Acta Dermatovener (Stockholm) 57: 159–163, 1977

PROVOCATION WITH COW'S MILK AND CEREALS IN ATOPIC DERMATITIS[1]

Hans Hammar

From the Department of Dermatology, Karolinska sjukhuset, Stockholm, Sweden

Abstract. Oral provocation test with cow's milk and cereals were performed in 81 children with atopic dermatitis, all below 5 years of age. In 15 of the children exacerbation of the dermatitis occurred on the second or third day after initiation of the tests. These patients, whose mean age was 13 months, underwent repeated provocation during the ensuring 18 months. Eleven children lost their sensitivity to the offending foods and 2 children became more tolerant during this period. Initial levels of serum IgE and specific reagins against the foods tested did not distinguish those patients who were positive to the provocation tests. In this group, however, reagins against egg white were significantly increased. The specific serum reagins were unaltered or increased in the majority of cases during the period of study. The radioallergosorbent test (RAST) was found of limited value as a screening tool in patients with atopic dermatitis in whom exacerbation of the condition appears to be caused by food sensitivity.

Key words: Dermatitis, atopic; Milk, cow; Cereals

The role of food reactivity in atopic dermatitis is a matter of controversy, but foods may well be involved in the exacerbation of the dermatitis, especially during the first years of life (3, 8). Immediate skin reactions have repeatedly been found to be only weakly correlated to provocation tests with the corresponding foods, and the therapeutic consequences of excluding possibly allergenic food have been disappointing (summarized by Rajka (7)). Hemagglutination titres of milk antibodies have been found in both healthy and atopic children and the antibody levels have not discriminated between these groups of patients (2, 5). Two of 25 patients with atopic dermatitis studied by Öhman (12) showed increased titres of reagins against milk tested by the radioallergosorbent test, RAST (10); the importance of such reagins in food allergy could not be established. An immunological mechanism might be involved in the adverse reactions to foods such as cow's milk and cereals. The present investigation was therefore undertaken to find out whether or not the titres of serum reagins against milk and the cereals wheat, oats and rye in patients with atopic dermatitis were correlated to the results of provocations with these foods. A positive provocation was defined as a flare-up of the skin lesions of atopic dermatitis. In patients found positive at such provocations the reaginic titre was followed for different lengths of time and related to the outcome of repeated provocation tests.

MATERIAL AND METHODS

During the period September 1972 through November 1973 all patients up to 5 years of age with a diagnosis of atopic dermatitis admitted to the dermatological ward for children at Karolinska sjukhuset were selected. One child had asthma besides the dermatitis. Ten of these 91 patients were excluded from the study, mostly for social

Table I. *Material*

Group of patients	No. of patients		
	Less than 5 yrs old	5 yrs and older	Total
Provocation done			
Atopic dermatitis	81[a]	43	124
Provocation not done			
Atopic dermatitis	4[a]	19	23
Non-atopic subjects	11	17	28
Total	96	79	175

[a] Initially 91 patients of whom 10 subjects were unable to participate in the provocation study.

[1] Presented at the Meeting of the Swedish Association of Allergology, March 19, 1975.

Table II. *Composition of the patient material and initial serological findings*

The material is divided according to results of the provocation tests and of analyses of serum IgE and of reagins against some food allergens

Group of patients	Number of patients	Age (months) (Mean±S.D.)	Serum IgE (U/ml) (expected at age 0)	Probability of finding a score 1–3 by the radioallergosorbent test, in percent				
				Cow's milk	Wheat	Oats	Rye	Egg white
Provocation positive (milk & cereals)								
Less than 5 yrs old (follow-up group)	15	13±11	166 (42)	26	6	30	6	53
5 yrs and older (no follow-up)	7	109±95	913 (255)	38	17	17	25	8
Provocation negative								
Less than 5 yrs old	66	19±16	62 (18)	13	2	5	4	17
5 yrs and older	36	95±29	373 (305)	9	1	10	9	8
Provocation not done								
Atopic dermatitis	23	87±41	240 (55)	12	4	2	9	8
Non-atopic subjects	28	75±44	85 (209)	10	0	5	0	1

reasons. In addition to the group of young children, 43 older children were also studied, together with 51 children in whom only serologic analyses were performed. The material is presented in Table I. Informed consent for carrying out food provocations was obtained from the parents of all children tested.

On admission, any lesions present were treated with a hydrocortisone ointment, antihistamines and, when necessary, with antibiotics. During this period of treatment the patients were given a formula free from cow's milk, cereals, fish, fruits, vegetables, eggs, spices, preservatives and food dyes. The diet was based on soy milk (Sobee) and for older infants and children it included the following: meat: veal, beef, pork or chicken; meal: rice, maize, potato or arrowroot; fat: milk-free margarine; blueberries, water or tea, sugar and salt were included when desirable. When the skin lesions had cleared the patients were tested with 100 g of cow's milk daily for 3 consecutive days, always at the midday meal. The cereals were tested on the following 3 days; 100 g of gruel or porridge made of equal parts of wheat, oats and rye meal being given. This was made with cow's milk if the previous test with cow's milk had been negative, and with water (soy milk) if it had been positive. If a reaction occurred, further provocations were postponed until the skin was clear again. In cases of intercurrent infections, tests were also delayed until the infection subsided.

Examination of positive reactions. Each patient was examined at the time of dressing and of undressing and at each meal. This meant 5–7 examinations per day, or more in some cases if the child became itchy or developed new lesions. The following symptoms and signs were recorded: pruritis, irritation or sleep disturbances, erythema or hives and lesions with peeling and/or papules. It was noted if lesions appeared on the old sites or on new ones. A test reaction was considered positive only if the signs of dermatitis included at least erythema and peeling. Papules were not present in all reactions. A positive reaction was followed by pruritus, irritation and often sleep disturbances. A test reaction was judged to be probably positive if erythema alone appeared in the old lesions. In these cases a further test was done a few weeks later to decide the matter. Urticaria was not regarded as a positive response in this series.

No adverse reactions, with flare-ups of the dermatitis, resulted from the basic diet. Some of the youngest children had diarrhoea when the change to the soy milk formula was made abruptly. This reaction could be avoided by changing the diet gradually over a 3–4 day period. On this schedule there were no reactions that could be attributed to the soy component of the diet.

Provocation tests were made on 124 patients (Table I). In 43 patients 5 years old or older, only one provocation test was done, although 7 of these were positive. Patients older than 59 months were excluded from the final series because of the age limit initially set in this study. In the group of young children 15 patients had a positive reaction to the first provocation test for cow's milk and/or cereals and these patients were subsequently followed up.

Measurement of reagins. Serum IgE was measured by the radioimmunosorbent technique (11) and the reagins against cow's milk, cereals and egg white were measured by the radioallergosorbent test (RAST) according to Johansson et al. (4). All tests were done at the Blood Centre, University Hospital, Uppsala by courtesy of Dr S. G. O. Johansson. Commercially available antigens were used for the tests. Serum IgE is expressed in units/ml. In the earlier part of the study the RAST scores were given from the laboratory in an 8-grade scale from 0 to 7 (4) but later in a 5-grade scale from 0+ to 4+. Since there was some overlapping between the boundaries of these scores our results were re-graded in a 4-grade scale: 0 (0–1; 0+), 1 (2–3; 1+); 2 (4–6; 2+, 3+) and 3 (7; 4+).

Statistical treatment. Measurements of statistical differences included χ^2 and *t*-tests. Serum IgE is expressed as the geometric mean. The RAST scores were analysed

Table III. *History of dermatitis following ingestion of cow's milk and results of oral milk provocations*
The number of patients less than 5 years old is given, together with the mean score in the radioallergosorbent test against milk (RAST, scores 0 through 3)

Provocation with oral cow's milk for 3 consecutive days	History of dermatitis following ingestion of cow's milk					
	Positive		Probable		Negative	
	No.	RAST	No.	RAST	No.	RAST
Positive	6	1.0±0.3	–		8	0.4±0.2
Negative	1	0	4	0	61	0.4±0.1[a]

[a] One value lost.

by using the binomial distribution: mean score: $\bar{x}_1 = x_i/n_1$; error of the mean: $\sqrt{\bar{x}_1 (N-\bar{x}_1)/N^2}$; and probability of scores 1, 2 or 3: $\bar{x}/N$: x_i individual score, n_1 number of patients in subgroup 1, and N number of scores, which was equal to 3. The t value calculated for testing two means binomially distributed, was obtained from the formula

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{1}{2}(\bar{x}_1+\bar{x}_2)\frac{1}{2N}(2N-\bar{x}_1-\bar{x}_2)\frac{2}{N}}}$$

with (n_1+n_2-2) degrees of freedom (9).

RESULTS

The serum IgE titres and the distribution of circulating reagins against cow's milk, wheat, oats, rye and egg white are summarized in Table II. In patients 5 years of age or older the serum IgE was at a higher level than in the younger age group but the probability of RAST scores being positive was generally low and mostly below 10%. In the age group below 5 years these probabilities were higher, especially for egg white.

The final series of children, below 5 years of age, comprised 81 children of whom 14 had positive provocations to cow's milk and 3 to one or several cereals. The history and results of the milk provocation in these patients are summarized in Table III. Among children who had a history of dermatitis following the intake of cow's milk there was a greater frequency of positive than of negative provocation tests. This was significant by a test of contingency, $\chi^2=25.4$, $P<0.001$. The mean RAST scores for milk are also given in Table III. The values found in the patients with a concordant history and provocation result differed between the positive and negative groups, but both differences were only statistically probable, since t-tests gave P values equal to 5%.

The 15 patients with positive reactions at the first provocation test were followed up for up to 18 months by serial IgE and RAST measurements and by repeated provocations. The age distribution is shown in Fig. 1. In the positive tests, the skin reactions to the offending food did not occur until the second and third day in most cases. Erythema and pruritus were present as primary symptoms mostly on old sites, and these were followed the next day by scaling and/or by a papular dermatitis, which was sometimes disseminated. Only in one

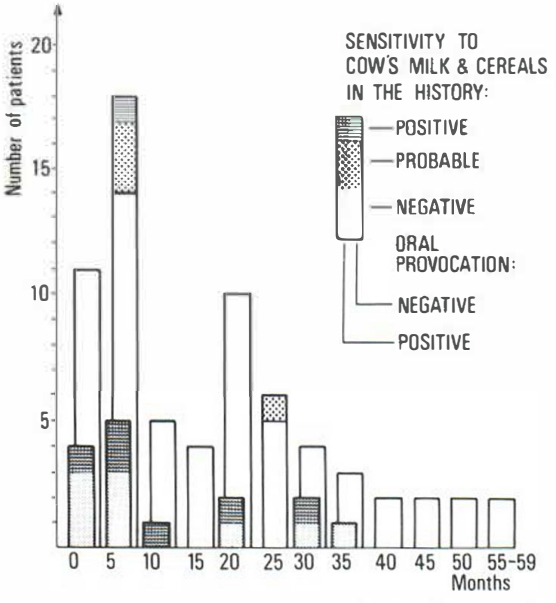

Fig. 1. Milk and/or cereal sensitivity in children with atopic dermatitis, less than 5 years old, admitted to the Dermatological ward, Karolinska sjukhuset, September 1972 to November 1973.

Table IV. *Results of repeated oral provocations and seriological tests*

Children found sensitive to cow's milk and/or wheat followed in time by repeated oral provocations. The number of patients, mean age (±S.E.M.), serum IgE (U/ml; geometric mean), and the reaginic titres obtained at the radioallergosorbent test (RAST, score 0 through 3) against cow's milk and wheat are given. The mean scores and their errors are given for the RAST titres

Provocation no.	Number of patients tested			Age at time of provocation (months)	Months after first provocation	Serum IgE and RAST scores grouped according to provocations (positive +; negative −)							
						Milk				Wheat			
	Positive	Negative	Not done			Test	No.	IgE	RAST	Test	No.	IgE	RAST
1	15	0	0	13.2±3.0	0	(+)	14	143	0.5±0.1[a]	(+)	3	402	0.7±0.4
						(−)	1	1 240	2.0	(−)	7	194	0
2	10	5	0	19.5±3.3	6.2±0.9	(+)	7	410	1.7±0.3	(+)	2	26	0
						(−)	6	85	0.2±0.2	(−)	11	286	0.3±0.2
3	5	4	1	25.2±4.5	13.3±1.5	(+)	3[a]	799	2.3±0.4	(+)	1	860	0
						(−)	4	92[a]	1.0±0.4	(−)	6[b]	215[a]	0.7±0.3
4	2[c]	2	1	28.3±6.0	18.5±2.7	(+)	2[c]	2 828	2.5±0.5	(+)	0		
						(−)	2	908	1.0±0.6	(−)	4	1 603	0.7±0.4[a]

[a] One value lost.
[b] Two patients not examined.
[c] No intolerance to milk boiled for 20 min.

patient did pruritus occur as early as during the first night after provocation.

Two patients dropped out from the follow-up studies and could not be traced. Two other patients, M. W. 21 months and M. R. 11 months, were still sensitive to 100 ml of cow's milk at the fourth provocation. M. W. was admitted with atopic erythroderma. In both of these children exacerbation of the dermatitis was seen at the first provocation following tests with 30 ml of undiluted milk and 30 ml of milk diluted 1 : 50, respectively. They were both sensitive to milk boiled for 20 min and given in amounts of 20–60 ml. With time, this reactivity towards boiled milk disappeared. The patient M. W. was also initially sensitive to wheat and rye. This reactivity was not present 10 months after the first provocation when she was 31 months old.

In all of the patients a formula free from milk and from the offending cereal improved their skin condition, but relapses occurred almost without exception. These generally consisted of intermittent dermatitis of a milder type than that present before the formula was given. When provocations had a negative result the patients were switched over to the regular diet. There was no initial exacerbation of the dermatitis during the following months.

About 17% of the patients in the younger age group were sensitive to cow's milk, about 4% to wheat and oats and 1% to rye. About 75% of the patients lost this sensitivity during the follow-up period, as seen in Table IV. It is evident that this sensitivity may be present for several years, as indicated by the few patients in the older age group (Table II). In the younger age group the initial RAST values for milk and for cereals did not differ between the patients with positive and those with negative test results (Table IV). The RAST score for egg white was, however, significantly different in this respect ($P<0.01$). During the period of study, those patients who remained sensitive to the foods tested showed increased reagin levels, both of the total IgE and of the foot-specific reagins. Variation in reaginic activity was not correlated to positive provocation reactions. In 10 out of 11 patients, who became negative during the course of the study, the same or increased RAST scores were seen at the last measurement. In only one patient did the score of milk RAST decrease, from 2 to 0, following the development of tolerance to the offending food.

DISCUSSION

This study revealed a high incidence of intolerance to cow's milk in 1-year-old children. This reactivity decreased in most of the children within the next 18 months. A similar frequency of milk sensitivity to

that found in this investigation has been reported previously (1). However, intolerance to milk is fairly common in a population of atopic children, as indicated by Halpern et al. (3), who found an incidence of about 2% in this age group.

Multiple reaginic activities were found in the patients with a positive reaction to the provocation test. This pattern has been pointed out by other authors, as summarized by Rajka (7). On the other hand, our patients were not studied for other immunological abnormalities which could be seen in some of these children (1, 6, 7).

In the preparation of specific IgE antibodies for RAST the various foods were used as antigens without prior treatment. It is possible that the specificity responsible for the recorded reactions following oral provocation needs the passage through the intestinal mucosa for full antigenic activity. Such material might thus be missed by the test system used. On the other hand, other immunological reactions including a cell-mediated reaction, might be involved. It may be noted that lymphocytes sensitized to milk, as measured by the lymphocyte transformation test (in collaboration with Dr B. Nilsson, M.D., Karolinska sjukhuset, Stockholm), were found in one of the two patients (M. R.) who was still positive to provocation on the fourth occasion. The other one (M. W.) had a negative reaction to milk with this system in spite of a positive provocation result. If an immunological reaction is responsible for the observed flare-ups of the atopic dermatitis, it might be speculated to be of the delayed type of allergic reaction. Without further proof this was suggested from the delay of one or two days after initiating the oral test before signs and symptoms from the skin were noticed.

From this study it appears that neither the initial IgE level nor the RAST score parallels the results of a positive oral provocation with the foods tested, but that a high RAST score might suggest such sensitivity if there is also a positive history of sensitivity to a specific food.

ACKNOWLEDGEMENTS

I am indebted to the Head Nurse, Mrs Caroline Häggmark, for help with the provocation tests. I wish to thank Dr S. G. O. Johansson, the Blood Centre, University Hospital, Uppsala, for providing all laboratory aids and for much helpful criticism.

This work was supported by the Finsen Foundation, Stockholm.

REFERENCES

1. Cohen, H. I.: Milk allergy in childhood. Cutis *14:* 667, 1974.
2. Farr, R. S.: Immunologic responses in infantile eczema. J Pediatr *66:* 230, 1965.
3. Halpern, S. R., Sellars, W. A., Johnson, R. B., Anderson, D. W., Sapersten, S. & Reisch, J. S.: Development of childhood allergy in infants fed breast, soy or cow milk. J Allergy Clin Immunol *51:* 139, 1973.
4. Johansson, S. G. O., Bennich, H. & Berg, T.: In vitro diagnosis of atopic allergy III. Quantitative estimation of circulating IgE antibodies by the radioallergosorbent test. Int Arch Allergy Appl Immunol *41:* 443, 1971.
5. Lorincz, A. L.: Immunologic responses in infantile eczema. J Pediatr *66:* 224, 1965.
6. Peterson, R.: Immunologic responses in infantile eczema. J Pediatr *66:* 225, 1965.
7. Rajka, G.: Atopic Dermatitis, pp. 65–70. W. B. Saunders, Co., London. Philadelphia, Toronto, 1975.
8. Sedlis, E.: Some challenge studies with foods. J Pediatr *66:* 236, 1965.
9. Weber, E.: Grundriss der biologichen Statistik, 6th ed., pp. 190–193. G. Fischer, Jena, 1967.
10. Wide, L., Bennich, H. & Johansson, S. G. O.: Diagnosis of allergy by an in vitro test for allergen antibodies. Lancet *II:* 1105, 1967.
11. Wide, L. & Porath, J.: Radioimmunoassay of proteins with the use of Sephadex-coupled antibodies. Biochim Biophys Acta *130:* 257, 1966.
12. Öhman, S.: Immunoglobulin E in atopic dermatitis. Thesis. University of Uppsala, 1971.

Received May 30, 1975

H. Hammar, M.D.
Department of Dermatology
Karolinska sjukhuset
S-104 01 Stockholm 60
Sweden