

Infant feeding patterns affect the subsequent immunological features in cow's milk allergy

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Summary

Background The first exposure to food antigens provokes an immune reaction in an infant, its type depending on the quantity and frequency of doses and the age at introduction, and also being influenced by genetic factors. Most infants develop tolerance to food antigens, but in a small minority they provoke adverse symptoms.

Objective To study the effects of breast and formula feeding and other environmental and genetic factors on the subsequent type of cow's milk allergy classified by the presence or absence of immunoglobulin (Ig) E antibodies to cow's milk.

Methods A cohort of 6209 infants was followed prospectively from birth for symptoms of cow's milk allergy. The infant-feeding regimen was recorded at the maternity hospital and at home. At a mean age of 6.7 months, a total of 118 infants (1.9%) reacted adversely to a challenge with cow's milk. Before the challenge, the response to a skin-prick test with cow's milk and serum IgE cow's milk antibodies was measured.

Results At challenge, 75 (64%) infants showed IgE-positive reactions to cow's milk, their most common symptom being acute-onset urticaria. Significant risk factors for the presence of IgE cow's milk antibodies in allergic infants were long breast-feeding (odds ratio [OR] 3.9, 95% confidence interval [CI] 1.6–9.8), exposure to cow's milk at the maternity hospital (OR 3.5, 95% CI 1.2–10.1) and breast-feeding during the first 2 months at home either exclusively (OR 5.1, 95% CI 1.6–16.4) or combined with infrequent exposure to small amounts of cow's milk (OR 5.7, 95% CI 1.5–21.6). Fifty infants had their first adverse symptoms during exclusive breast-feeding, and 32 infants were sensitized during exclusive breast-feeding. Most of the infants in both cases were IgE-positive: 37 and 23, respectively.

Conclusions In infants who are prone to developing cow's milk allergy, prolonged breast-feeding exclusively or combined with infrequent exposure to small amounts of cow's milk during the first 2 months of life induces development of IgE-mediated response to cow's milk.

Keywords: breast milk, cow's milk, food allergy, IgE, infant feeding

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Introduction

Cow's milk proteins are usually the first foreign antigens encountered by the newborn infants in their diet. Most infants tolerate these proteins readily, but in a small minority they provoke adverse symptoms which usually develop shortly — within a few days to a few months — after introduction of cow's milk formula [1]. The symptoms are

diverse, involving generalized anaphylactic reactions, cutaneous, gastrointestinal or respiratory symptoms, and often a combination of these [2]. The time at which adverse symptoms appear after ingestion of cow's milk varies from minutes to hours [3], or even days [4,5]. In atopic immune reactions to dietary antigens, specific immunoglobulin (Ig) E antibodies may be found in the circulation, or those that are fixed to skin mast cells can be measured by a skin-prick test. A cell-mediated delayed-type immune reaction to dietary antigens may be responsible for symptoms in infants who show no evidence of an IgE-mediated reaction, but

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currently no good tests are available to detect such a hypersensitivity reaction [2].

Our aim was to examine the effects of different feeding patterns and other environmental and genetic factors on the development of cow's milk allergy with and without evidence of an IgE-mediated reaction. We prospectively followed a large group of unselected healthy, term newborn infants for symptoms of cow's milk allergy. The infant-feeding regimen was carefully recorded both at the maternity hospital and at home.

Subjects and methods

To study the effects of different types of supplementary milk given soon after delivery on the subsequent incidence of cow's milk allergy, we recruited 6209 healthy, full-term newborn infants in three maternity hospitals between August 1994 and November 1995 [6]. While in hospital, 824 (13%) infants were breast-fed exclusively, but 5385 (87%) were given supplementary milk. Of these infants, 1789 received adapted liquid cow's milk formula (Tutteli[®], Valio, Finland), 1859 pasteurized human milk (a mixture of milk from multiple donors expressed 1–6 months after delivery) and 1737 extensively hydrolysed whey-based formula (Pepti-Junior[®], Nutricia, The Netherlands). The supplements were changed monthly; the three hospitals each using a different supplement, and thus the supplement that an infant received depended on the month of birth and the hospital where it was born. The time and amount of every feeding was recorded in the delivery ward. The infants were kept in the hospital for a mean of 4 days.

When at home, the mothers were asked to record the infant-feeding regimen daily during the first 8 weeks, and then return the records. They were advised to supplement breast-feeding with adapted cow's milk formula when required and to start solid foods between the ages of 4 and 6 months. Data on infant feeding regimen were also recorded at 6 and 12 months of ages.

Before leaving hospital, the mothers were given written information about symptoms suggestive of cow's milk allergy (urticaria, atopic dermatitis, vomiting, diarrhoea, poor gain in weight, allergic rhinitis or wheezing). If an infant had any of these symptoms, the parents were asked to call one of the authors (KMS). The well-baby clinics of the area, in which every infant is seen eight times during the first 12 months of life, were also informed of the study.

If, after a comprehensive interview, the symptoms were compatible with cow's milk allergy, cow's milk was eliminated from the infant's diet. Those who required supplementary milk were given extensively hydrolysed formula. If the symptoms did not subside in 2–4 weeks, hydrolysed formula was replaced with amino acid-based formula (Nutri-Junior[®], Nutricia, The Netherlands). If the infant's

symptoms had appeared during exclusive breast-feeding and the mother continued to breast-feed, she was advised to avoid cow's milk herself.

After the symptoms had subsided, an open cow's milk challenge was done at the outpatient clinic. Breast-fed infants were challenged when they regularly needed supplementary milk. Before the challenge, mothers were carefully interviewed about the history of the infants' symptoms and feeding-regimen. A blood sample was drawn from the infant and skin-prick tests with liquid cow's milk formula [7] and with commercial allergen extract of egg (Soluprick[®], Allergologisk Laboratorium, Hørsholm, Denmark) were performed according to a standard technique [8]. Histamine dihydrochloride (10 mg/mL) served as a positive and 50% glycerol solution (Soluprick[®], ALK, Hørsholm, Denmark) as a negative control. A weal diameter ≥ 3 mm greater than the negative control was taken as a positive response. Serum samples were stored frozen at -20°C until analysed. Total IgE and specific IgE cow's milk antibodies were measured by the Pharmacia CAP system (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden) [9]. Values for specific IgE cow's milk antibodies $\geq 0.7 \text{ kU}_A/\text{L}$ were considered positive. A serum sample was available for 116 infants.

If the skin-prick test with cow's milk was positive the challenge was started with drops of cow's milk placed on the volar side of the wrist, the cheek and the lips, followed by cow's milk formula given orally in quantities of 1, 10, 50 and 100 mL at intervals of 30–60 min. Infants who were without symptoms next day continued to receive formula at home. The parents were advised to record all adverse symptoms and the daily amounts of cow's milk formula consumed. Every infant was examined for delayed symptoms on the fifth day of the challenge, which was considered positive if one or more of the following symptoms had appeared in the interim: anaphylaxis, urticaria, exanthema, atopic dermatitis, vomiting, diarrhoea, wheezing or allergic rhinitis. Infants reacting adversely to the challenge were given a milk-free diet and those with no symptoms received cow's milk formula. One month later, all infants were examined to confirm the challenge result. The infants with cow's milk allergy are being followed up and re-challenged every 6–12 months until clinical tolerance to cow's milk has developed.

A total of 622 (10%) mothers contacted the authors because their infants had symptoms at home. Of those put on a cow's milk-free diet, 247 infants recovered. Of them, 118 (48%) reacted adversely to the cow's milk challenge, giving a cumulative incidence of 1.9% for cow's milk allergy in the whole cohort. The allergic infants were grouped according to the presence of specific IgE cow's milk antibodies: those with a positive skin-prick test and/or positive by CAP were assigned to the IgE-positive group and those with both tests negative to the IgE-negative group.

While in the maternity hospital, mothers filled in a questionnaire about the family history of atopy defined as asthma, atopic dermatitis, allergic rhinitis or conjunctivitis. The family history of atopy was considered positive if one or both parents and/or one sibling or more had asthma, or symptoms from two different organs. Data on maternal smoking, and numbers of siblings and furred household pets were also recorded.

Pearson's chi-square test and Student's *t*-test were used in statistical comparisons. The data with skewed distribution were analysed after logarithmic transformation. In infants with cow's milk allergy, multivariate logistic regression analysis was used to study the independent contribution of genetic and environmental factors to the risk of having IgE cow's milk antibodies detectable in the skin and/or in the circulation. For this purpose, continuous variables were categorized dichotomously, with the median as the cut-off point. All risk factors were included in the model and the significance of each one was tested with control for all other factors. Analyses were performed using a SPSS (version 7.5 for Windows, SPSS, Chicago, IL, USA) software package.

The ethics committee of each hospital approved the study protocol. Written informed consent was obtained from the mother of each infant entering the study.

Results

At the time of the positive challenge, 75 (64%) of the 118

infants hypersensitive to cow's milk had a positive skin test with cow's milk (weal ≥ 3 mm) and/or were positive by CAP for specific circulatory IgE cow's milk antibodies (≥ 0.7 kU_A/L) and thus were included in the IgE-positive group, although these tests may be inadequate for distinguishing the different reaction types [1,10]. Of the IgE-positive infants, 72 (96%) had a positive skin-prick test and 52 (69%) had increased serum IgE cow's milk antibodies. The IgE-negative group comprised 43 infants with both tests negative. The challenge was performed at mean (95% CI) ages of 7.0 (6.5–7.4) and 6.4 (5.6–7.2) months in the IgE-positive and -negative groups. The sex ratio was the same in the two groups: 53% for boys and 47% for girls.

The mothers noticed their infants' first adverse symptoms to cow's milk at mean (95% CI) ages of 2.9 (2.5–3.3) and 2.8 (2.3–3.4) months in the IgE-positive and -negative groups. The IgE-positive infants more often showed cutaneous symptoms and vomiting immediately (<2 h) after ingestion of cow's milk, whereas, in the IgE-negative group, respiratory symptoms and continuous regurgitation were more prevalent (Table 1). Three IgE-positive infants with anaphylactic reactions at their first exposure to cow's milk at home were admitted to hospital; two of them had been exposed to cow's milk at the maternity hospital.

Elimination diets in the IgE-positive and -negative groups were started before the challenge at mean (95% CI) ages of 5.5 (5.0–6.0) and 5.4 (4.6–6.1) months. Two infants in each group were given amino acid-based formula. From the

Table 1. Symptoms suggestive of cow's milk allergy reported by parents and symptoms at challenge in the IgE-positive and -negative groups of infants with cow's milk allergy

Symptom	Number (%)			
	Symptoms suggestive of cow's milk allergy		Symptoms at challenge	
	IgE-positive <i>n</i> = 75	IgE-negative <i>n</i> = 43	IgE-positive <i>n</i> = 75	IgE-negative <i>n</i> = 43
Urticaria and/or exanthema	50 (67)	4 (9)***	57 (76)	4 (9)***
Atopic dermatitis	68 (91)	38 (88)	21 (28)	31 (72)***
Vomiting	33 (44)	18 (42)	7 (9)	13 (30)**
Immediate vomiting (<2 h)	25 (33)	5 (12)**	7 (9)	4 (9)
Continuous regurgitation	8 (11)	13 (30)**	0	9 (21)***
Diarrhoea	14 (19)	10 (23)	0	10 (23)***
Rhinorrhoea	18 (24)	18 (42)*	9 (12)	7 (16)
Wheezing and/or cough	4 (5)	8 (19)*	1 (1)	6 (14)**
Allergic conjunctivitis	4 (5)	2 (5)	4 (5)	1 (2)
Anaphylactic reaction	3 (4)	0	1 (1)	0
Multiple symptoms	48 (64)	28 (65)	14 (19)	13 (30)

Difference between IgE-positive and -negative groups, χ^2 test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 2. Reaction time and volume of milk consumed from the start of challenge until the positive reaction in relation to IgE positivity or negativity in infants with cow's milk allergy

Challenge variable	Number (%)	
	IgE-positive <i>n</i> = 75	IgE-negative <i>n</i> = 43
<i>Reaction time***</i>		
< 2 h	51 (68)	7 (16)
2–24 h	19 (25)	14 (33)
> 24 h	5 (7)	22 (51)
<i>Cumulative dose of milk***</i>		
< 20 mL	44 (59)	6 (14)
20–200 mL	24 (32)	16 (37)
> 200 mL	7 (9)	21 (49)

*** $P < 0.001$ for the difference between IgE-positive and -negative groups, χ^2 test.

beginning of the successful elimination, the symptoms started to subside after mean intervals (95% CI) of 4 (3–5) and 5 (3–6) days in the IgE-positive and -negative groups. The geometric means (95% CI) for the total duration of the elimination diets were 26 (21–32) and 24 (20–29) days, respectively. Thirteen (11%) infants required no supplements and had no adverse symptoms when breast-fed, although their mothers consumed cow's milk.

At the challenge, a smaller volume of cow's milk elicited adverse symptoms in the IgE-positive than in the IgE-negative infants and the symptoms appeared earlier (Table 2). Urticaria and exanthema were more prevalent in the IgE-positive group, whereas in the IgE-negative group eruption of atopic

dermatitis, gastrointestinal and respiratory symptoms predominated (Table 1). One infant had an anaphylactic reaction at the challenge after ingesting a total of 161 mL of cow's milk formula. The reaction subsided in 1 h. Her skin test with milk was positive (weal 8 mm, histamine dihydrochloride 5 mm) and her value of IgE cow's milk antibodies was increased (11.25 kU_A/L). A drop of milk on the skin elicited urticaria and/or exanthema in 31/68 (46%) IgE-positive infants; six of them were not given milk orally at challenge because their symptoms had been severe.

The skin-prick test with egg was positive in 48 (64%) IgE-positive and 12 (28%) IgE-negative infants ($P < 0.001$), the geometric means (95% CI) for serum total IgE being 48 (34–67) kU/L and 10 (7–14) kU/L, respectively ($P < 0.001$).

The percentage of IgE-positive infants exposed to cow's milk at hospital tended to be greater than that of IgE-negative infants ($P = 0.15$) (Table 3). During the first 8 weeks at home, however, the IgE-positive infants were exposed to cow's milk less frequently and those who were exposed were given smaller amounts of cow's milk during a shorter period. Fifty infants developed their first adverse symptoms to cow's milk during exclusive breast-feeding, 18 of them had been given cow's milk at maternity hospital. Thus 32 infants were sensitized during exclusive breast-feeding. The IgE-positive infants were breast-fed significantly longer: all of them were breast-fed for at least 3.5 months, whereas 15 (35%) IgE-negative infants had been weaned by that age. Also, more IgE-positive infants were still being breast-fed at the time of diagnosis: 62 (83%) vs 14 (33%) ($P < 0.001$). Solid foods were introduced later in the IgE-positive group.

The prevalence of a family history of atopy was 40% in the IgE-positive and 44% in the IgE-negative group; 13 (17%) and 13 (30%) of the mothers, 15 (20%) and seven

Table 3. Infant feeding patterns in the IgE-positive and -negative groups. Continuous variables are expressed as geometric means (95% CI)

Feeding pattern	IgE-positive <i>n</i> = 75	IgE-negative <i>n</i> = 43
Number (%) exposed to cow's milk at maternity hospital	31 (41)	12 (28)
Number (%) exposed to cow's milk at home before 8 weeks of age	30 (40)	26 (61)*
Total duration of exposure (weeks)	1.3 (0.8–2.0)	4.0 (2.9–5.5)***
Daily dose of formula/infant (L)	0.04 (0.03–0.07)	0.15 (0.10–0.24)***
Total volume of formula/infant (L)	0.40 (0.21–0.77)	4.28 (2.10–8.75)***
Cumulative number (%) exposed to cow's milk during the first 8 weeks of age	46 (61)	28 (65)
Number (%) sensitized during exclusive breast-feeding	23 (31)	9 (21)
Number (%) showing symptoms during exclusive breast-feeding	37 (49)	13 (30)*
Duration of total breast-feeding (months)	8.4 (7.8–9.1)	4.5 (3.5–5.7)***
Age at introduction of solid foods (months)	4.0 (3.8–4.2)	3.4 (3.2–3.7)***

Difference between IgE-positive and -negative groups: * $P < 0.05$, χ^2 test. *** $P < 0.001$, Student's *t*-test.

Table 4. Effects of dietary and other environmental and genetic factors on the risk of showing an IgE-positive reaction to cow's milk at the time of diagnosis in infants with cow's milk allergy, according to multivariate logistic-regression analysis†

Variable	Reference category	Odds ratio (95% CI)	P-value
Exposure to cow's milk while in maternity hospital	None	3.5 (1.2–10.1)	<0.05
<i>Total amount of cow's milk formula at home during the first 8 weeks</i>			
No exposure	> 1.5 L	5.1 (1.6–16.4)	<0.01
0.01 to 1.5 L	> 1.5 L	5.7 (1.5–21.6)	<0.05
Breast-feeding for 7.5 months or longer	< 7.5 months	3.9 (1.6–9.8)	<0.01

†Factors tested but found not to be significant were age at first exposure to cow's milk at home, a family history of atopy, maternal smoking and the presence of siblings and furred household pets.

(16%) of the fathers, and six (8%) and four (9%) of the siblings were atopic, respectively. In the IgE-negative group, more mothers smoked: seven (16%) vs two (3%) ($P < 0.01$). The prevalences of siblings and furred household pets were similar in both groups (data not shown).

The effect of diet and other environmental and genetic factors on the development of an IgE-positive reaction to cow's milk in infants with cow's milk allergy was studied using multivariate logistic regression analysis (Table 4). The risk was increased by exposure to cow's milk at hospital, and by no exposure or exposure to only small amounts of cow's milk at home during the first 8 weeks of life. Also, long breast-feeding increased the risk. Factors tested but found not to be significant were age of first exposure to cow's milk at home, a family history of atopy, maternal smoking and the presence of siblings and furred household pets.

Discussion

This prospective study on an unselected group of infants with cow's milk allergy shows that different feeding patterns during the first few months of life modulate the subsequent hypersensitivity reaction to cow's milk. In hypersensitive infants, breast-feeding exclusively or combined with infrequent exposure to small amounts of cow's milk during the first 2 months of life was associated with the development of IgE cow's milk antibodies, whereas infants showing reactions not associated with specific IgE antibodies had been exposed to larger volumes of cow's milk more frequently. The latter infants had received on average almost four times as much cow's milk daily, and the cumulative volume was 10 times as great. In animal studies, feeding small amounts of antigen-stimulated IgE production [11], whereas the specific IgE response was suppressed by a larger antigen dose, the degree of suppression depending on the dose [12]. Previously, in a group of 23 infants with cow's milk allergy,

the highest concentrations of specific IgE antibodies were found in those who were exposed to the smallest volumes of cow's milk formula before diagnosis [13]. In the same study, infrequent exposure to cow's milk was associated with development of IgE cow's milk antibodies [13]. Similarly, we found that, of the infants who were exposed to cow's milk during the first 2 months of life at home, those who were IgE-positive had received formula only one-third as frequently as those who were IgE-negative.

The presence of skin-fixed and circulating specific IgE cow's milk antibodies was assessed at the time of diagnosis (at a mean age of 7 months). However, classification of the cow's milk-provoked reactions as IgE- and non-IgE-mediated according to these tests may not be entirely valid, since the sensitivity of these tests is lower in infants under 1 year of age [1,5]. Recently, moreover, a local duodenal IgE-mediated response to cow's milk was found in biopsy specimens taken during a challenge from infants with cow's milk allergy of delayed type and in whom skin and blood tests for specific IgE antibodies were negative [10].

Intestinal absorption of macromolecules is greatest during first 2 months of life [14], which is the critical period for induction of a specific immune response to dietary antigens [15]. Two-thirds of the infants in both groups were given cow's milk during the first 2 months of life, but those in the IgE-positive group tended to be exposed earlier. Previously we showed that exposure to cow's milk at maternity hospital increases the risk of cow's milk allergy [6], as also observed earlier [16]. In the present study, hospital exposure to cow's milk increased the risk of developing IgE cow's milk antibodies when followed by exclusive breast-feeding or infrequent exposure to small amounts of cow's milk formula, whereas the infants who received larger volumes of cow's milk more frequently after hospital exposure developed specific IgE antibodies less frequently.

Fifty infants showed their first symptoms during exclusive breast-feeding, but 18 of these had been exposed to cow's milk at hospital. Thus, at least 32 infants (0.5% of the whole study population) were probably sensitized to cow's milk proteins shown to be present in breast milk [17]. However, other ways of sensitization, like inhalant exposure to food antigens, are also possible [18]. A similar incidence (0.4%) of sensitization to cow's milk during exclusive breast-feeding was reported in another study [19]. In contrast, in yet another study, all the infants who developed cow's milk allergy during exclusive breast-feeding had been exposed to cow's milk at maternity hospital [16]. Maternal avoidance of antigens is known to reduce development of specific IgE antibodies in breast-fed infants [20]. In the present study, some mothers eliminated cow's milk from their diet after the infant's symptoms had developed, but none avoided cow's milk initially. We infer that cow's milk antigens in breast milk are capable of both sensitizing and triggering the allergic immune reaction to cow's milk; the reaction in both cases being more frequently IgE-mediated.

Parental atopy was a significant risk factor for cow's milk allergy in the present cohort [6], as also reported earlier [5,19]. In the present study, no association was found between a family history of atopy and the immunological reaction type to cow's milk in infants with cow's milk allergy. In another study, a positive atopic heredity prevailed in infants with IgE-mediated cow's milk allergy [21]. Maternal smoking was more prevalent in the IgE-negative group. Previously, it has been related to wheezing in nonatopic infants [22].

As in earlier studies [4,21,23], most infants in the IgE-positive group showed symptoms at challenge within 2 h, the most frequent rapidly developing symptoms being urticaria and exanthema. Similar percentages of infants in the two groups reacted to the challenge between 2 and 24 h but their symptoms differed: of the IgE-positive infants, 68% had urticaria or exanthema, whereas, of the IgE-negative infants 71% exhibited a morbilliform rash as an early symptom of atopic dermatitis. Most delayed reactions were seen among the IgE-negative infants and were associated with deterioration of atopic dermatitis and gastrointestinal symptoms, as described earlier [4,21,23]. However, five infants in the IgE-positive group reacted only after 24 h, all with eruption of atopic dermatitis, corresponding with the findings of Hill *et al.* [4]. Five infants with multiple symptoms at challenge developed allergic conjunctivitis, which is a rare but recognized symptom of cow's milk allergy [2]. Almost half of the IgE-positive infants had immediate contact urticaria to cow's milk, as is commonly seen in infants with food allergy [24]. Anaphylactic reactions to foods are rare [25] but may occur on exposure to cow's milk [26], as also shown in the present study. Therefore, in six infants we based our diagnosis on a

positive skin-prick test combined with a history of serious symptoms. One further infant unexpectedly had an anaphylactic reaction at challenge. For practical reasons, an open cow's milk challenge, which is shown to be an adequate method in this age group [27], was used for the diagnosis.

We conclude that, in infants prone to developing cow's milk allergy, short exposure to cow's milk at the maternity hospital and prolonged breast-feeding exclusively or combined with infrequent intake of small amounts of cow's milk stimulates specific IgE antibody production, whereas frequent feeding of larger volumes of cow's milk induces development of the non-IgE-mediated delayed type hypersensitivity to cow's milk.

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