egg and cow's milk hypersensitivity in exclusively breast-fed infants with eczema, and detection of egg protein in breast milk. *Br Med J* 1985;291:932-5.
23. Jacobsson I, Lindberg T, Benediktsson B, Hansson BG. Dietary bovine beta-lactoglobulin is transferred to human milk. *Acta Paediatr Scand* 1985;74:342-5.
24. Gerrard JW, Perelmutter L. IgE-mediated allergy to peanut, cow's milk, and egg in children with special reference to maternal diet. *Ann Allergy* 1986;56:351-4.
25. Brandtzaeg P. The B cell system. In: Brostoff J, Challacombe SJ, eds. *Food allergy and intolerance*. London-Philadelphia: Bailliere Tindall, 1987:119-55.