Cord blood lymphocyte responses to food antigens for the prediction of allergic disorders

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Abstract

Proliferative responses of cord blood lymphocytes (CBLs) to food antigens and cord blood IgE concentrations were measured in 37 full term newborn infants for the prediction of allergic disorders. In these 37 infants who were followed up for two years, allergic history of the family was found in four (sensitivity 57.1%) and cord blood IgE concentrations were greater than 0.5 IU/ml in three (sensitivity 42.9%) of seven infants who developed allergic disorders. When CBLs were stimulated twice by ovalbumin or bovine serum albumin, the value of the stimulation index in proliferative responses of CBLs to ovalbumin or bovine serum albumin was greater than 1.5 in six (sensitivity 85.7%) of seven infants who developed allergic disorders. The specificity of the responses of CBLs in the prediction of the development of allergic disorders was 93.3%. The proliferative responses of CBLs to food antigens were useful in the prediction of not only development of allergic disorders but also offending allergens. These observations provide further evidence that sensitisation is occurring in utero. This would appear to be increasingly important in the genesis of early atopic problems. As our follow up is only two years, in utero sensitisation is a prediction for the early development of atopic disease but only longer follow up will show whether this holds good for allergic disorders at any age.

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Allergy often begins early in life,^{1 2} in many cases during the first year. Patients who suffer from allergic diseases are very difficult to cure completely. Prevention of allergic disorders in infants has been attempted by means of food and aeroallergen avoidance.³ The detection of a newborn at high risk of allergy is therefore important. Michel *et al*,⁴ and Magnusson⁵ reported that the IgE concentrations of cord blood appeared to be a good predictor of the development of allergy during infancy. However, it is not sufficient to predict 'high allergic risk newborns' on the basis of increased IgE concentrations of cord blood alone, because a raised cord blood IgE concentration is specific but not sensitive in the prediction of the development of allergic disorders.⁵⁻⁷ Moreover, Ruiz *et al* report that maternal blood may have contaminated cord blood in 7% of their cases.⁸ Allergic disorders that begin early in life are often atopic dermatitis and food allergy such as hens' egg and cows' milk sensitivities. We previously

reported that the combination of the radioallergen sorbent test and the detection of proliferative responses of peripheral blood lymphocytes to each food allergen is very useful in the diagnosis of hypersensitivity in children with atopic dermatitis who are sensitive to each food.⁹ In this study, cord blood IgE concentrations and proliferative responses of cord blood lymphocytes (CBLs) to each food antigen were measured for the prediction of the development of allergy during infancy.

Subjects and methods

SUBJECTS

Thirty seven full term newborn infants were investigated. Cord blood was collected immediately after birth by direct needle aspiration of the umbilical vein.

CORD BLOOD IgE CONCENTRATIONS

Cord blood IgE concentrations were measured by the enzyme linked immunoassay as previously described by Pene *et al* using a rabbit anti-IgE immunoglobulin (Dako), a murine antihuman IgE monoclonal antibody (Medical Biological Laboratory), and a goat antimouse immunoglobulin coupled to alkaline phosphatase (Sigma).¹⁰ A standard IgE serum (Behring) was used as a reference. The limit of the sensitivity of the assay was 0.1 IU/ml.

CELL PREPARATION AND CULTURE

CBLs were isolated from heparinised cord blood by gradient centrifugation in Ficoll-Paque (Pharmacia). CBLs were suspended to give a density of 10⁶/ml in the culture medium consisting of Roswell Park Memorial Institute 1640 medium supplemented with 15% pooled human AB⁺ serum, L-glutamine (2 mM), penicillin (100 units/ml), and streptomycin (100 µg/ml). In the case where CBLs were stimulated once by each food antigen, CBLs (2 × 10⁵) were cultured at 0.2 ml per well in round bottomed microtest plates (A/S Nunc) with or without various concentrations of food antigens (0.25, 2.5, 25 µg/ml, respectively) at 37°C for four days in a humidified atmosphere containing 5% carbon dioxide.⁹ In the case where CBLs were stimulated twice by each food antigen, CBLs (10⁶/ml) were preincubated at 2 ml per tube in the culture test tube with or without each food antigen (2.5 µg/ml) at 37°C for various numbers of days in a humidified atmosphere containing 5% carbon dioxide. Thereafter, cells were washed three times with phosphate buffered

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saline and resuspended to give a density of $10^6/\text{ml}$ in the culture medium. Subsequently, the CBLs (2×10^5) were cultured at 0.2 ml per well in round bottomed microtest plates with or without various concentrations of the same food antigen (0.25 , 2.5 , $25 \text{ } \mu\text{g/ml}$, respectively) at 37°C for various numbers of days in a humidified atmosphere containing 5% carbon dioxide.

PROLIFERATIVE RESPONSES

DNA synthesis was measured by adding $0.5 \text{ } \mu\text{Ci}$ [^3H] thymidine (specific activity: 43 Ci/mmol , Amersham) per well four hours before harvesting onto glass fibre filters. ^3H incorporation was measured by liquid scintillation counting, and the results were expressed as the mean of a triplicate. To compare responses between individuals, we also expressed the results as a stimulation index (SI; cpm, counts/minute).

$$\text{SI} = \frac{\text{Mean cpm in cultures stimulated with food antigens}}{\text{Mean cpm in unstimulated cultures}}$$

ANTIGENS

Ovalbumin and bovine serum albumin were purchased from Wako Junyaku (Japan). The purity of ovalbumin or bovine serum albumin used was confirmed by sodium dodecylsulfate polyacrylamide gel electrophoresis as a single band, 45 K or 66 K , respectively.

CONDUCT OF THE STUDY

Cord blood IgE concentrations and proliferative responses of CBLs to each food antigen were measured at birth. The allergic history of the family (parents and siblings) was also investigated at that time.

All 37 newborn infants were followed up for two years. Allergic disorders or symptoms (atopic dermatitis, bronchial asthma, allergic rhinitis, food allergy, wheezy bronchitis) which developed in the infants were investigated. Clinical criteria that defined allergy were chosen

according to those of Orgel *et al.*,¹ and Kjellman.¹¹ Diagnosis of atopic dermatitis was defined by the criteria of Hanifin.¹² The severity of atopic dermatitis was graded by the food challenge symptom scores for skin of Bock *et al.*¹³ Diagnosis of food allergy was defined on the basis of clinical history and double blind placebo controlled food challenges.¹³ Consequently, the relationships among allergic history of the family (parents and siblings), cord blood IgE concentrations, proliferative responses of CBLs to each food antigen, and the development of allergic disorders in the infant for two years were investigated.

STATISTICAL ANALYSIS

Frequencies were compared by Fisher's exact test (2×2 contingency tables).

Results

PROLIFERATIVE RESPONSES OF CBLs TO EACH FOOD ANTIGEN

CBLs stimulated twice by ovalbumin or bovine serum albumin exhibited proliferative responses in some subjects. As the peak proliferation was seen under the culture conditions of five day preincubation and a subsequent three day culture with a concentration of 2.5 or $25 \text{ } \mu\text{g/ml}$ of each antigen (figure), cultures were routinely harvested on these culture days. The maximal proliferative responses of CBLs stimulated once and twice by ovalbumin or bovine serum albumin for the whole series of all 37 subjects are shown in table 1, in the order of the values of the SI in responses to ovalbumin or bovine serum albumin. Although CBLs stimulated once by ovalbumin or bovine serum albumin showed no proliferative responses in any subject, CBLs stimulated twice exhibited proliferative responses in some subjects. For convenience, subjects were divided into two groups according to the values of the SI: greater than 1.5 and less than 1.5 . SI values greater than 1.5 in either ovalbumin stimulation or bovine serum albumin stimulation were seen in eight (21.6% , subjects 1–8) of 37 subjects, as shown in table 1.

CORD BLOOD IgE CONCENTRATIONS AND ALLERGIC HISTORY OF THE FAMILY

Table 1 also shows cord blood IgE concentrations and the allergic history of the family for each subject. According to the report previously described by Michel *et al.*,⁴ subjects were divided into two groups according to the cord blood IgE concentrations: greater than 0.5 IU/ml and less than 0.5 IU/ml . Cord blood IgE concentrations were greater than 0.5 IU/ml in 10 (27.0%) of 37 subjects. There was allergic history of the family (parents and siblings) in 11 (29.7%) of 37 subjects.

RELATIONSHIP BETWEEN VARIABLES FOR PREDICTION

There was allergic history of the family (parents and siblings) in four (40.0%) of 10 subjects whose cord blood IgE concentrations were

A time response study of proliferative responses of CBLs stimulated by ovalbumin. (A) Proliferative responses of CBLs stimulated once by ovalbumin. The X axis shows culture periods; $\circ$ — $\circ$: $25 \text{ } \mu\text{g/ml}$ of ovalbumin, $\bullet$ — $\bullet$: $2.5 \text{ } \mu\text{g/ml}$ of ovalbumin, $\triangle$ — $\triangle$: $0.25 \text{ } \mu\text{g/ml}$ of ovalbumin. (B) Proliferative responses of CBLs stimulated twice by ovalbumin. The X axis shows culture periods after three, five, or seven day preincubation with a concentration of $2.5 \text{ } \mu\text{g/ml}$ of ovalbumin; $\bullet$ — $\bullet$: cultures with $2.5 \text{ } \mu\text{g/ml}$ of ovalbumin after three day preincubation, $\circ$ — $\circ$: cultures with $25 \text{ } \mu\text{g/ml}$ of ovalbumin after five day preincubation, $\bullet$ — $\bullet$: cultures with $2.5 \text{ } \mu\text{g/ml}$ of ovalbumin after five day preincubation, $\triangle$ — $\triangle$: cultures with $0.25 \text{ } \mu\text{g/ml}$ of ovalbumin after seven day preincubation.

Table 1 Cord blood IgE concentrations, proliferative responses of CBLs stimulated once or twice by ovalbumin or bovine serum albumin, allergic history of the family, and allergic disorders developed in infants who were followed up for two years

Subjects	Allergic history of the family	Cord blood IgE (IU/ml)	Proliferative responses (SI*) of CBLs stimulated				Allergic disorders developed in infants
			Once		Twice		
			Ovalbumin	Bovine serum albumin	Ovalbumin	Bovine serum albumin	
1	B(AD)	1.1†	0.59	1.24	1.04	3.29†	AD (severe, cows' milk, 4 months)
2		0.2	1.23	1.51	2.81†	1.17	AD (moderate, hens' egg and soybean, 11 months)
3		<0.1	1.79	0.54	2.45†	2.15†	AD (mild, hens' egg, 8 months), WH (1.3 years)
4		2.3†	1.03	1.03	1.28	2.25†	
5	M(BA)	0.2	0.94	0.71	1.07	2.07†	AD (moderate, cows' milk, 4 months)
6	F(AR)	<0.1	0.78	0.95	1.82†	2.00†	AD (moderate, hens' egg, 1 year), WH (1 year)
7		2.7†	0.80	0.58	1.82†	1.27	AD (severe, hens' egg, 11 months)
8		0.7†	0.97	1.25	1.10	1.79†	
9	M(AR), B(AD)	0.3	0.72	0.69	1.44	1.16	
10		<0.1	0.97	0.77	0.99	1.34	
11	F(AR)	0.8†	0.92	0.84	0.94	1.31	
12		<0.1	0.66	1.01	1.27	0.83	
13		0.2	0.56	0.67	1.23	1.17	
14		<0.1	1.10	0.83	1.23	1.04	
15		<0.1	1.00	1.02	1.14	1.16	
16		<0.1	1.03	0.97	0.69	1.16	
17	F(AR)	0.3	0.73	0.84	0.97	1.14	
18		<0.1	0.77	0.70	0.92	1.11	
19		<0.1	1.26	1.25	0.95	1.09	
20		0.5†	0.71	1.15	0.69	1.01	
21	M(AD)	<0.1	1.03	0.73	0.90	0.97	
22		1.5†	0.61	0.43	0.67	0.94	
23		2.3†	0.98	0.79	0.88	0.90	
24	M(AR)	3.6†	0.59	0.41	0.83	0.90	
25	S(AD)	2.7†	0.84	0.87	0.89	0.85	AD (severe, soybean, 1.7 years)
26		<0.1	0.91	1.12	0.63	0.89	
27		<0.1	1.13	0.97	0.81	0.68	
28		0.2	0.95	0.87	0.79	0.69	
29	M(AR), B(AD)	<0.1	0.77	1.17	0.78	0.73	
30		<0.1	0.61	0.64	0.72	0.77	
31	F(BA)	<0.1	0.93	0.79	0.67	0.66	
32		0.2	1.09	0.63	0.61	0.54	
33		0.2	1.18	0.96	0.43	0.51	
34		<0.1	0.42	0.47	0.43	0.50	
35		0.2	0.93	0.71	0.39	0.50	
36		<0.1	0.87	0.72	0.48	0.38	
37		0.2	0.90	0.81	0.46	0.45	

B, brother; S, sister; F, father; M, mother; AD, atopic dermatitis; AR, allergic rhinitis; BA, bronchial asthma; WH, wheezy bronchitis.
*Mean of triplicate.
†≥0.5 IU/ml for cord blood IgE concentration or ≥1.5 for SI of proliferative responses of CBLs.

greater than 0.5 IU/ml and in three (37.5%) of eight subjects whose SI was greater than 1.5 in proliferative responses of CBLs stimulated twice by ovalbumin or bovine serum albumin (table 2A). In four (50.0%) of eight subjects whose SI was greater than 1.5 in proliferative

responses of CBLs stimulated twice by ovalbumin or bovine serum albumin cord blood IgE concentrations were greater than 0.5 IU/ml (table 2B).

Table 2 Relationship between allergic history of the family, cord blood IgE concentrations, and proliferative responses of CBLs stimulated twice by ovalbumin or bovine serum albumin

(A) Allergic history of the family	Cord blood IgG concentrations (IU/ml)		Proliferative responses of CBLs to ovalbumin or bovine serum albumin (SI)	
	<0.5	≥0.5	<1.5	≥1.5
Negative (26)*	20	6	21	5
Positive (11)*	7	4	8	3
Total	27	10	29	8

(B) Cord blood IgE concentrations (IU/ml)	Proliferative responses of CBLs to ovalbumin or bovine serum albumin (SI)	
	<1.5	≥1.5
<0.5 (27)†	23	4
≥0.5 (10)†	6	4
Total	29	8

*The numbers in parentheses are the numbers of infants in whom allergic history of the family was negative or positive.
†The numbers in parentheses are the numbers of infants whose cord blood IgE concentrations were less than 0.5 IU/ml or greater than 0.5 IU/ml.

ALLERGIC DISORDERS AND SYMPTOMS IN INFANTS

All 37 newborns were followed up for two years. As shown in table 1, atopic dermatitis or atopic dermatitis and wheezy bronchitis developed in seven (18.9%) of 37 subjects. Allergic manifestations in these seven infants are shown in table 1.

RELATIONSHIP BETWEEN VARIABLES FOR PREDICTION AND ALLERGIC DISORDERS IN INFANTS

This is summarised in table 3. The sensitivity and specificity of each variable in the prediction of the development of allergic disorders are also shown in table 3. There was a history of allergy in the family in four (sensitivity 57.1%) of seven infants who developed allergic disorders. Cord blood IgE concentrations were greater than 0.5 IU/ml in three (sensitivity 42.9%) of seven infants who developed allergic disorders. SI was greater than 1.5 in proliferative responses of CBLs stimulated twice by ovalbumin or bovine serum albumin in six of seven infants who developed allergic disorders (sensitivity 85.7%,

$p<0.0005$ compared with non-allergic group) (table 3). In comparison, among the 30 infants of the non-allergic group, seven (23.3%) had positive allergic history of the family, seven (23.3%) had cord blood IgE concentrations greater than 0.5 IU/ml, and only two (6.7%) had an SI greater than 1.5 in proliferative responses of CBLs stimulated twice by ovalbumin or bovine serum albumin. The specificities of positive allergic history of the family, cord blood IgE concentration greater than 0.5 IU/ml, and an SI greater than 1.5 in proliferative responses of CBLs stimulated by ovalbumin or bovine serum albumin were 76.7%, 76.7%, and 93.3%, respectively. As shown in table 4, in all seven infants who developed allergic disorders, cord blood IgE concentrations were greater than 0.5 IU/ml and/or the SI was greater than 1.5 in proliferative responses of CBLs stimulated twice by ovalbumin or bovine serum albumin. The sensitivity and specificity of this combination for prediction were 100% ($p<0.0005$ compared with the non-allergic group) and 76.7%, respectively (table 4).

Moreover, as shown in table 1, four infants (subjects 2, 3, 6, and 7) with atopic dermatitis who are sensitive to hens' egg had proliferative responses of CBLs, at least, to ovalbumin, and two infants (subjects 1 and 5) with atopic dermatitis who are sensitive to cows' milk had

proliferative responses of CBLs to bovine serum albumin but not to ovalbumin. One patient (subject 25) with atopic dermatitis who is sensitive to soybean had no proliferative responses of CBLs to either ovalbumin or bovine serum albumin (table 1).

Discussion

Several investigators have shown that newborn infants with raised IgE concentrations of cord blood are at increased risk of developing allergic diseases.⁴⁻⁶ Michel *et al*⁴ and Magnusson⁵ reported that the IgE concentrations of cord blood appeared to be more predictive for the development of allergy in infancy than the family history. Moreover, Iikura *et al* reported that the measurement of specific IgG and IgG₄ in cord blood could be useful in the prediction of the development of allergic diseases and of offending foods.¹⁴ However, several reports showed that a raised cord blood IgE concentration is specific but not sensitive in the prediction of the development of allergic disorders.⁵⁻⁷ Kjellman and Croner reported that although the specificity was 97%, the sensitivity was 28%,⁷ and Magnusson reported that although the specificity was 93%, the sensitivity was 68%.⁵ These data indicate that a low cord blood IgE concentration does not exclude the development of allergic diseases.

Allergic disorders that begin early in life are often atopic dermatitis and food allergy such as hens' egg and cows' milk sensitivities. We previously reported that in children with atopic dermatitis who are sensitive to certain foods, there is no significant correlation between the proliferative responses of peripheral blood lymphocytes and radioallergosorbent test to each food allergen, and that the proliferative responses of peripheral blood lymphocytes to each food allergen is very useful in the diagnosis of hypersensitivity in food sensitive atopic dermatitis.⁹

In humans, sensitisation in utero was suggested by several investigators.^{4, 15} In this study, proliferative responses of CBLs to food antigens were measured. Although CBLs stimulated once by ovalbumin or bovine serum albumin showed no proliferative responses in any subject, CBLs stimulated twice by ovalbumin or bovine serum albumin exhibited proliferative responses in some subjects. We utilised the optimum double stimulation times of five and three days with a concentration of 2.5 or 25 µg/ml of each antigen. Our observations provide further evidence that sensitisation is occurring in utero. This would appear to be increasingly important in the genesis of early atopic problems. It may be due to the weakness of in vivo sensitisation by offending allergens in the maternal uterus that CBLs responded to ovalbumin or bovine serum albumin only in double stimulations. The specificity (93.3%) and the sensitivity (85.7%) of the proliferative responses of CBLs in the prediction of the development of allergic disorders were greater than those of the raised cord blood IgE concentrations or the allergic history of the family, respectively. Moreover, the sensitivity was

Table 3 Relationship between allergic history of the family, cord blood IgE concentrations, proliferative responses of CBLs stimulated twice by ovalbumin or bovine serum albumin, and the numbers of infants who developed allergic disorders for two years

	Allergic history of the family		Cord blood IgE concentrations (IU/ml)		Proliferative responses of CBLs to ovalbumin or bovine serum albumin (SI)	
	Negative	Positive	<0.5	≥0.5	<1.5	≥1.5
Allergic disorders developed in infants:						
Non-allergic (30)*	23	7	23	7	28	2
Allergic (7)*	3	4	4	3	1	6†
Total	26	11	27	10	29	8
Positive rate (%)	29.7 (11/37)		27.0 (10/37)		21.6 (8/37)	
Sensitivity (%)†	57.1 (4/7)		42.9 (3/7)		85.7 (6/7)‡	
Specificity (%)†	76.7 (23/30)		76.7 (23/30)		93.3 (28/30)	
Accuracy (%)†	73.0 (27/37)		70.3 (26/37)		91.9 (34/37)	

*The numbers in parentheses are the numbers of infants who did not develop or developed allergic disorders for two years.
†Sensitivity: true positives/(true positives+false negatives); specificity: true negatives/(false positives+true negatives); and accuracy: (true negatives+true positives)/total number of cases.
‡ $p<0.0005$ compared with non-allergic group.

Table 4 Relationship between the numbers of infants who developed allergic disorders for two years and the combination of cord blood IgE concentrations and proliferative responses of CBLs stimulated twice by ovalbumin or bovine serum albumin

	Cord blood IgE: <0.5 IU/ml and/or proliferative responses of CBLs: SI<1.5	Cord blood IgE: ≥0.5 IU/ml and/or proliferative responses of CBLs: SI≥1.5
Allergic disorders developed in infants:		
Non-allergic (30)*	23	7
Allergic (7)*	0	7‡
Total	23	14
Positive rate (%)	37.8 (14/37)	
Sensitivity (%)†	100.0 (7/7)‡	
Specificity (%)†	76.7 (23/30)	
Accuracy (%)†	81.1 (30/37)	

*The numbers in parentheses are the numbers of infants who did not develop or developed allergic disorders for two years.
†Sensitivity: true positives/(true positives+false negatives); specificity: true negatives/(false positives+true negatives); and accuracy: (true negatives+true positives)/total number of cases.
‡ $p<0.0005$ compared with non-allergic group.

increased by combining the cord blood IgE concentrations and proliferative responses of CBLs to food allergens. The measurement of proliferative responses of CBLs was also able to predict offending allergens. However, Ruiz *et al* report that maternal blood may have contaminated cord blood in 7% of their cases.⁸ In a further experiment, proliferative responses of CBLs to each food allergen will be compared with the responses of maternal peripheral blood lymphocytes and the responses of peripheral blood lymphocytes at seven days in the subjects whose CBLs responded to the allergen. Thus, the possibility of maternal blood contamination and the effects on the responses of CBLs will be investigated.

Taken together, our results indicate that the detection of the proliferative responses of CBLs to food antigens is useful in the prediction of not only the development of allergic disorders but also offending allergens. However, as our follow up is only two years, in utero sensitisation is a prediction for the early development of atopic disease but only longer follow up will show whether this holds good for allergic disorders at any age.

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