

Value of lymphoblast transformation test in cow's milk protein intestinal intolerance

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Summary

Lymphoblast transformation tests were carried out in the presence of α -lactalbumin and β -lactoglobulin. In patients with cow's milk protein intestinal intolerance in seventeen of forty-five (37.8%) the lymphoblast transformation tests were positive. Sensitization in the first month of life seemed to favour lymphoblast transformation. In control children in only four of forty-three (9.5%) the lymphoblast transformation tests were positive, the difference from intolerant patients being significant $0.01 < P < 0.001$. Lymphoblast transformation tests were negative in the seven children with active coeliac disease. Although a negative test does not exclude cow's milk protein intolerance, lymphoblast transformation tests can be considered a useful aid in diagnosis because of its specificity.

Introduction

Cow's milk protein intestinal intolerance often requires numerous clinical, biochemical and histological investigations. Its diagnosis must be early and accurate. Lymphoblastic transformation tests (LTT) were done in order to know whether they could contribute more to the diagnosis of cow's milk allergy than detection of antibodies, thus being a reliable and innocuous *in vitro* test. The present study examines this question and is compared with previous data reported by Borrone *et al.* (1970), Despres *et al.* (1971) and Polonovski *et al.* (1975).

Patients and methods

Patients with cow's milk protein intolerance

Forty-four patients, from 1 to 34 months old, were diagnosed as suffering from cow's milk protein intolerance. They were divided into three groups (Despres *et al.*, 1975; Rey & Frezal, 1974).

(a) Group I (twenty-one patients, Table 1): ingestion of cow's milk was rapidly followed by one or several acute manifestations: anaphylactic shock, pallor, cyanosis,

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Table 1. Clinical data and LTT results in patients of group I presenting with acute manifestations

Case no.	Sex	Age at onset of troubles (months)	Age at LTT (months)	Duration of cow's milk free diet (months)	LTT: blasts %			
					Control	α -LA	β -LG	Conclusion
1	F	<1	1	1/3	2.0	5.6	7.2	+ LG
2	F	<1	1½	1/10	1.4	6.3	1.5	+ LA
3	F	1½	2	1/3	3.0	3.9	8.5	+ LG
4	M	2	2 3/4	1/4	0.6	ND	4.5	+ LG
5	F	2	3	1	4.8	4.7	3.7	neg.
6	M	2½	3 1/4	1	0.1	0.1	0.1	neg.
7	M	1½	3½	Cow's milk fed	12.1	ND	14.6	neg.
8	F	1	4	½	1.2	2.8	2.4	neg.
9	F	1	4	2	1.3	4.1	0.8	+ LA
10	M	<1	5	3	1.4	1.0	3.1	neg.
11	F	<1	5	2	6.8	14.2	8.2	+ LA
12	F	1	5	4	5.1	12.0	4.8	+ LA
13	M	3	5	2	9.1	14.0	13.0	neg.
14	M	<1	5	1½	1.9	10.0	3.5	+ LA
15	M	2	5 3/4	2	6.9	10.9	5.9	neg.
16	F	<1	6 1/4	5	1.5	4.8	3.0	+ LA
17	M	<1	6 1/4	5	1.2	1.2	0.6	neg.
18	F	<1	6 1/4	4	3.8	9.2	8.4	neg.
19	F	6	7½	1½	6.8	9.1	3.2	neg.
20	F	9	9	3	0.6	0.3	0.6	neg.
21	F	<1	8	7	0.4	ND	2.4	neg.
22	F	<1	18	16½	0.5	0.5	2.3	+ LG

Table 2. Clinical data and LTT results in patients of group II presenting with chronic digestive troubles recurring after milk challenge

Case no.	Sex	Age at onset of troubles (months)	Age at LTT (months)	Duration of cow's milk free diet (months)	LTT: blasts %			
					Control	α -LA	β -LG	Conclusion
1	F	<1	1½	1/3	1.6	5.9	5.9	+ LA + LG
2	F	<1	2 1/4	½	2.6	5.6	9.2	+ LG
3	M	<1	3½	1½	1.4	1.0	4.0	+ LG
4	M	<1	4	2	9.0	28.0	17.0	+ LA + LG
5	F	<1	4½	1	0.3	2.6	2.6	neg.
6	M	<1	4½	4	5.7	5.7	1.8	neg.
7	M	<1	5 3/4	5	1.8	2.9	2.8	neg.
8	F	<1	10	2/3	1.9	1.2	2.4	neg.
9	F	2½	11	2	0.4	0.5	0.7	neg.
10	F	<1	11 3/4	11	1.0	3.0	3.0	neg.
11	F	<1	24	23	1.6	2.5	1.4	neg.

Table 3. Clinical data and LTT results in patients of group II presenting with chronic digestive troubles without conclusive provocation test

Case no.	Sex	Age at onset of troubles (months)	Age at LTT (months)	Duration of cow's milk free diet (months)	LTT: blasts %			
					Control	α -LA	β -LG	Conclusion
1	F	<1	2	1	6.1	6.0	3.8	neg.
2	M	1½	2 ¾	½	6.1	10.6	3.1	neg.
3	M	<1	4	3	11.1	9.8	11.5	neg.
4	F	2	4 ¼	1	5.2	6.5	9.9	neg.
5	F	<1	4½	1/10	0.7	2.9	3.4	+ LA
6	F	<1	4½	1	1.5	4.8	ND	neg.
7	F	1	5	1½	0.3	1.3	2.1	+ LG
8	F	4	8½	Cow's milk	3.6	7.4	4.6	neg.
9	M	3	10	fed 2½	7.0	7.6	ND	neg.
10	F	6	12	1/4	2.9	ND	8.3	neg.
11	F	<1	18	Cow's milk fed	3.0	2.3	ND	neg.
12	M	4	34	Cow's milk fed	1.0	0.9	0.9	neg.

unconsciousness, dyspnoea, vomiting, bulky and sometimes bloody diarrhoea. These symptoms might be associated with those encountered in Group II.

(b) Group II (eleven patients, Table 2): chronic digestive troubles with diarrhoea, which when persistent, led to a malabsorption syndrome. Withdrawal of cow's milk resulted in clear clinical improvement. Symptoms recurred after voluntary or involuntary milk challenge.

(c) Group III (twelve patients, Table 3): symptoms were the same as in group II, but re-exposure to cow's milk was not as conclusive, essentially because of intercurrent illness or because the provocation test was performed when cow's milk protein intolerance had already disappeared.

In several cases in each of the three groups, provocation tests were not done because of medical and/or ethical reasons.

Control patients

Forty-three children, from 20 days to 14 years old, having tolerated cow's milk well, were considered as control patients. They were divided into two groups: the first one consisted of twenty-five patients, amongst whom fourteen had unspecific and transient diarrhoea, seven had coeliac disease with partial (three cases), subtotal (three cases) and total (one case) intestinal villous atrophy, the remaining four children having cystic fibrosis, sucrose intolerance, Crohn's disease and abetalipoproteinaemia. The second group consisted of eighteen children without any digestive problem; eleven of them were either premature or dysmature infants.

Methods

Lymphoblastic transformation tests (LTT) were carried out according to Papiernik's technique (1970). Plasma rich in lymphocytes was collected and distributed into four tubes: (a) 0.5 ml plasma + 1.5 ml TC 199 (Pasteur Institute, Paris); (b) 0.5 ml plasma +

1.5 ml TC 199 + 50 µg phytohaemagglutinin (PHA) (Bacto Phyto hemagglutinin M, Difco Laboratories, Detroit); (c) 0.5 ml plasma + 1.5 ml TC 199 + 0.50 mg α -lactalbumin (α -LA) (Pasteur Institute, Paris); (d) 0.5 ml plasma + 1.5 ml TC 199 + 0.50 mg β -lactoglobulin (β -LG) (Pasteur Institute, Paris).

Antigens were diluted 1/1000 in normal saline solution. Antigenic concentrations represented a mean value as compared to literature data (Despres *et al.*, 1971; Baudon & Mougenot, 1973) and experimental data (Halpern, 1971). Because of the small amount of blood available, a large range of antigenic concentrations were not used. The cells were cultured for 4 days. Results were estimated cytologically by two observers, counting, as far as possible, 1000 cells per slide. Cells were considered as blasts if they were greater than 30 µm in diameter, hyperbasophilic with a reticulum pattern of the chromatin and two or more nucleoli. The LTT were considered positive when the slide with antigen showed a significant increase in lymphoblast transformation as compared to the control slide (confidence limit $P = 0.05$). In all the tests normal stimulation occurred with PHA.

In patients with cow's milk protein intolerance forty-five LTT were performed: forty-two cultures with α -LA, forty-three with β -LG were interpretable. In control children, among the forty-three LTT, forty-three cultures with α -LA and forty with β -LG were available. A χ^2 test was performed for evaluation of statistical signification.

Results

In patients with cow's milk protein intolerance seventeen of forty-five (37.8%) LTT were positive, two in presence of both α -LA and β -LG, seven in presence of α -LA and eight in presence of β -LG (Table 4).

The incidence of positive LTT was 45.5% in group I with acute manifestations, 36.4% in group II and 25% in group III with chronic manifestations. Yet this decrease was not statistically significant. In twenty-nine patients troubles had begun in the first month of life; fourteen of twenty-nine LTT (48.3%) were positive. In contrast, only three of sixteen LTT (15.8%) were positive when troubles had begun later (Table 5). The difference was significant ($P = 0.05$). The lapse of time after withdrawal of cow's milk did not seem to influence results of LTT: one patient with positive LTT had not been fed cow's milk for 16.5 months.

In control patients, four of forty-three LTT (9.30%) were positive, three in the presence of α -LA, one in the presence of β -LG (Table 4); the difference from those intolerant to cow's milk was significant ($0.01 < P < 0.001$); Three patients with positive LTT were less than 3 months old and were either premature or dysmature, one of them

Table 4. Numbers of positive lymphoblast transformation tests in intolerant patients and control children

LTT	Global positive results number and %	α -LA and β -LG	α -LA alone	β -LG alone
Intolerant patients	17/45 (37.8)	2	7	8
Control children	4/43 (9.3)	0	3	1

Table 5. Influence of precocity of sensitization on results of lymphoblast transformation tests in intolerant patients

Age at onset of troubles	≤ 1 month	> 1 month
Total number of LTT	29	16
Positive LTT	14	3

had unspecific and transient diarrhoea. The fourth one, 8.5 months old, started frequent diarrhoea in his first month. LTT in the seven patients with coeliac disease and villous atrophy were all negative.

Spontaneous lymphoblast transformation was studied in the eighty-eight control cultures without antigen. In intolerant patients, spontaneous lymphoblast transformation exceeded 4% in fifteen of forty-five LTT (33%) while in control patients this occurred in five of forty-three cases (11.6%); the difference was significant ($P < 0.025$).

Comments

In patients with cow's milk protein intestinal intolerance, seventeen of forty-five (37.8%) LTT were positive. The incidence is lower than that obtained by Borrone *et al.* (1970), Despres *et al.* (1971) and Polonovski *et al.* (1975), respectively, ten of twenty-one (48%), seven of nine (77%) and twenty-two of thirty-two (69%). Our strict criteria of positive results may explain these discrepancies as well as the fact that in five patients LTT were carried out when cow's milk allergy had already clinically disappeared: in patient 11 of group II, LTT immediately preceded reintroduction of cow's milk; in group III patients 8, 11 and 12 had already been fed with cow's milk as well as patient 7 of group I (3.5 months old) where, for social reasons, cow's milk had been reintroduced gradually 6 weeks before. These data may be related to the negative results of case no. 9 in group I at the age of 17 months; cow's milk feeding was from then on uneventful. Finally, the use of a single antigenic concentration possibly reduced the number of positive LTT.

Lymphoblast transformation was induced equally by α -LA and β -LG. At variance with this finding Freier (1974), Kletter *et al.* (1971b) Lebenthal (1975) for antibodies, and Polonovski *et al.* (1975) for L.T.T. had reported β -LG as the most antigenic cow's milk protein. However, β -LG could not be considered as a non-specific mitogen in disagreement with Asquith, Housley & Cooke (1970).

Clinical features of cow's milk protein intolerance did not seem to alter results of LTT. Thus, cellular hypersensitivity is likely to be involved in patients with acute and/or chronic symptoms. Indeed, cell-mediated immunity, also demonstrated by positive leucocyte migration tests (Brostoff, 1971), does not exclude antibody responses, increases in the numbers of IgA and IgM (Savilahti, 1973) and IgE containing cells (Kilby, Walker-Smith & Wood, 1975) with mast cell degranulation (Shiner, Ballard & Smith, 1975) have been shown after milk challenge in jejunal mucosa.

Results of LTT were influenced neither by age nor by length of time without cow's milk. In contrast, early sensitization seemed more important, in agreement with the findings of Baudon & Mougenot (1973) and with the increased antibody response in infants reared on the bottle since birth (Kletter *et al.*, 1971a).

In control children, early age and precocity of sensitization may account for the

only four positive LTT. Yet forty-two among our control children were likely to have increased permeability to food antigens (Freier, 1973; Walker, 1975; Walker-Smith, 1975) because of young age, alteration in mucosal barrier or abnormal intraluminal digestion. Yet it appears that the seven patients with coeliac disease and villous atrophy had negative LTT, in agreement with the findings of Borrone *et al.* (1970) and Baudon & Mougenot (1973), and in contrast with the frequency of milk antibodies in evolutive coeliac disease (Baudon & Mougenot, 1973; Kenrick & Walker-Smith, 1970).

Spontaneous lymphoblast transformation was significantly higher in patients with cow's milk allergy than in control children. There is no linear relationship between time since withdrawal of cow's milk and spontaneous lymphoblast transformation. Yet in sensitive children recent antigenic stimulation *in vivo* may account, at least partially, for this fact (Borrone *et al.*, 1970).

In conclusion, while there is a general agreement about the poor diagnostic value of serum and copro-antibodies to milk, LTT seems more reliable. False positive tests are uncommon. Although our results show that a negative test does not exclude cow's milk protein intolerance, LTT can be considered as a useful aid in the diagnosis of this food intolerance.

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References

- ASQUITH, P., HOUSLEY, J. & COOKE, W.T. (1970) Lymphocyte stimulation by bovine β -lactoglobulin. *Nature*, **228**, 462.
- BAUDON, J.J. & MOUGENOT, J.F. (1973) Anticorps sériques anti-lait et test de transformation lymphoblastique aux antigènes lactés en pathologie digestive du premier âge. In: *Journées Parisiennes de Pédiatrie*, p. 289. Flammarion Médecine Sciences. Paris.
- BORRONE, C., DAGNA-BRICARELLI, F., MASSIMO, L., FOSSATI-GUGLIELMONI, A. & DURAND, P. (1970) Lymphocyte transformation in milk allergy. *Acta paediatrica scandinavica*, **59**, 449.
- BROSTOFF, J. (1971) Cellular hypersensitivity in milk allergy. *Acta allergologica*, **26**, 478.
- DESPRES, P., GENDREL, D., PLAINFOSSE, B., SARRAF-CHIRAZI & SERINGE, P. (1975) L'intolérance aux protéines du lait de vache. Nouvelle étude à propos de 33 observations personnelles. *Annales de Pédiatrie*, **22**, 375.
- DESPRES, B., PLAINFOSSE, B., PAPIERNIK, M. & SERINGE, P. (1971) Les intolérances digestives aux protéines du lait de vache chez l'enfant. Etude de 15 cas. *Annales de Pédiatrie*, **18**, 464.
- FREIER, S. (1973) Paediatric gastrointestinal allergy. In: *Clinical Immunology-Allergy in Paediatric Medicine* (Ed. by J. Brostoff), p. 107. Blackwell Scientific Publications, Oxford.
- FREIER, S. (1974) Allergic mechanisms in intestinal cow's milk intolerance. *Acta paediatrica scandinavica*, **63**, 651.
- HALPERN, B. (1971) Personal communication.
- KENRICK, K.C. & WALKER-SMITH, S.A. (1970) Immunoglobulins and dietary protein antibodies in childhood coeliac disease. *Gut*, **11**, 635.
- KILBY, A., WALKER-SMITH, S.A. & WOOD, C.B.S. (1975) Small intestinal mucosa in cow's milk allergy. *Lancet*, **i**, 531.
- KLETTER, B., GERY, I., FREIER, S. & DAVIES, A.M. (1971a) Immune responses of normal infants to cow milk. II. Decreased immune reactions in initially breast-fed infants. *International Archives of Allergy and Applied Immunology*, **40**, 667.
- KLETTER, B., GERY, I., FREIER, S., NOAH, Z. & DAVIES, M.A. (1971b) Immunoglobulin E antibodies to milk proteins. *Clinical Allergy*, **1**, 249.

- LEBENTHAL, E. (1975) Cow's milk protein allergy. *Pediatric Clinics of North America*, **22**, 827.
- PAPIERNIK, M. (1970) Le test de transformation lymphoblastique chez le fœtus, le prématuré et l'enfant: micro et macro technique. *Pathologie et Biologie*, **18**, 1119.
- POLONOVSKI, C., BAUDON, J.J., FONTAINE, J.L., MOUGENOT, J.F., NAVARRO, J. & LAPLANE, R. (1975) Intolerance digestive aux protéines du lait de vache. Données immunologiques et histologiques. *XXIV^e Congrès de L'association des Pédiatres de Langue Française*, p. 338. Expansion Scientifique Française, Paris.
- REY, J. & FREZAL, J. (1974) L'intolérance aux protéines du lait. Conception actuelle, critères de diagnostic et tentative de classification. *Archives Françaises de Pédiatrie*, **31**, 933.
- SAVILAHTI, E. (1973) Immuno-clinical study of the malabsorption syndrome with cow's milk intolerance. *Gut*, **14**, 491.
- SHINER, M., BALLARD, J. & SMITH, M.E. (1975) The small intestinal mucosa in cow's milk allergy. *Lancet*, **i**, 136.
- WALKER, W.A. (1975) Antigen absorption from the small intestine and gastro-intestinal disease. *Pediatric Clinics of North America*, **22**, 731.
- WALKER-SMITH, J. (1975) Cow's milk protein intolerance transient food intolerance of infancy. *Archives of Disease in Childhood*, **50**, 347.

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