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MILK ALLERGY

II. Skin Testing of Allergic and Normal Children with Purified Milk Proteins

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A FUNDAMENTAL criticism of the role of skin testing in the diagnosis of milk allergy is that in past studies the definition of the milk allergic patient did not include the reproduction of symptoms as a result of oral challenge with milk. However, in a recent study,¹ milk allergy was proven in a group of allergic children by reproduction of symptoms upon feeding milk and/or isolated milk proteins. As a part of that investigation the isolated milk proteins used for oral challenge were also used as skin testing antigens in the milk allergic patients. To ascertain further the specificity of these skin tests, normal children and children allergic to non-milk substances were also skin tested with the same milk antigens.

MATERIALS AND METHODS

Four milk proteins, casein, bovine serum albumin (BSA), beta-lactoglobulin and alpha-lactalbumin, were prepared and tested for purity as described previously.¹ Skin testing preparations* were made as follows. The proteins were solubilized at

pH 7.0-7.2 and diluted to final concentration with 0.8% saline solution. These solutions were sterilized by filtration through a Morton sintered glass filter. All intradermal solutions were standardized to 1,000 and 10,000 PNU/ml. For scratch testing, the sterile solubilized protein was diluted with Hollister Stier's glycerol saline solution to a concentration of 10,000 PNU/ml. These protein solutions and diluting fluids were distributed to each of the 17 physicians participating in this study.

Scratch tests were done by the scarification technique. Intradermal tests were done with approximately 0.02 ml of each protein solution and control diluting fluid. Reactions were observed in 20 minutes. The diameters of each wheal and erythema were measured in millimeters. Tests were judged positive when the wheal diameter was 2 mm. or more greater than the control, or if the erythema diameter was 5 mm or more greater than the control. Reactions of 2+, 3+, or 4+ were recorded when the wheal diameter was 4, 6, or 8 mm greater than the control wheal, or when the erythema diameter was 10, 15 or 20 mm greater than the control erythema.

* Skin testing solutions were made in the laboratories of the Pharmaceutical Division, The Borden Company.

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PEDIATRICS, October 1963

Selection of Patients

Skin testing of a normal group of children was done by one of us (A.S.G.). The basis of selection was a negative personal and family history for allergy and a normal physical examination. Only intradermal skin tests at a concentration of 10,000 PNU/ml were used.

Skin testing of this group of children allergic to substances other than milk was done by one of us (A.S.G.). Each child was selected on the basis of the presence of an allergic disorder such as allergic rhinitis, bronchial asthma, atopic dermatitis or urticaria; no improvement following milk elimination; no increase in symptoms following milk ingestion; proof of sensitivity to another allergen by improvement following elimination of it, and symptoms following oral or inhalent challenges with it. Only intradermal tests at a concentration of 10,000 PNU/ml were used.

Each child in the allergic to milk group was selected on the basis of improvement following milk elimination and the appearance of symptoms following oral ingestion of milk or isolated milk protein. The establishment of milk allergy for this group has been described.¹ When possible, skin testing was done before or soon after milk elimination. Usually the initial skin tests were by the scratch method. If the scratch tests were negative or weakly positive, intradermal tests were done. Each physician tested his patients. However, the results of all skin tests were reviewed by two of the investigators (W.A.S. and A.S.G.) who agreed independently upon the grading of each skin test.

RESULTS

One hundred and two normal children were skin tested, and six had a positive skin test. All reactions were one-plus only, and each of the six children gave a positive test to just one of the proteins. One of the positive tests occurred in the group of 24 newborns, two positive tests occurred in the group of 38 infants 2 weeks to 1 year of

age, and three of the positive tests occurred in the group of 40 children over 1 year of age.

The results of skin testing the 31 children allergic, but not to milk, are shown in Table I. Twenty-one (68%) of these children had a positive skin test to one or more of the proteins. In 17 of the 21 children, positive skin tests were obtained to two or more of the proteins. Of the 124 separate skin tests applied to this group, there were 16 positive tests to casein, 5 to alpha-lactalbumin, 15 to beta-lactoglobulin and 15 to BSA. There were 40 one-plus reactions, and 11 two-plus reactions.

As described elsewhere,¹ the group allergic to milk consisted of 89 children, who were divided as to whether or not they were challenged orally with both milk and purified proteins (Group A), or challenged with milk only (Group B). Accordingly, the skin testing results have been reported for each of the 45 patients in Group A in Table II and for each of the 40 patients in Group B in Table III. Eighty-five of the 89 combined Group A and Group B patients were skin tested, and 50 (59%) of these patients had positive skin tests to one or more of the proteins. In 30 of these 50 patients, positive skin tests were obtained to two or more of the proteins.

Of 340 separate tests applied to these 85 milk allergic patients, 34 tests were positive to casein, 22 were positive to alpha-lactalbumin, 20 were positive to beta-lactoglobulin, and 21 to BSA (Table IV). A total of 296 intradermal and 44 scratch tests were applied. The intensity of the separate skin tests as elicited by the intradermal and the scratch methods are shown in Table V.

On the basis of two-plus or greater scratch tests, and three-plus or greater intradermal tests, 20 of the 97 positive tests were considered "strongly positive." Seventeen of these strongly positive tests occurred in the Group A patients. Except for the greater number of strongly positive tests in the Group A patients, no significant differences were found in the results of the skin tests for both groups.

For Group A patients, the skin test results were compared with the results of oral challenge with the same protein. No correlation was found. However, as seen in Table VI, close agreement was found between the strongly positive skin tests and the positive oral challenge with the corresponding protein. Fourteen of the 15 skin tests for which comparisons could be made agreed with the oral challenge results.

The relationship of positive skin tests to the preceding time on a milk-free diet was examined. Arbitrarily, 47 milk allergic patients on a milk-free diet less than 50 days, mean of 17 days, were compared to 38 milk allergic patients on a milk-free diet for a longer period, mean of 145 days. Sixty-two per cent of the first group and 58% of the latter group had positive tests, a difference of no statistical significance.

TABLE I
SYMPTOMS AND ALLERGENS OF CHILDREN ALLERGIC BUT NOT TO MILK, AND
SKIN TESTS WITH PURIFIED MILK PROTEINS

Patient	Age (yr)	Symptoms	Allergen*	Skin Tests†			
				Casein	Alpha-lactalbumin	Beta-lactoglobulin	BSA
1	9	Asth., rhin.	House dust	0	0	1+	1+
2	12	Asth., rhin.	House dust	2+	1+	1+	2+
3	11	Asth., rhin.	House dust	0	0	1+	0
4	12	Asth., rhin.	Fish	1+	0	1+	0
5	10	Asth., rhin.	Tomato	0	0	0	0
6	4	Asth., rhin.	Grass pollen	0	0	0	0
7	7	Asth., rhin.	House dust, alternaria	0	0	1+	0
8	6½	At. derm.	House dust, alternaria	2+	1+	0	0
9	14	Rhin.	Wheat	1+	1+	1+	2+
10	11	Asth., rhin.	House dust, alternaria	0	0	0	0
11	13	Asth., rhin.	Chocolate	1+	0	0	1+
12	13	Asth., rhin.	House dust, alternaria	2+	0	2+	1+
13	5	Asth., rhin.	House dust, alternaria	2+	1+	2+	2+
14	9	Asth., rhin.	House dust, alternaria	1+	0	1+	0
15	9	Asth., rhin.	Egg	0	0	0	0
16	5	Rhin.	House dust	1+	0	1+	0
17	1½	Asth., at. derm.	Egg, wheat, chocolate	0	0	0	0
18	1½	Asth., at. derm.	Egg, wheat, chocolate	0	0	2+	1+
19	5	Urt.	Tomato	0	0	0	0
20	8	Asth., rhin.	Egg	1+	0	0	1+
21	1½	Dia., rhin.	Lamb	1+	0	1+	1+
22	7	Asth., rhin.	House dust	1+	1+	1+	1+
23	3	At. derm.	House dust	0	0	0	0
24	6	Asth.	House dust	0	0	0	0
25	5	Asth.	House dust	0	0	0	1+
26	3	Urt., rhin.	Rice	1+	0	0	2+
27	13	Asth.	Chocolate	1+	0	1+	1+
28	9	Asth.	Chocolate, dog hair	1+	0	0	0
29	13	Asth.	Chocolate	0	0	0	0
30	11	Rhin.	House dust	1+	0	1+	1+
31	9	Asth., rhin., at. derm.	Egg	0	0	0	1+

* Sensitivity to non-milk substance was determined by oral or inhalent testing.

† Intradermal tests with concentrations of 10,000 PNU/ml were used. Reactions were observed in 20 minutes. Asth. = asthma, Rhin. = rhinitis, Urt. = urticaria, At. Derm. = atopic dermatitis, Dia. = diarrhea.

TABLE II
SKIN TESTS WITH PURIFIED MILK PROTEINS AND TIME ON MILK-FREE DIET BEFORE
TESTING MILK ALLERGIC CHILDREN IN GROUP A*

Patient	Age	Months off Milk	Skin Tests			
			Casein	Alpha- lactalbumin	Beta- lactoglobulin	BSA
1	7 mo	None	1+	2+	1+	0
2	12 mo	1	1+†	1+	2+†	1+
3	2½ mo	2	2+	2+	0	2+
4	3 mo	2	0	0	0	0
5	5 mo	4	0	0	0	0
6	6 mo	None	0	1+	0	0
7	5 mo	4½	0	0	0	0
8	4 mo	3	2+	0	0	2+
9	2½ mo	1½	1+	0	0	1+
10	10 mo	6	1+	0	0	0
11	6 yr	2	0	1+	1+	1+
12	6 yr	12	0†	0†	0†	0†
13	3½ mo	11 da	2+	2+	0	2+
14	5 mo	3	3+	1+	0	0
15	14 mo	None	0	0	0	0
16	4 mo	1	1+	0	0	0
17	1 mo	None	0†	0†	0†	0†
18	3½ mo	1½	1+	0	0	0
19	5½ mo	5	0†	0†	2+†	0†
20	5½ mo	3½	0	0	0	0
21	16 mo	1½	0†	0†	0†	0†
22	23 mo	1	0	0	0	0
23	7½ mo	4½	2+	2+	3+†	1+†
24	3 yr	3	0	0	0	0
25	14 mo	None	0†	0†	0†	0†
26	15 mo	1	0	0	0	0
27	11 mo	4	3+†	1+	2+†	1+
28	2½ mo	1	1+	0	0	1+
29	2½ mo	1½	2+	0	1+	0
30	5 mo	1	0	0	0	0
31	4 mo	1	0	0	1+	0
32	5½ mo	½	0	0	0	0
33	26 mo	24	0†	4+†	2+†	2+†
34	3 mo	2	3+	0	3+	0
35	3 mo	10 da	0	0	0	0
36	10 mo	3	0	0	0	0
37	6 mo	2	4+	4+	4+	4+
38	12 mo	8	0	1+	0	0
39	2 mo	½	1+	1+	0	0
40	17 mo	2	0	0	0	0
41	24 mo	1	1+	0	0	0
42	5 mo	1	0	0	0	1+
43	4 mo	2½	2+	2+	2+	2+
44	2 wk	None	0	0	0	0
45	12 mo	5	0	3+	1+	4+

* Patients were challenged orally with purified milk proteins.

† Indicates scratch tests. All other tests are by the intradermal method.

TABLE III
SKIN TESTS WITH PURIFIED MILK PROTEINS AND TIME ON MILK-FREE DIET BEFORE
TESTING MILK ALLERGIC CHILDREN IN GROUP B*

Patient	Age	Months off Milk	Skin Tests			
			Casein	Alpha-lactalbumin	Beta-lactoglobulin	BSA
1	24 mo	2	1+	0	0	0
2	3½ yr	None	0†	0†	0†	0†
3	5½ mo	3	0	0	0	0
4	3 mo	1	2+	0	2+	0
5	11 mo	3	0	0	1+	0
6	16½ mo	6	0	1+	0	3+
7	18 mo	21 da	0	0	0	0
8	3 mo	None	0	0	0	0
9	5 mo	3	1+	0	0	1+
10	7 mo	nd	nd	nd	nd	nd
11	13 yr	None	1+	0	0	0
12	14½ mo	None	1+	0	0	0
13	18½ mo	14 da	2+	0	0	0
14	12 mo	2	1+	0	1+	1+
15	9 mo	nd	nd	nd	nd	nd
16	3½ mo	1	2+	0	0	0
17	7 mo	None	0	1+	0	0
18	18 mo	None	4+†	1+†	0†	0†
19	24 mo	None	1+	0	0	0
20	12 mo	None	0	0	0	0
21	12 mo	9	0	0	0	0
22	33 mo	None	0†	0†	0†	0†
23	19 mo	10	0	0	1+	0
24	1½ mo	nd	nd	nd	nd	nd
25	12 yr	4	0	0	0	0
26	11½ mo	1	0	0	2+	1+
27	3½ mo	2	0	0	0	0
28	5 yr	7 da	1+	0	0	0
29	4 yr	None	0	0	0	0
30	12 mo	1	1+	2+	0	0
31	10 mo	1	1+	0	0	1+
32	6 mo	14 da	1+	1+†	2+†	0
33	10 mo	3	0	0	1+	0
34	3 mo	10 da	0	0	0	0
35	7 mo	3	0	0	0	0
36	1 mo	None	0	1+	0	1+
37	9 mo	1	0	2+	0	1+
38	6 mo	2	0	0	0	0
39	11 yr	None	1+	0	0	0
40	10 yr	2	0	0	0	0
41	7 mo	1	0	0	0	0
42	2 mo	nd	nd	nd	nd	nd
43	3 mo	2	0	0	0	0
44	5 mo	3½	0	0	0	0

* Patients were challenged orally with milk only.

† Indicates scratch tests. All other tests are by the intradermal method.

TABLE IV
POSITIVE SKIN TEST RESULTS WITH PURIFIED MILK PROTEINS IN MILK ALLERGIC PATIENTS

<i>Patients*</i>	<i>Separate Tests (no.)</i>	<i>Positive Tests (no.)</i>			
		<i>Casein</i>	<i>Alpha-lactalbumin</i>	<i>Beta-lactoglobulin</i>	<i>Serum Albumin</i>
Group A*	180	16	15	13	14
Group B	160	15	8	7	7
Total	340	31	23	20	21

* Group A were challenged orally with milk and purified milk proteins. Group B were challenged orally with milk only.

Correlation coefficients were calculated for the various skin testing results, types of symptoms, age of onset of hypersensitivity and features of the challenge reactions. No correlations were found.

COMMENT

The participation of a large number of physicians was not the ideal method for evaluating skin testing of milk allergic children with purified milk proteins. However, since each participant contributed measurements as well as interpretations of each skin test reaction and had patients with negative as well as positive tests, it is believed that this data is valid and can be compared with

other skin testing results obtained from control groups of patients.

The frequency of positive skin tests in the milk allergic children described here is higher than previously reported.^{2,3} However, the results of past studies are not comparable. In this study, four purified milk protein antigens were used separately and at equal concentration, while in other studies a commercial antigen made from whole milk protein or homogenized milk was used. Since casein comprises about 85% of the total milk protein, it is questionable if enough whey protein is present in these latter two types of skin testing materials to give a positive reaction to alpha-lactal-

TABLE V
INTENSITY OF INTRADERMAL AND SCRATCH SKIN TESTS IN PATIENTS ALLERGIC TO MILK

<i>Testing Method</i>	<i>Patients*</i>	<i>Tests Applied (no.)</i>	<i>Number of Tests by Intensity</i>				
			<i>0</i>	<i>1+</i>	<i>2+</i>	<i>3+</i>	<i>4+</i>
Intradermal	Group A	150	99	26	16	4	5
	Group B	146	114	24	7	1	0
	Total	296	213	50	23	5	5
Scratch	Group A	30	20	2	5	2	1
	Group B	14	10	2	1	0	1
	Total	44	30	4	6	2	2

* Group A were challenged orally with milk and purified milk proteins. Group B were challenged orally with milk only.

bumin, beta-lactoglobulin, or BSA. Also, we used both intradermal and scratch tests, whereas in past studies^{2,3} only scratch tests were used.

It is clear that negative as well as positive skin test reactions to milk proteins occur in the milk allergic patient and neither are associated with any particular symptom, age of onset of the hypersensitivity, severity of reaction, or other features of the oral challenge reactions.

In spite of the high frequency of positive skin tests in the milk allergic group (59%), it is obvious that positive skin tests have little diagnostic significance because the group of children who were allergic, but not to milk, have as great as, if not a greater, frequency of positive reactions (68%). It might be postulated that these children allergic, but not to milk, were sensitive to milk at an earlier age, lost the clinical sensitivity, but retained the ability to synthesize skin fixing antibodies. However, this premise is unsupported by the past histories of these patients. Further it is improbable that the frequency of milk allergy in allergic children would have been as high as indicated by the skin test results. Further elucidation of this is in progress by means of the skin testing of infants who are allergic, but not to milk.

Lack of correlation between the dermal and systemic reactivity of the milk proteins in the milk allergic child is further indicated in that no systemic reactions resulted from skin testing of the patients who were allergic to milk. However, it must be emphasized that serious systemic reactions to milk skin tests have been reported⁴ in an infant who previously had an anaphylactic reaction following milk ingestion.

Skin tests to milk proteins have limited diagnostic value. However, *strongly* positive skin tests to purified milk proteins were limited to milk allergic children, and such tests correlated closely with the results of oral challenge with the same protein. The phenomenon of the limitation of strongly positive skin tests to milk allergic patients suggests that the titer of this antibody may be greater in that group, or that a different and more specific skin fixing antibody may be present in the milk allergic child. Skin testing more allergic children not sensitive to milk will be necessary to verify this point.

It is difficult to determine why these skin sensitizing antibodies persist long after elimination of antigens or after the disappearance of circulating antibodies as detected by immunodiffusion, hemagglutination and passive cutaneous anaphylaxis.⁵

TABLE VI
COMPARISON OF STRONGLY POSITIVE SKIN TESTS WITH MILK PROTEINS IN MILK ALLERGIC PATIENTS
TO THE RESULTS OF ORAL CHALLENGE WITH THE SAME PURIFIED PROTEIN

Patient	Skin Tests				Oral Challenge†			
	Casein	Alpha-lactalbumin	Beta-lactoglobulin	BSA	Casein	Alpha-lactalbumin	Beta-lactoglobulin	BSA
2	..	..	2+*	..	..	..	Pos.	..
14	3+	..	..	..	Neg.	..	..	..
19	..	..	2+*	..	..	..	Pos.	..
23	..	..	3+*	..	..	..	nd	..
27	3+*	..	2+*	..	Pos.	..	Pos.	..
33	..	4+*	2+*	2+*	..	Pos.	Pos.	Pos.
34	3+	..	3+	..	Pos.	..	Pos.	..
37	4+	4+	4+	4+	Pos.	Pos.	Pos.	Pos.
45	..	3+	..	4+	..	nd	..	Pos.

* Skin testing by the scratch method. All other skin tests are by the intradermal method.

† Pos. = positive reaction to oral challenge; neg. = negative reaction to oral challenge; nd = not done.

The following possibilities are suggested. Milk antigens may remain in the tissues and continue to act as a stimulus for antibody formation. The skin sensitizing antibody may have a longer life span than "normal" antibody. The production of these allergic antibodies may be independent of antigens.

Since few allergic patients were skin tested in the first two months of life, no information was obtained regarding the presence or absence of skin sensitizing antibodies during the period which precedes the production of gamma globulin antibodies of 160,000 molecular weight. Although allergic manifestations have been reported in agammaglobulinemic patients, reagenic antibodies have seldom been demonstrated in the antibody deficiency syndromes.⁶ The relationship between the skin sensitizing antibodies and the gamma globulin antibodies remains unsettled, but skin testing and antibody studies of milk allergic children in the first 2 months of life may provide clarification.

Other means of detecting milk antibodies were utilized in these milk allergic patients. The results of the antibody studies are presented elsewhere⁵ and are compared to the results of skin testing and oral challenge with milk proteins.

SUMMARY

Skin testing separately and with equal concentrations of the purified milk proteins, casein, alpha-lactalbumin, beta-lactoglobulin, and BSA was done in 85 milk allergic children. For control purposes, intradermal tests with the same antigens were done in 102 normal children and 31 children allergic to non-milk substances.

Six per cent of the normal children had weakly positive reactions. However, positive skin tests occurred in 68% of the children allergic, but not to milk, and in 59%

of the children allergic to milk. In the milk allergic children, the incidence of positive reactions to each milk protein was similar. The incidence of positive skin tests in milk allergic children was higher than previously appraised. Positive skin tests in the milk allergic child were obtained long after the dietary elimination of milk antigens and after the disappearance of circulating milk antibodies as detected by a variety of techniques.

In contrast to the control groups, strongly positive skin tests occurred only in milk allergic patients. Although no correlation was found between the results of all skin tests and the results of oral challenges with the same milk protein, a close correlation was found between the results of strongly positive skin tests and the results of oral challenge with the same milk protein. It appears that weakly positive skin tests with purified milk proteins have a little diagnostic value, but strongly positive skin tests have diagnostic significance.

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