

Elevated serum immunoglobulin E in T cell-deficient infants fed cow's milk

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Serum IgE levels at various ages during infancy were related to the number of T cells assessed at the age of 1 mo and type of feeding. Cow's milk-fed babies with low T cell counts had higher IgE at the ages of 3 and 6 mo than breast-fed babies with low T cell counts. Of the babies fed cow's milk, those with low T cell counts had higher IgE levels than those with normal T cell counts. Onset of cow's milk feeding before the age of 3 mo in babies with low T cell counts was associated with continuously elevated IgE during the first year of life, as compared with babies with normal T cell counts. However, when cow's milk feeding was instituted after the age of 3 mo such a difference was not noted. It is concluded that in T cell-deficient infants there might exist a critical period during which onset of cow's milk feeding is associated with subsequently increased IgE synthesis.

Recently it has been proposed that a physiologic mechanism preventing the development of atopic allergy depends on the supply of sufficient amounts of antigens for activation of lymphocytes with suppressive properties on the formation of IgE antibodies.¹ According to this hypothesis, individuals with atopy have a deficiency in a particular subpopulation of T lymphocytes (T cells) that suppress IgE antibody synthesis. The antithesis of this claims that atopy is a consequence of overstimulation by antigens during a critical vulnerable period in early life.² Individuals with transiently lower levels of serum IgA would, according to this hypothesis, be at high risk to develop atopy when stimulated with antigens during the period of IgA deficiency. These hypotheses initiated a prospective study of infants in whom the development of serum IgE was followed up with respect to number of T cells and feeding with small and large amounts of antigen, i.e., breast milk and cow's milk, respectively.

MATERIAL AND METHODS

All parents of newborn infants born during the period April to September 1977 at the Obstetric Clinic, University Hospital (Umeå, Sweden) were requested to participate with their babies in this prospective study. During this period there were about 400 deliveries at the clinic. A total of 68 parents gave their written consent to participate. Of these 68 participants 38 had a family history positive of atopic allergy in parents or older siblings of the infants.

Fifty infants were at the age of 1 mo subjected to assessment of T cells by rosette formation with sheep erythrocytes. Of these 31 had a family history positive of atopic allergy. In the test fetal calf serum was included. The incubation time for the rosette formation was 2 to 3 hr in the cold. The method used and the results of the assay have been presented in detail elsewhere.³ As has also been described previously, the occurrence of low T cell count was correlated with heredity for atopic allergy.³ The distribution of the T cells showed a bimodal pattern; i.e., one group had $\leq 45\%$ (12 infants), and another group had $>45\%$ (38 infants). In the group with low numbers of T cells 10 had a family history of atopic allergy. In this presentation $\leq 45\%$ is considered low, and $>45\%$ normal.

Serum samples obtained by finger punctures 3, 6, and 12 mo after birth were tested for IgE concentrations using Phadebas paper radioimmunosorbent test (PRIST) commercial test kit system (Pharmacia Diagnostics AB, Uppsala, Sweden). The IgE concentrations in the total material correlated neither to feeding with breast or cow's milk nor to heredity for allergy at the ages of 3 and 6 mo. In 12-mo-old babies the highest values were recorded in those with heredity for allergy and longest duration of breast-feeding. These results are presented in detail elsewhere.⁴ In the 50

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TABLE I. Serum IgE (geometric means $\pm$ 1 SD) at different ages in relation to low or normal T cell count determined at the age of 1 mo

T cell count	Age (mo)								
	3			6			12		
	Number of subjects	Geometric means	1 SD*	Number of subjects	Geometric means	1 SD	Number of subjects	Geometric means	1 SD
Low	11	2.3	1.0-5.5	11	5.9	1.8-19.1	12	5.3	2.0-13.6
Normal	37	1.2	0.4-3.4	32	3.2	3.1-10.0	34	3.3	0.8-13.2
Total	48	1.3	0.5-3.6	43	3.6	1.1-11.6	46	4.4	1.1-17.0

*1 SD = range of $\pm$ 1 SD.**TABLE II.** Coefficients of correlation between serum IgE levels and T cell counts in 3- and 6-mo-old infants with respect to feeding with breast or cow's milk

	Age (mo)			
	3		6	
	Number of subjects	Coefficient of correlation	Number of subjects	Coefficient of correlation
Breast milk or breast and cow's milk	29	$r = 0.08$, NS	17	$r = 0.14$, NS
Cow's milk	19	$r = -0.75$, $p < 0.001$	26	$r = -0.61$, $p < 0.001$

NS = not significant.

babies in whom T cells were assessed serum IgE concentrations were determined at the ages of 3 (mean 3, range 2.5 to 3.75), 6 (mean 6.1, range 5.75 to 6.75), and 12 mo (mean 12.3, range 11.5 to 13.5).

The times of onset of feeding with cow's milk and cessation of breast-feeding (i.e., in the following weaning) were carefully registered. One infant received a soybean-based formula from the age of 2 wk until 8 mo. All other infants receiving formula were given commercially available cow's milk-based preparations. At the age of 2 mo fruit juices were introduced in the diet, and at 3 mo solid foods, i.e., fruits and potatoes, were given. Mothers who were still breast-feeding their babies at the age of 3 mo without any addition of formula were advised to continue breast-feeding until the age of 6 mo, with the exclusion of dairy products, cereals, fish, and egg. Breast-feeding continued at least to 6 mo in 17 babies, and of them 10 had not been given any food containing cow's milk, cereals, fish, or egg. The median time for onset of feeding with cow's milk was 2.75 mo and for weaning was 5 mo. At the age of 12 mo four babies were still being breast-fed. At the age of 1 mo 50 infants were included in this part of the study, but of them four dropped out of the study at the age of 12 mo.

Statistical analysis

For analysis of serum IgE between different groups the two-tailed Mann-Whitney U test was used. Pearson correlation coefficient was used for calculation of correlations be-

tween numbers and serum IgE levels. Serum IgE values are given as geometric means $\pm$ standard deviation.

RESULTS

Relationship between T cell count and development of serum IgE

When the babies were divided into two groups according to T cell counts ($\leq 45\%$ and $>45\%$, respectively) it was found that IgE levels were highest in the group with low T cell counts at 3, 6, and 12 mo after birth (Table I). The differences observed, however, were not statistically significant.

Influence of feeding on serum IgE levels with regard to T cell count

In infants with low T cell count exclusive feeding with cow's milk was associated with significantly higher levels of IgE than was exclusive feeding with breast milk (Fig. 1, A and B). This finding was evident both when 3- and 6-mo serum specimens were compared ($p = 0.02$ and $p < 0.02$, respectively). Babies fed both cow's and breast milk had IgE levels intermediate to those found in those fed cow's milk or breast milk.

In contrast to the findings obtained in infants with low T cell counts, cow's milk feeding was not asso-

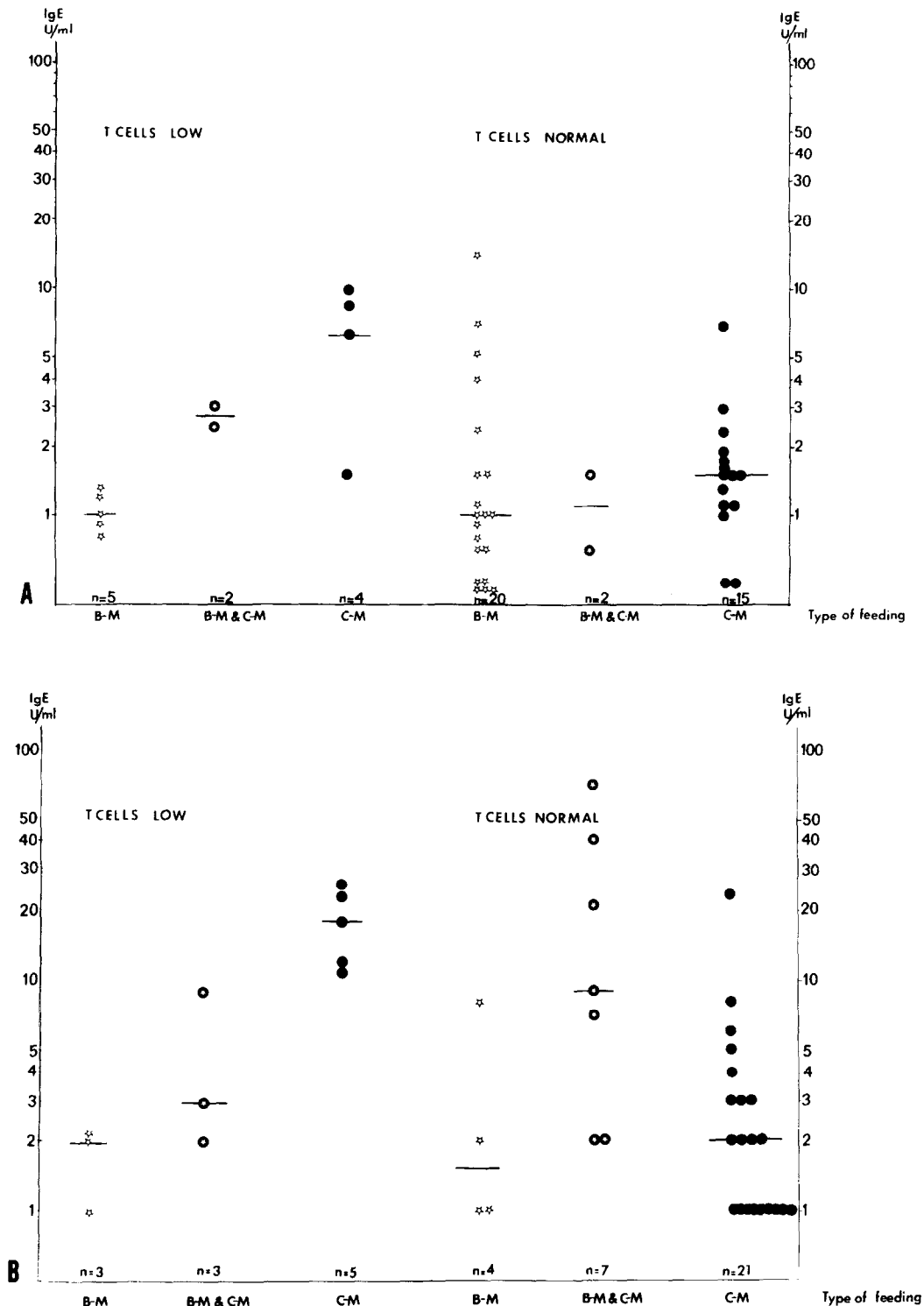


FIG. 1. Relationship of serum IgE in (A) 3-mo-old and (B) 6-mo-old infants to T cell count with regard to feeding with breast and cow's milk. Horizontal bars indicate median values. B-M, breast-milk; C-M, cow's milk.

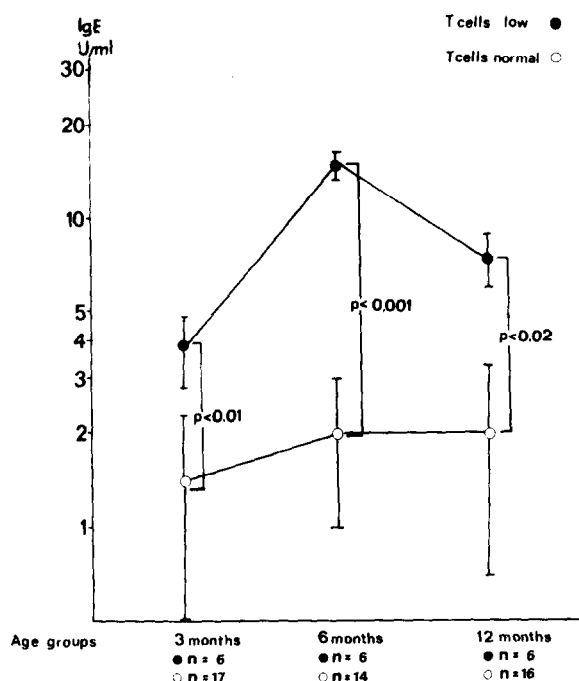


FIG. 2A. Development of serum IgE during first year of life in infants with early onset of cow's milk feeding (before 3 mo) with regard to T cell count. IgE values are depicted as geometric means $\pm$ 1 SD.

ciated with higher IgE levels in infants who had normal T cell counts. Instead, in this group the highest IgE levels were recorded in 6-mo-old infants fed both cow's and breast milk. As shown in Fig. 1, B, the mean IgE level in the latter group was significantly higher than in both the cow's milk ($p < 0.01$) and breast-fed ($p < 0.05$) groups.

In babies fed only cow's milk there was an evident association between low T cell counts and high IgE levels (Fig. 1, A and B). Thus the IgE levels in T cell-deficient infants were significantly higher than in infants with normal T cell counts both at 3 ($p < 0.05$) and at 6 mo ($p < 0.002$) of age. This finding in babies fed cow's milk, described as coefficients of correlation between number of T cells and serum IgE levels, showed significant negative correlations in 3- and 6-mo-old subjects ($r = -0.75$, $p < 0.001$ and $r = -0.61$, $p < 0.001$, respectively) (Table II). In breast-fed babies or those fed breast and cow's milk there was no such correlation (Table II).

Development of serum IgE levels in infants given cow's milk at an early age in relation to T cell count

Of the 50 infants participating in the study 23 were given cow's milk before the age of 3 mo. Of these six had low and 17 had normal T cell counts. When the two groups were compared it was evident that IgE

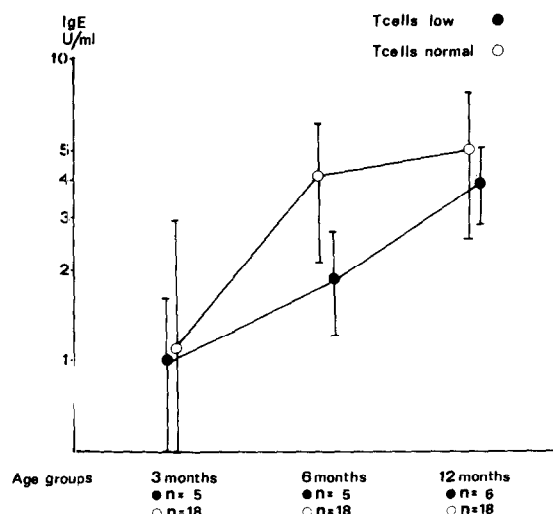


FIG. 2B. Development of serum IgE during first year of life in infants with late onset of cow's milk feeding (after 3 mo) with regard to T cell count. IgE values are depicted as geometric means $\pm$ 1 SD.

levels were consistently higher in babies with low T cell counts throughout the first year of life (Fig. 2A). In infants given cow's milk after 3 mo there were no significant differences in IgE levels with respect to number of T cells (Fig. 2B).

Relationship of IgE levels in 12-mo-old babies to number of T cells and time of cessation of breast-feeding

The results described above indicate that low T cell count and early onset of cow's milk feeding predispose to development of high IgE levels. This kind of relationship was further demonstrable when the children were divided into four groups defined by low or normal T cell counts and by cessation of breast-feeding before or after 6 mo. As shown in Fig. 3, in children weaned before 6 mo low T cell counts were associated with higher levels of IgE than were normal T cell counts ($p < 0.002$). In contrast there was no such difference in babies weaned after 6 mo. In infants with normal T cell counts and in whom breast-feeding continued after the age of 6 mo IgE was higher than in infants also with normal T cell counts but in whom breast-feeding was discontinued before that age ($p < 0.05$). In babies with low T cell counts the difference was the opposite, because early weaning was associated with higher IgE than was late weaning ($p < 0.02$).

DISCUSSION

We have recently shown that heredity for allergy in newborn babies is associated with a relative T cell deficiency.³ This finding suggests that the T cell

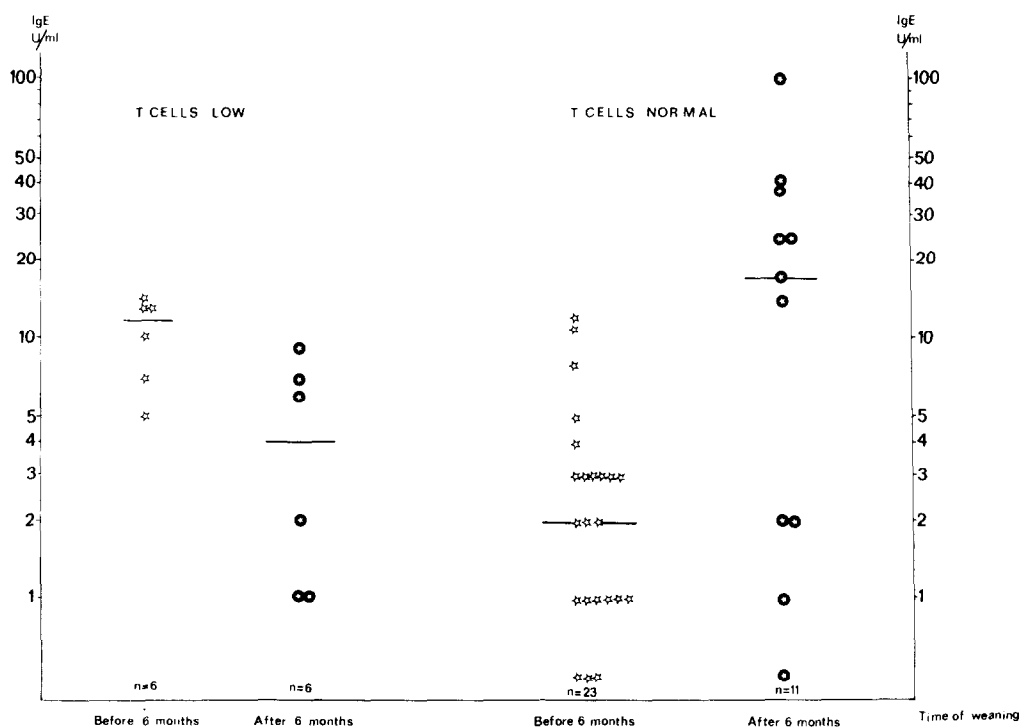


FIG. 3. Relationship of serum IgE in 12-mo-old infants to T cell count and cessation of breast-feeding before and after 6 mo of age. Horizontal bars indicate median values.

deficiency associated with atopic disease (reviewed by Strannegård and Strannegård⁵) is primary and of causal importance in the development of the disease. If this is the case, it would be expected that a T cell deficiency demonstrable at an early age should predispose to development of hyperresponsiveness as regards IgE antibody levels and possibly later reagin-mediated allergic disease. In this report data are presented to show that infants with a relative T cell deficiency do develop raised IgE levels when subjected to antigenic stimulation in the form of cow's milk. The findings of an association between heredity for allergy, low T cell counts, and hyperproduction of IgE strengthens the idea that the genetically determined basis for atopic disease is a relative T cell deficiency.

In animal experiments it has been demonstrated that the production of IgE antibodies is unusually sensitive to influences by helper and suppressor T cells.^{1, 6, 7} The difference between "high responder" and "low responder" strains of inbred mice in terms of their ability to mount IgE responses is probably that the former have low and the latter high activity of antigen nonspecific suppressor T cells.^{1, 6, 7} Thus in mice the "allergic" phenotype appears to be characterized by a relative deficiency of certain types of suppressor T cells. Results of clinical studies suggest that in manifest atopic disease there is also a

deficiency of suppressor cells,⁸ and in particular a deficiency of IgE-class specific regulatory T cells.⁹ These results suggest that a similar mechanism may operate in infants.

Although the nature of the defective T cells has not been directly studied in this investigation, there is indirect evidence for an IgE regulatory T cell deficiency in infants with low T cell counts. Evidence for this is based on the influence of cow's milk feeding on the IgE levels. Thus introduction of large amounts of antigen (cow's milk) in infants with low T cell counts results in increased IgE levels, as compared with in infants with normal T cell counts but similar feeding. In babies with low T cell counts those receiving cow's milk had at the ages of 3 and 6 mo higher IgE than those being fed breast milk. This kind of difference was not observed in infants at the same ages with normal T cell counts. These differences suggest that the physiologic mechanism is disturbed in babies with low T cell counts. This suggestion is in agreement with the hypothesis proposed by Jarret,¹ who has pointed out that introduction of large amounts of antigen leads to activation of IgE-suppressing mechanisms in animals. Also in agreement with this hypothesis is the finding in 6-mo-old infants that feeding with moderate doses of antigen (both breast and cow's milk) was associated with higher IgE levels than feeding with large doses of

antigen (cow's milk) in infants with presumed intact suppressor function (normal T cell counts).

The time of onset of feeding with cow's milk and of weaning appears to be crucial for the development of IgE responses. Thus T cell-deficient babies developed higher IgE levels than babies with normal T cell counts when the onset of feeding with cow's milk was early. This rise of IgE persisted throughout the study period. When the onset of cow's milk feeding occurred later this type of difference was not noted. These findings may suggest the existence of a critical period during early life when antigenic stimulation leads to excessive production of IgE antibodies. A tentative explanation for this phenomenon is that the maturation of T cells with suppressive properties on IgE antibody synthesis is delayed in individuals with low T cell counts in early life.

It may seem paradoxical that in babies with normal T cell counts late weaning was associated with higher IgE levels at the age of 12 mo than early weaning. This, however, may merely be a reflection of the kinetics of IgE antibody formation. Thus the babies weaned late conceivably were in the stimulatory phase of IgE response. Those weaned early probably had passed that phase. Prolonged and massive stimulation by antigens might have suppressed their IgE antibody synthesis. Again this mechanism should be assumed to be operative only in individuals with intact suppressor function.

In conclusion, the findings obtained in this study support the concept that small amounts of antigen

initiate and facilitate the syntheses of IgE antibodies and that large amounts inhibit this synthesis, provided the T cell function as regards control of IgE synthesis is normal. In individuals with low T cell count the early onset of feeding with cow's milk is followed by elevated IgE, which may indirectly suggest an impaired T cell suppression of IgE synthesis.

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