

Influence of type of feeding on lymphocyte function and development of infantile allergy

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Summary

Symptom scores indicating infantile allergy were recorded longitudinally in a group of infants during the first year of life. The symptom scores were related to duration of breast-feeding. Lymphocyte function, measured as the proliferative responsiveness to PHA-stimulation, was related to feeding with breast-milk or cow's milk. Babies with a short duration of breast-feeding; i.e. 1 month or less had significantly higher symptom scores at the ages of 1 month ($P < 0.05$), 3 months ($P < 0.05$), 6 months ($P < 0.001$) and 12 months ($P < 0.05$), than babies who were breast-fed for more than 1 month. The lymphocyte responsiveness to PHA was significantly higher at 3 and 6 months in babies who were fed cow's milk compared with those who were solely or partially breast-fed ($P < 0.05$ and $P < 0.01$ respectively). There was no correlation between symptom scores and lymphocyte responsiveness. It is concluded that infantile allergy is more common in babies with a short duration of breast-feeding and that feeding with cow's milk, compared to breast-feeding, causes a changed lymphocyte reactivity to PHA, which latter finding indicates an altered T-cell function between the two feeding groups.

Introduction

Several studies have shown that early weaning to artificial feeding is more likely to lead to an increased risk of developing atopic allergy in childhood than when breast-feeding is continued for the first months of life (Blair, 1977; Matthew *et al.*, 1977; Chandra, 1979; Saarinen *et al.*, 1979). This allergy-preventing effect of breast-feeding has initiated conflicting hypotheses about the allergic sensitization by dietary antigens. It has been proposed that a transient period of IgA deficiency in early life may represent a period of vulnerability to antigenic exposure (Taylor *et al.*, 1973; Soothill *et al.*, 1976). Such a transient defect combined with early feeding with cow's milk would permit excessive stimulation of IgE-secreting plasma cells at the mucosal surface leading to

sensitization. Animal experiments, however (Jarret, 1977), and a study of infants (Juto, 1980) have shown that excessive amounts of antigen suppress rather than facilitate IgE responses, provided the T-cell function is normal. Recently evidence has accumulated favouring the concept that atopic allergy is a consequence of a disturbed balance between different subpopulations of T cells (Strannegård & Strannegård, 1978). The purpose of this investigation was to examine the possibility that early weaning to cow's milk could induce changes in lymphocyte function possibly leading to symptoms of atopic allergy.

Materials and methods

Participants

All parents of infants born in April–May and July–September 1977 at the Obstetric Clinic of University Hospital, Umeå, were requested to participate in a longitudinal study for a year. During this period there were about 400 deliveries at the Clinic. Of these, parents of sixty-eight gave their written consent to participate. The parents of two infants born in June asked by their own initiative to participate and their babies entered the study when the babies were 3 months old.

The infants were examined by the same physician (P.J.) at the Out-patient Clinic of Pediatrics four times during the study period; i.e. at the ages of 1 month (mean 1 month, range 3–5 weeks), 3 months (mean 3.1 months, range $2\frac{1}{2}$ – $3\frac{3}{4}$ months), 6 months (mean 6.3 months, range $5\frac{3}{4}$ –8 months) and 12 months (mean $12\frac{1}{2}$ months, range $11\frac{1}{2}$ – $14\frac{1}{2}$ months). The numbers of babies who attended at the different ages were: 1 month, sixty-eight; 3 months, sixty-nine; 6 months, fifty-seven; and 12 months, sixty-five.

Type of feeding

The times for onset of bottle-feeding and cessation of breast-feeding (in the following defined as weaning) were recorded. The bottle-fed infants were all, except one, given cow's-milk-based formulas. This particular infant was given a soybean based formula. The median time of weaning was 5 months after birth.

Laboratory studies

Collection of blood samples. The infants were bled by finger-punctures at each visit to the Clinic. By this technique about 1.0–1.5 ml blood was collected. When insufficient volume of blood was obtained the IgE analysis was given priority to the lymphocyte stimulation test.

Serum-IgE concentrations. Serum-IgE concentrations were analysed by using Phadebas PRIST® test-kit system (Pharmacia Diagnostics AB, Uppsala, Sweden). Blood samples of sixty-eight babies at 3 months, fifty-six babies at 6 months and sixty-five babies at 12 months after birth were analysed.

Lymphocyte-stimulation test. The lymphocyte stimulation test was made on a whole blood microculture technique with purified phythemagglutinin (PHA, Leucoagglutinin®, Pharmacia Diagnostics) in a final dilution of 5.0 µg/ml. Heparinized blood of 3-month samples was initially diluted 1:8 and because of lower cell density diluted 1:4 for 6- and 12-month samples in Roswell Park Memorial Institute medium 1640 (RPMI) with 20% pooled human serum, streptomycin 100 µg/ml, penicillin 100 u/ml, and 1.0% glutamin (200 mmol/ml). Portions of 0.1 ml diluted blood were then

delivered to six wells in flat-bottomed Falcon Microtiter II Plates®. To the first three wells was then added 0.1 ml RPMI with antibiotics and to the other three 0.1 ml RPMI with antibiotics and PHA, at a concentration of 10 µg/ml. The plates were incubated for 96 hr in humidified air with 5.0% CO₂ at +37°C. The last 6 hr of incubation the 3-month cultures were pulsed with 0.05 Ci ¹⁴C-thymidine (specific activity 55 mCi/mmol, Radiochemical Centre, Amersham, U.K.) in a volume of 0.25 ml. Because of changed routines in the laboratory the cultures of 6- and 12-months-old subjects were pulsed with 1.0 µCi ³H-thymidine (specific activity 25 Ci/mmol, Radiochemical Centre). The cultures were then harvested onto glass-fibre filters, dried, solubilized in Insta-Flour® and finally the degree of incorporation of traced thymidine was measured in a liquid scintillation counter.

Clinical assessment

The parents at the first visit to the Clinic were advised to notify all kinds of possible symptoms the babies presented. At the visits to the Clinic at 1, 3 and 6 months the parents were questioned by one of us (P.J.) according to a standardized form (Table 1).

Table 1. Clinical symptoms requested for at 1, 3 and 6 months of age. Presence of a symptom yielded 1 scoring point at each age. The scores presented at 3 and 6 months are cumulative

Colic daytime
Colic night
Persistent nasal stuffiness
Persistent rattling respiration
Wheezing
Otitis media
Eczema in the face/neck
Eczema on the trunk/extremities
Itch
Dry skin
Immediate reaction to foods
(colic, urticaria, diarrhoea or wheezing)

Presence of a listed symptom rendered a single scoring point at each of the visits. The recorded scores after each visit were summed up in a total score, and separate scores for symptoms from the skin, gastrointestinal and respiratory tracts respectively.

When the infants were 12-months-old the parents were interviewed and the infants examined by two of us (S.E. and C.M.) without any knowledge of the results of the laboratory investigations, the feeding practice of the babies and family history of atopy.

In the assessment certain symptoms were included giving various scores (Table 2). Prior to the examination the parents were informed of the design of this clinical assessment and were instructed not to give any further information on the infants than that requested. The criteria of eczema were those of Kjellman and Johansson (1976).

Table 2. Clinical symptoms assessed at the age of 12 months

Symptoms	Scoring point
Eczema	
Possible (2), moderate (4), generalized (6)	2-6
Urticaria	
Possible (1), obvious (2)	1-2
Chronic rhinitis	2
Persistent rattling respiration	2
Wheezing	
Once (1), twice (2), ≥ 3 times (4), asthma (6)	1-6
Diarrhoea	
Two weeks (1), > 1 month (2), > 1 month with weight loss (4), positive jejunal biopsy and positive provocation to cow's milk (6)	1-6
Otitis media	
≥ 3 times	2
Chronic otosuppingitis	1
Colic or diarrhoea of certain foods	
Once (1), twice (2)	1-2
Syndroma fatigue	1
Fever	
Three times without any other signs of infection	1

Statistical analyses

The results of symptom scores (Table 3a,b) and lymphocyte responsiveness (Table 4a,b) are presented as arithmetic means $\pm$ 1 s.d., and of IgE concentrations (Table 5) as geometric means $\pm$ 1 s.d. Statistical analysis was performed by using analysis of variance. This analysis of IgE concentrations between different groups was performed with log-transformed values.

Results

Relationship between symptom scores and duration of breast-feeding

When the duration of breast-feeding was related to the symptom scores, it was evident that the critical duration as regards the development of infantile allergy was the first month of life. Thus when breast-feeding ceased at the first month there were significantly higher symptom scores at all the times of assessment than when breast-feeding was continued after that age (1 month, $P < 0.05$; 3 months, $P < 0.05$; 6 months, $P < 0.001$ and 12 months, $P < 0.05$) (Fig. 1). Symptom scores recorded from the respiratory tract and the skin yielded significantly higher scores in the 6-month cumulative score in infants weaned in the first month, compared to those weaned later ($P < 0.05$ and $P < 0.05$ respectively) (Table 3a). The same pattern of symptom scores was found at the ages of 1 and 3 months. These differences were, however, not statistically significant (data not shown). At the age of 12 months, only respiratory symptoms differed significantly between the two groups ($P < 0.001$) (Table 3b). Probable or obvious eczema was more common, however, not significantly at the

Table 3a and b. Symptom scores of gastro-intestinal and respiratory tract and the skin at 6 (3a) and 12 months (3b) in relation to time of weaning. The figures indicate means (± 1 s.d.).

Table 3a

Symptom	Time of weaning		P-value
	The first month <i>n</i> = 9	After the first month <i>n</i> = 48	
Gastro-intestinal	1.6 (± 1.6)	0.6 (± 0.9)	NS
Respiratory	2.9 (± 1.8)	1.6 (± 1.0)	< 0.05
Skin	2.2 (± 3.3)	0.6 (± 0.9)	< 0.05
All symptoms	6.7 (± 3.4)	2.7 (± 1.7)	< 0.001

Table 3b

Symptom	Time of weaning		P-value
	The first month <i>n</i> = 11	After the first month <i>n</i> = 54	
Gastro-intestinal	0.3 (± 0.6)	0.4 (± 1.1)	NS
Respiratory	1.3 (± 2.1)	0.06 (± 0.3)	< 0.001
Skin	1.6 (± 2.4)	0.9 (± 1.3)	NS
All symptoms	3.2 (± 4.5)	1.4 (± 1.8)	< 0.05

12-month assessment in the group with 1-month duration of breast-feeding (45%) than in the group with prolonged time of breast-feeding (22%).

Relationship between lymphocyte responsiveness and feeding with breast-milk and/or cow's milk

The thymidine-incorporation of unstimulated cultures did not differ significantly when related to feeding with breast-milk and/or cow's milk (Table 4a). In contrast in PHA-stimulated cultures the thymidine incorporation was greater in cow's-milk-fed than breast-fed subjects (Table 4b). This difference was statistically significant at 3 and 6 months ($P < 0.05$ and $P < 0.01$ respectively).

Relationship between symptom scores and laboratory parameters

Serum-IgE concentrations were higher in babies with symptom scores above ± 1 s.d., compared to those with symptom scores below ± 1 s.d., however, not to a degree of statistical significance (Table 5). Babies with obvious eczema ($n = 6$) had at the age of 12 months serum-IgE concentrations of 10.6 ± 4.8 u/ml, babies with probable eczema ($n = 11$) 5.4 ± 5.1 u/ml and babies without eczema ($n = 48$) 3.8 ± 3.7 u/ml. None of these differences were statistically significant from each other. The lymphocyte responsiveness to PHA was not correlated to the symptom scores. There was either no correlation between lymphocyte responsiveness and serum IgE (data not shown).

Table 4a and b. Lymphocyte responsiveness of unstimulated (4a) and PHA stimulated (4b) (5.0 µg/ml) whole blood microcultures at various ages of infants fed breast-milk and/or cow's milk. The figures indicate means ($\pm$ 1 s.d.) of thymidin-incorporation.

Table 4a

Age (months)	Type of feeding					
	BM*	n	BM & CM	n	CM†	n
3	179 ($\pm$ 63)	28	137 ($\pm$ 59)	5	175 ($\pm$ 63)	21
6	719 ($\pm$ 229)	8	905 ($\pm$ 604)	17	866 ($\pm$ 344)	32
12			723 ($\pm$ 167)	4	1146 ($\pm$ 718)	58

Table 4b

Ages (months)	Type of feeding					
	BM	n	BM & CM	n	CM	n
3	3634 ($\pm$ 3305)	28	4876 ($\pm$ 3913)	5	6011 ($\pm$ 3537)	21
6	5311 ($\pm$ 3017)	8	8250 ($\pm$ 4460)	17	11565 ($\pm$ 6713)	32
12			5882 ($\pm$ 4148)	4	10857 ($\pm$ 9411)	58

* Breast-milk

† Cow's milk

Statistically significant differences of PHA-stimulated cultures at 3 months between groups fed BM and CM ($P < 0.01$) and BM + BM and CM and CM ($P < 0.05$) and at 6 months between groups fed BM and CM ($P < 0.05$) and BM + BM and CM and CM ($P < 0.01$).

Table 5. Serum-IgE concentrations of 3-, 6- and 12-month-old infants in relation to symptom scores above and below +1 SD at the same ages. The figures indicate geometric means (GM) ($\pm$ 1 s.d.).

Symptom score	Months					
	3		6		12	
	n	GM	n	GM	n	GM
> +1 s.d.	14	2.4 ($\pm$ 3.9)	8	8.8 ($\pm$ 2.7)	5	12.1 ($\pm$ 4.0)
< +1 s.d.	54	1.7 ($\pm$ 0.5)	3	3.8 ($\pm$ 3.6)	60	4.1 ($\pm$ 3.9)
P value		NS		NS		NS

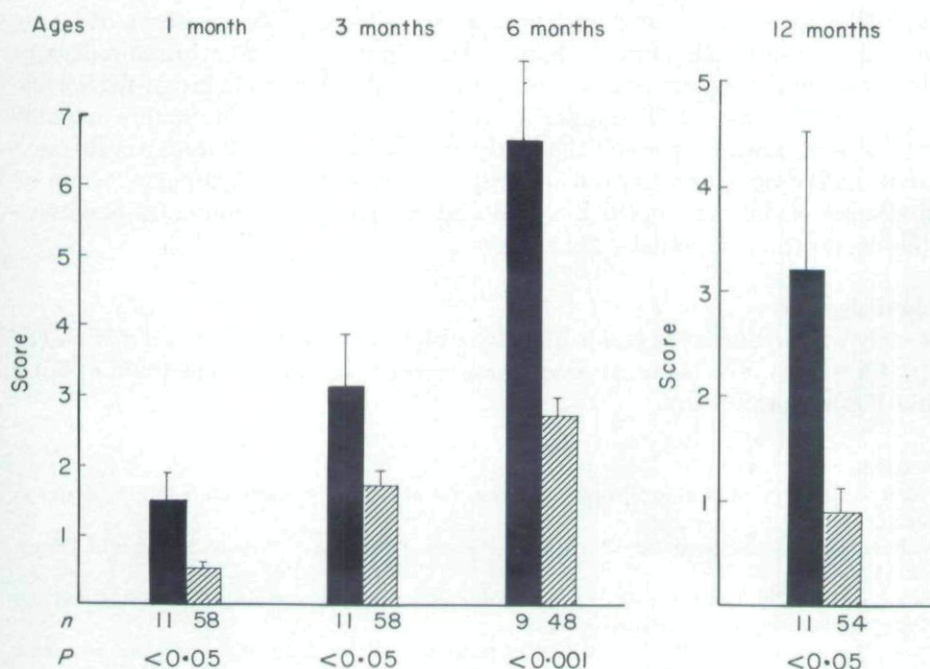


Fig. 1. Relationship between symptom scores indicating infantile allergy recorded at various ages and the time of cessation of breast-feeding. The histograms indicate mean values and the vertical bars ± 1 s.e. mean. Note the different scoring systems at 1, 3, 6 and 12 months. ■, Weaned in first month; ▨, weaned after first month.

Discussion

The results of our study, showing that a short duration of breast-feeding is associated with an increased incidence of infantile allergic symptoms, are consistent with previous studies (Blair, 1977; Matthew *et al.*, 1977; Chandra, 1979; Saarinen *et al.*, 1979; Stintzing & Zetterström, 1979). From the present, and the previous studies it was found that the duration of breast-feeding resulting in most of the allergy-preventing effect was fairly short; i.e. from 1 week to 2 months of breast-feeding.

The diagnosis of infantile allergy may be difficult because some of the symptoms recorded may have been caused by infections. On the other hand these symptoms are also typical for food allergies, particularly allergy to cow's milk in this age group (Bahna, 1978). Food allergy in infants and children is not associated with raised total IgE or presence of specific IgE-antibodies to the same extent as eczema, asthma or rhino-conjunctivitis later in childhood (Dannaeus *et al.*, 1977; Foucard, 1973; Ohman & Johansson, 1974). This might explain the rather poor correlation between the symptom scores recorded and the IgE concentrations.

Since PHA stimulates T cells mainly (Chess *et al.*, 1976) the finding of significantly higher PHA-responsiveness of cow's-milk-fed than breast-fed infants indicates that this T-cell activity differs between the two groups. It may be argued that lipids in breast-milk may interfere and mediate the depression (Morse, Witte & Goodman, 1979). Studies in which PHA-stimulated lymphocytes were cultured in the presence of breast-milk showed a profound decrease of the lymphocyte proliferation (Ogra &

Ogra, 1978), as we also found in lymphocytes cultured in the presence of either breast-milk or cow's milk (unpubl. observ.). Thus the lipid content in breast-milk may not have caused the observed depression of PHA-responsiveness in breast-fed babies. The greater response of T-cell proliferation in cow's-milk-fed infants remains unknown. It is, however, possible that early weaning to cow's milk causes a changed reactivity and co-operation between different subpopulations of T lymphocytes and/or macrophages leading to altered humoral and cell-mediated immune responses to ubiquitous protein compounds, e.g. in food.

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