

A study of serum antibodies to isolated milk proteins and ovalbumin in infants and children

**C. D. MAY, LINDA REMIGIO, JOAN FELDMAN, S. A. BOCK
and R. I. CARR**

National Jewish Hospital and Research Center, and University of Colorado Medical School, Denver, Colorado, U.S.A.

Summary

Serum antibodies to cow milk proteins and ovalbumin were measured quantitatively. Food hypersensitivity of the immediate type was determined to be present or absent by skin tests and double-blind food challenges. Elevated levels of antibodies to milk proteins in sera characteristic of infants fed cow milk were found to decline with age, so that sera from children who were 6 to 15 years of age (inclusive), not hypersensitive to food, had significantly lower levels than the infants. In contrast, sera from age-matched children, who were shown to have hypersensitivity to some food, were found to have levels of antibodies to milk proteins as elevated as in infancy. Hypersensitivity was not necessarily to milk but often to some other food. This persistence of greater antibody production to milk throughout childhood in those hypersensitive to some food indicates a fundamental difference from those without hypersensitivity to food, either in permeability, in immunological reactivity of the gut or in development of immunological unresponsiveness. Implications for pathogenesis of clinical disorders are discussed.

Introduction

In conjunction with a comprehensive objective study of hypersensitivity to food in children (May, 1976a), serum antibodies to five milk proteins and to ovalbumin were measured by the quantitative antigen-binding method of Farr (Farr, 1971; Minden & Farr, 1973). One purpose was to compare serum antibodies in children hypersensitive to food and those not hypersensitive to food, and to correlate serum antibodies with other laboratory and clinical observations. Another purpose was to extend the observations by Farr and associates (Rothberg & Farr, 1965; Barrick & Farr, 1965; Korenblatt *et al.*, 1968) pertaining to the incidence and amounts of serum antibodies to milk proteins in health and disease. In their studies, bovine serum albumin and alpha-lactalbumin were employed as antigens. The present study included three additional major milk proteins and ovalbumin to quantify antibody responses to a wider variety of antigens.

An unanticipated finding was that serum antibodies to cow milk proteins remained

Correspondence: Dr Charles D. May, National Jewish Hospital and Research Center, 3800 East Colfax Avenue, Denver, Colorado 80206, U.S.A.

elevated beyond infancy in children hypersensitive to food, while levels declined in children not hypersensitive to food. The hypersensitivity was not necessarily to cow milk but sometimes to other food.

Persistence of elevated antibodies to milk proteins in sera of children with hypersensitivity to food indicates a fundamental difference to non-hypersensitive children in immunological reactivity, similar to findings in allergic versus non-allergic adults (Barrick & Farr, 1965).

The bearing of the results of the present investigation on unsettled questions about the role of serum antibodies to food proteins in the pathogenesis of hypersensitivity disorders will be considered in the discussion of the results.

Materials and methods

Definition of terms

The expression 'hypersensitive to food' as used herein means that reaginic (IgE) sensitization to antigen from food was demonstrable, but symptoms did not necessarily occur upon ingestion of the corresponding food.

Asymptomatic hypersensitivity. This indicates that immunological sensitization to a food was demonstrable, but symptoms did not occur upon double-blind challenge the with food.

Symptomatic hypersensitivity. This applies to sensitized subjects who reacted to a food in a double-blind challenge. Symptomatic hypersensitivity is equivalent to clinically significant hypersensitivity in common parlance.*

Subjects

The 136 subjects studied were infants and children from 5 months up to 15 years (inclusive) of age. They were divided into two groups: eighty-three were not hypersensitive to food and fifty-three were hypersensitive to food. The latter group sometimes exhibited additional evidence of hypersensitivity in lungs, skin, etc., but other illnesses and non-immunological gastrointestinal disorders were absent.

Hypersensitive to food. Subjects were assigned to this group on the basis of significant net weal reactions (3 mm or greater) in puncture skin tests with verified extracts of one or more among the twenty foods under study. That 3 mm wheals in puncture tests are indicative of true immunological hypersensitivity to the food extracts used was verified in a preceding study (Bock *et al.*, 1977), which included comparison of skin tests with histamine release from leucocytes and double-blind food challenges.

* The justification of this classification is set forth in a chapter on Adverse Reactions to Foods in 'Allergy: Principles and Practice'; by E. Ellis, E. Middleton and C. Reed, to be published by Mosby, St Louis in 1977.

* (+) Positive; (–) negative; MP, cow-milk proteins; OV, chicken ovalbumin; X, another food extract.

† FC, double-blind food challenge: M, positive to cow milk; E, to egg; O, to other food; (–) negative.

‡ High spontaneous release of histamine: (+) present, (–) not present (discussed in text).

§ 1:10 dilution of serum. BSA, bovine serum albumin; CAS, casein; ALA, alpha-lactalbumin; BLG, beta-lactoglobulin; BGG, bovine gamma-globulin; OV, ovalbumin, chicken.

¶ Months in parentheses.

Table 1. Infants and children hypersensitive to food

No.	Age (years)	Skin tests*					Antigen bound by serum (%)§					
		MP	OV	X	FC†	HSR‡	BSA	CAS	ALA	BLG	BGG	OV
1	1	+	+	+	M	-	88	67	59	54	9	0
2	1 8/12	+	+	-	-	-	89	61	17	16	56	5
3	2 6/12	+	+	-	-	-	23	15	5	0	6	60
4	3	+	+	+	M	+	58	23	10	11	15	42
5	5	+	+	+	-	-	41	24	0	6	10	59
6	5	+	+	-	-	-	7	45	7	22	5	20
7	5 8/12	+	-	+	-	-	93	10	22	4	1	13
8	6 6/12	+	+	+	O	+	100	65	16	12	30	61
9	6 6/12	+	+	+	O	+	83	57	44	26	20	68
10	7	-	+	+	-	+	55	42	40	11	5	25
11	7	+	-	-	M	-	60	29	15	15	47	86
12	7	+	+	-	-	+	68	4	10	9	21	54
13	8 1/2	+	+	+	O	+	100	91	71	46	76	97
14	9	+	-	+	-	-	36	0	11	19	58	53
15	9	+	+	+	E	+	4	40	10	11	2	0
16	9	-	-	+	O	-	50	37	6	1	95	100
17	9 1/2	+	+	+	E	+	25	48	2	0	0	0
18	10	+	-	+	-	+	6	5	0	5	61	4
19	10	+	+	+	-	-	15	18	2	0	9	17
20	10	+	+	+	-	+	60	30	39	2	66	30
21	10	+	+	+	-	+	7	0	0	0	0	4
22	10 1/2	+	+	+	O	+	65	8	77	46	34	67
23	11	+	+	+	O	+	14	63	19	6	37	49
24	11	+	+	+	-	-	10	32	32	14	6	54
25	11	-	-	+	-	-	100	26	14	3	59	9
26	11	-	-	+	-	-	4	16	2	0	8	1
27	11	+	-	+	-	-	47	0	9	9	0	4
28	11	-	-	+	-	-	33	1	2	10	20	67
29	11	+	-	+	-	+	68	43	12	9	67	67
30	11 1/2	-	-	+	-	-	26	30	0	3	49	53
31	11 7/12	+	+	+	-	-	19	54	17	14	15	10
32	12	+	+	+	-	-	4	6	0	2	1	0
33	12	+	+	+	-	-	0	1	0	0	0	20
34	12	+	+	+	-	-	3	0	0	13	6	34
35	12	+	-	+	-	-	97	40	17	20	25	65
36	12	+	+	+	-	-	80	0	0	0	6	16
37	13	+	+	+	-	-	3	9	18	17	0	0
38	13	+	+	+	-	-	98	76	48	7	15	81
39	13	+	+	+	E	+	35	35	20	20	10	71
40	13	+	+	+	-	+	8	46	7	18	2	23
41	13	+	+	+	-	-	31	9	10	6	1	13
42	13 1/2	+	+	+	O	+	27	62	47	77	97	52
43	14	+	+	+	-	-	23	8	4	24	56	64
44	14	+	+	+	-	-	4	35	2	5	6	32
45	14	+	-	+	-	-	10	4	1	1	11	31
46	14	+	+	+	-	-	3	6	0	1	7	18
47	14	+	+	+	O	+	98	22	30	7	75	38
48	14	+	+	+	-	-	22	29	4	8	15	43
49	14	+	+	+	-	+	32	8	1	4	8	15
50	14	+	+	+	O	+	1	2	0	0	4	26
51	15	+	-	+	-	+	5	29	2	10	80	31
52	15	+	+	+	E	+	5	47	9	0	0	77
53	16	-	+	+	E	+	12	6	16	0	66	26

The food extracts, used and interpreted as described, did not give false positive or false negative results, but indicated the presence or absence of immunological hypersensitivity of the immediate type.

A subject was considered hypersensitive to food if a reaction in a skin test with any of the foods was significantly positive. Whether the subject reacted to milk proteins, ovalbumin or some other food is shown in Table 1.

Food antigens to which symptomatic hypersensitive subjects reacted are shown in Table 1. All the reactions were of the immediate type. Comparisons of the findings in symptomatic versus asymptomatic subjects will be given in the Results section.

Infants and children known to be hypersensitive to food (Table 1) were patients at the National Jewish Hospital and Research Center. All studies were carried out according to a basic protocol approved by the Committee on Studies Involving Humans. The conduct of the basic study is described in a previous report (May, 1976a).*

Not hypersensitive to food. Subjects were included in this group only if careful inquiry or prolonged observation revealed no evidence of manifestations of adverse or hypersensitivity reactions to foods; namely, eczema, urticaria, rhinitis, asthma, gastrointestinal disturbances or malabsorption.† In addition, the majority of the children were skin-tested with extracts of ten to twenty of the foods under study and were found to give no significant reactions with concentrations up to 1:100 w/v intradermally. Thus there was no evidence of either asymptomatic or symptomatic hypersensitivity to food of the immediate type.

Antigen-binding by sera

Antibodies to milk proteins and ovalbumin in sera were assayed according to the antigen-binding procedure of Farr (Farr, 1971; Minden & Farr, 1973).

The antigens were isolated cow milk proteins obtained from commercial sources (May, 1976a). Bovine serum albumin, casein, alpha-lactalbumin and beta-lactoglobulin were used without further purification, as was the chicken ovalbumin. Bovine gamma-globulin was further purified by passage through a DEAE-cellulose column.

Antigens were labelled with ^{125}I by conventional methods: chloramine-T (McConahey & Dixon, 1966) or lactoperoxidase (Marchalonis, 1969) for milk proteins, and Bolton-Hunter for ovalbumin (Bolton & Hunter, 1973). The nitrogen content of stock solutions was determined for milk proteins. Because of contamination with gelatin in the Hunter-Bolton procedure for labeling with ^{125}I , the nitrogen content of ovalbumin could not be measured in the stock solution and so a dilution suitable for the binding procedure was determined by trial.

Antigen-binding by sera was carried out essentially as described by Farr (1971), the following details being noteworthy: stock solutions of ^{125}I -labelled milk proteins were diluted to 0.04 μg antigen N/ml for use in the assay. A dilution of the stock ovalbumin solution was selected as already described. The reaction mixture contained: 75 μl 1:10 dilution of subject's serum and 75 μl antigen dilution. The precipitants used were: saturated ammonium sulphate (SAS) for BSA and ovalbumin, 15% polyethylene

* We are grateful to Dr Jerome Buckley of Denver, Colorado, for referral of several patients from his private practice.

† We are indebted to Dr Samuel Fomon, University of Iowa Medical School, for generous provision of sera of all the infants and many of the children in this group. They were under long-term close observation during his studies of infant nutrition.

glycol (PEG) for BGG, 20% PEG for CAS, and 40% PEG for ALA and BLG. (final concentrations were half these values). The PEG (mol. wt. 6000 to 7500) was dissolved in borate buffer. The reaction between antigen and serum was allowed to proceed at 4°C. 150 μ l precipitant was added (immediately when PEG was used, and at the end of 18 hr when SAS was the precipitant). Precipitates were washed by SAS or PEG at half the concentration of the precipitant as originally used. The use of PEG in the manner described was developed, and the efficiency of selective precipitation of antibody was established in the laboratory of one of the authors (RIC).

Expression of results

Antigen-binding is expressed in the Tables and throughout this paper as the per cent of ^{125} I-labelled antigen bound by 1:10 dilution of the subjects' serum. For milk proteins the amount of antigen incubated with serum in the assay system was 0.003 μ g antigen N (75 μ l of 0.04 μ g antigen N/ml in a volume of 150 μ l).

Statistical analyses were carried out with the generous guidance of Dr Robert Kinsman.

Skin tests

The techniques and interpretation of skin testing are fully described in a previous study (Bock *et al.*, 1977). In that study the extracts of foods were shown to be reliable for detection of immediate-type hypersensitivity and did not give non-specific, false irritant reactions. The extracts of foods were stock commercially prepared products.* Subjects suspected of hypersensitivity to food were tested with extracts of twenty foods: whole cow milk, whole chicken egg, peanut, walnut, almond, cashew, fish mix, filbert, peas, soybean, corn, chocolate, pepper, shellfish mix, wheat, orange, tomato, potato, beef and pork.

These were applied by puncture test in concentrations of 1:20 w/v or intradermally at 1:1000 and 1:100 w/v, progressively, along with buffer and histamine controls, until a significant net weal of 3 mm diameter or greater, or no reaction, was elicited.

In addition, milk proteins (MP) and chicken ovalbumin (OV) were injected as intradermal skin tests in concentrations from 10 to 1000 μ g/ml, progressively, along with buffer and histamine controls, until a significant net 3 mm weal, or no reaction, was elicited. The milk proteins contained a mixture of equal parts of bovine serum albumin, casein, alpha-lactalbumin, beta-lactoglobulin and bovine gamma-globulin; the sources and preparation have been previously described (May, 1976a). There was almost complete agreement in results of skin tests using these purified proteins with results using the extracts of whole cow milk and whole egg. Because the purified proteins were utilized in *in vitro* studies of histamine release, the skin test results with these proteins are listed in Table 1 for comparison with other data.

Food challenges

The procedure for conducting a double-blind food challenge is fully described elsewhere (May, 1976a).

Spontaneous histamine release from leucocytes

The procedures for determination of spontaneous *in vitro* release of histamine from leucocytes of persons hypersensitive to food has been described (May, 1976b). A release of 25% or more of the histamine content of a leucocyte suspension, without the

* Obtained from Greer Laboratories, Lenoir, North Carolina.

addition of antigen, is considered as a high spontaneous release in contrast to the usual 5–10% spontaneous release.

Results

Subjects not hypersensitive to food

The binding of cow milk proteins and ovalbumin by sera from infants and children not hypersensitive to food is presented in Table 2. The mean amount of antigen bound differed among the various antigens; antibodies to ALA and BLG were apparently, produced less abundantly. As can be ascertained from the ranges of values, the sera of a few individuals had a relatively large content of antibodies to one antigen or another, without evidence of symptomatic or asymptomatic immediate-type hypersensitivity to food. The mean antibody content of the sera appears to become progressively less as the ages of the subjects advance. The infants fed cow milk developed relatively high levels of serum antibodies to the milk proteins. Apparently, the level of antibodies later decline, even though cow milk products are continued in the diet, because in the present study serum antibodies to cow milk proteins were found to be significantly lower in the 6 to 15-year-old group of subjects not hypersensitive to food. The number of subjects between 2 and 6 years of age was too small to follow the trend of the decline more precisely, or to make comparisons.

Subjects hypersensitive to food

All the data concerning infants and children proven to be hypersensitive to food are given in Table 1. Of the entire group of fifty-three infants and children shown to be hypersensitive to food, only three were demonstrated by double-blind challenge to manifest symptomatic hypersensitivity specifically to cow milk, and five to egg.

Table 2. Infants and children not hypersensitive to food

Ages	No. of subjects		Antigen bound by serum (%)*					
			BSA	CAS	ALA	BLG	BGG	OV
(a) 20-30 (weeks)†	30	Mean:	35.7	26.3	10.3	18.0	28.9	0
		s.d.:	42	16.8	10.1	16.9	28.7	
		Range:	0-100	0-56	0-36	0-54	0-87	
(b) 2-6 (years)‡	5	Mean:	33.6	15.2	6.6	2.2	29.4	33.8§
		s.d.:	23.4	9.6	8.4	2.2	20.8	26.9
		Range:	1-56	4-26	0-21	0.5	0-47	18-74
(c) 6-15 (years)‡	48	Mean:	12.4	16.1	4.3	4.1	20.9	7.7¶
		s.d.:	11.7	16.4	4.3	4.8	23.6	10.3
		Range:	0-48	0-65	0-16	0-21	0-100	0-31
Binding by sera (c) less than (a)**			<i>P</i> < 0.01	<i>P</i> < 0.01	n.s.	<i>P</i> < 0.01	<i>P</i> < 0.05	—

* 1:10 dilution of serum. See Table 1 for abbreviations.

† Diets contained cow milk 8 weeks or more before serum samples obtained. No egg in diets.

‡ Diets contained cow milk and eggs.

§ Four subjects.

¶ Ten subjects.

** Significance shown by Duncan's multiple range test.

Table 1 shows that high percentages of binding of cow milk proteins or ovalbumin by sera of some subjects were observed, even when skin tests to these antigens were negative. However, extracts of other food(s) evoked positive skin tests in these subjects, indicating hypersensitivity to some other food(s). All subjects in Table 1 were hypersensitive to one or more of the twenty foods under study.

More detailed analysis of the data in Table 1 and comparisons with the findings in subjects not hypersensitive to food are provided in subsequent Tables. For reasons given in preceding paragraphs, children of 6–15 years of age are treated as a separate group to the infants and children who were less than 6 years old.

Incidence of detectable serum antibodies versus amounts of antibodies

In Tables 1 and 2 the percentage binding of antigen reflects the amount of antibody in the sera. Table 3 gives the incidence of detectable antigen binding by the sera of children 6–15 years of age who are hypersensitive and not hypersensitive to food; that is, the percentage of each group whose sera bound 5% or more of the antigens. It is clear the two groups do not differ remarkably in the percentage of sera with detectable serum antibodies to cow milk proteins and ovalbumin. The data in Table 4 shows that this contrasts significantly with the greater mean amounts of antigen bound by children hypersensitive to food. This distinction between the incidence of detectable antibody and the amount of antibody in the sera must be firmly grasped if the significance of the findings of the present study is to be appreciated.

Positive versus negative skin tests

In Table 5, the results of skin tests are compared with serum antibodies for the children who were 6–15 years of age and were hypersensitive to food. The mean amounts of serum antibodies to the milk proteins and ovalbumin were not significantly greater in those having positive reactions to skin tests with the corresponding antigens. These results are not incompatible as skin tests depend on a small concentration of specific IgE antibody, while antigen-binding by all serum antibodies reflects the relatively large concentrations of other antibodies present in the serum (IgG, IgM, IgA, etc.).

Table 3. Comparisons of antigen binding by sera from children who were 6 to 15 years (inclusive) of age

Groups	No.		Antigen bound by serum (%)*				
			BSA	CAS	ALA	BLG	BGG
Not hypersensitive to food (from Table 2)	48	Mean:	12.4	16.1	4.3	4.1	20.9
		s.d.:	11.7	16.4	4.3	4.8	23.6
Hypersensitive to food (from Table 1)	46	Mean:	36.0	26.5	14.9	11.1	27.7
		s.d.	33.6	23.5	18.7	14.5	29.4
Binding by sera of hypersensitive subjects greater than not hypersensitive†			<i>P</i> < 0.01	<i>P</i> < 0.01	<i>P</i> < 0.01	n.s.‡	n.s.

* 1:10 dilution of serum. See Table 1 for abbreviations.

† Significance shown by Duncan's multiple range test.

‡ n.s. = Not significant.

Table 4. Incidence of detectable antigen-binding* by 1:10 dilution of sera from children who were 6 to 15 years (inclusive) of age

Group	BSA†	CAS	ALA	BLG	BGG
Not hypersensitive to food; significant binding: (No.)‡	33	34	17	18	35
(%)	68.8	70.8	35.4	37.5	72.9
Hypersensitive to food; significant binding: (No.)§	37	36	27	29	35
(%)	80.4	78.3	58.7	63.0	76.1

* 5% or more of antigen bound.

† Abbreviations explained in Table 1.

‡ Total = 48.

§ Total = 46.

Table 5. Comparison of results of skin tests with antigen-binding by sera from children who were 6 to 15 years (inclusive) of age and hypersensitive to food*

Skin test results†	No. of subjects		Antigen bound by serum (%)‡					
			BSA	CAS	ALA	BLG	BGG	OV
Milk proteins positive	39	Mean:	35.3	27.2	15.5	12.4	25.0	—
		s.d.:	34.2	24.7	19.5	15.3	28.2	—
Milk proteins negative	7	Mean:	40.0	22.6	11.4	4.0	43.1	—
		s.d.:	32.3	15.5	14.0	4.6	33.5	—
Ovalbumin positive	33	Mean:	—	—	—	—	—	35.9
		s.d.:	—	—	—	—	—	26.8
Ovalbumin negative	13	Mean:	—	—	—	—	—	43.8
		s.d.	—	—	—	—	—	33.2

Sera from subjects with positive skin test to milk proteins do not have significantly greater antigen-binding (shown by Duncan's multiple range test). Sera from subjects with positive skin test to ovalbumin do not have significantly greater antigen-binding (shown by independent *t*-test).

* Subjects shown in Table 1.

† Technique and interpretation given in methods section.

‡ 1:10 dilution of serum. Abbreviations explained in Table 1.

Positive versus negative food challenges

Only the most highly sensitized children (3 mm or larger weal to skin test by the puncture technique using 1:20 w/v concentration of food extract) have had objectively demonstrated reactions in double-blind food challenges (May, 1976a; Bock *et al.*, 1977).

Subjects who had positive reactions in food challenges had significantly greater amounts of serum antibodies to milk proteins and ovalbumin, except for BLG (Table 6). Note particularly that the positive food challenges were not necessarily with milk or egg, but sometimes to some other food antigen. In other words, a high degree of sensitivity to some food, identified by a positive food challenge, was accompanied by a greater mean content of serum antibodies to milk proteins and ovalbumin.

Table 6. Comparison of results of food challenges with antigen-binding by sera from children who were 6 to 15 years (inclusive) of age and hypersensitive to food*

Groups	No.		Antigen bound by serum (%)†					
			BSA	CAS	ALA	BLG	BGG	OV
Food challenge positive:‡	15	Mean:	45.3	40.8	25.5	17.8	39.5	54.5
		s.d.:	36.9	25.0	23.9	22.5	34.7	31.5
Food challenge negative:	31	Mean:	31.5	19.6	9.8	7.9	22.0	30.3
		s.d.:	31.5	19.6	13.2	6.8	25.2	23.7
Positive FC greater than negative FC:§			<i>P</i> < 0.05	<i>P</i> < 0.01	<i>P</i> < 0.05	n.s.	<i>P</i> < 0.05	<i>P</i> < 0.01

* Subjects shown in Table 1.

† 1:10 dilution of serum. Abbreviations explained in Table 1.

‡ Positive to some food, not necessarily cow milk or egg (see Table 1).

§ Significance shown by Duncan's multiple range test.

High versus usual spontaneous release of histamine

In our experience, high spontaneous release of histamine from leucocytes *in vitro* has been observed thus far only with leucocytes from persons hypersensitive to food (May, 1976b). All individuals found to have positive reactions in double-blind food challenges have also exhibited high spontaneous histamine release from their leucocytes. Some persons whose leucocytes revealed high spontaneous release of histamine did not have positive reactions in food challenges; however, only one or two foods were tested. It seems that only the persons with high degree of hypersensitivity to food are prone to exhibit high spontaneous release of histamine from their leucocytes. As seen in Table 7, there appeared to be some tendency for greater mean amounts of serum

Table 7. Comparison of spontaneous release of histamine from leucocytes with antigen-binding by sera from children who were 6 to 15 years (inclusive) of age and hypersensitive to food*

Group	No.		Antigen bound by serum (%)†					
			BSA	CAS	ALA	BLG	BGG	OV
High spontaneous histamine release‡	22	Mean:	39.9	34.2	21.5	14.5	34.6	40.2
		s.d.:	35.0	25.2	22.6	19.2	32.6	27.8
Usual spontaneous histaminerelease	24	Mean:	32.4	19.4	8.9	8.0	21.5	36.3
		s.d.:	32.6	19.8	11.7	7.4	25.3	29.7
High spontaneous release greater than usual spontaneous release			n.s. $P < 0.05$		n.s.	n.s. $P < 0.05$		n.s.

* Subjects shown in Table 1.

† 1:10 dilution of serum. Abbreviations explained in Table 1.

‡ Defined in text.

Table 8. Category of hypersensitivity versus antigen-binding by sera from children who were 6 to 15 years (inclusive) of age

Groups	No.		Antigen bound by serum (%)				
			BSA	CAS	ALA	BLG	BGG
(a) Not hypersensitive to food (from Table 2)	48	Mean:	12.4	16.1	4.3	4.1	20.9
		s.d.:	11.7	16.4	4.3	4.8	23.6
(b) Hypersensitive to food (from Table 1)	46	Mean:	36.0	26.5	14.9	11.1	27.7
		s.d.:	33.6	23.5	18.7	14.5	29.4
(c) Hypersensitive to inhalants only	6	Mean:	9.5	14.8	3.5	6.3	29.3
		s.d.:	12.4	12.6	1.9	9.0	36.7

* 1:10 dilution of serum. Abbreviations explained in Table 1.

antibodies to milk proteins in children whose leucocytes were found to exhibit a high spontaneous release of histamine.

Hypersensitivity to inhalants versus hypersensitivity to foods

The question arose whether greater amounts of serum antibodies to milk proteins occurred in individuals who had evidence of any variety of immediate-type hypersensitivity, or was this true only for hypersensitivity to food? A small number of children were discovered who exhibited positive skin tests exclusively to inhalants (Timothy, ragweed, *Alternaria*) and to none of the twenty foods under study. The findings in this group are compared in Table 8 with a group having no positive skin tests to the inhalants or the foods. By the averages, the sera of the group hypersensitive to inhalants bound only a small percentage of the milk proteins, as did a comparable group shown to be not hypersensitive to the inhalants or to the foods. This is in sharp contrast to the greater mean antigen-binding by sera from the group of children hypersensitive to food.

Discussion

The findings in the present investigation on incidence and amount of serum antibodies to food proteins, as determined by the antigen-binding procedure of Farr, are in general agreement with the studies by Farr and associates, although there are some additions which may be considered of fundamental importance.

About 10 years ago, Barrick & Farr (1965) reported an interesting observation of greater incidence of detectable serum antibodies to bovine serum albumin among allergic (36% of 30) than non-allergic individuals (12% of 75) who were 16 or more years of age. Manifestations in the allergic group were asthma and/or rhinitis and positive skin tests to a variety of inhalant allergens. No history of allergic reactions to milk and beef products was obtained in the allergic group. The non-allergic group exhibited no reactions to the assortment of inhalant allergens tested. Of the twenty-eight subjects with detectable antibody to BSA, half had positive skin tests with BSA and half did not; no other food antigens were applied in skin tests. There are no data in their paper on the amounts of serum anti-BSA found in those with detectable BSA in the serum in the allergic and non-allergic groups.

In an earlier study, Rothberg & Farr (1965) presented data on thirty-one

allergic infants and children from 0 to 15 years (inclusive) of age, 84% of whom had detectable serum antibody to BSA, and an average of 56% of the amount of BSA in the assay was bound by their sera. Data from a corresponding group of 158 'well' subjects were: 69% with detectable binding of BSA and an average of 53% of the amount of BSA used was bound by the sera. The findings with ALA were similar. The sera of 'well' children were collected from school children for an unrelated study and were apparently not examined or tested for allergy. In subsequent papers, Farr and associates (Barrick & Farr, 1965; Korenblatt *et al.*, 1968) refer to these data as showing that 'the incidence and amount of anti-BSA in allergic children was not significantly higher than in the non-allergic'; actually the figures on amount of antigen bound they referred to were derived from a grouping composed of both infants and children from 0 to 15 years (inclusive) of age with a wide variety of disorders.

In the study (Korenblatt *et al.*, 1968) seeking to explain the lower incidence of detectable antibodies to BSA in sera from non-allergic adults, various possibilities were tested. Of fundamental importance is the stimulating suggestion set forth on the basis of their experiments that 'acquired immunological tolerance' or 'prolonged minimal antigenic stimulation via the oral route may be an important factor that in some way is responsible for the hyporesponsiveness of adults to BSA.' Or, as stated in an earlier paper (Barrick & Farr, 1965), 'the higher incidence of detectable anti-BSA among allergic adults suggests they may differ from normal persons with respect to having less effective mechanisms for reducing anti-BSA production during adult life.'

Our findings on serum antibodies in infants fed cow milk (Table 2) are in agreement with various aspects of many previous studies in which other methods of measuring antibodies were used (Gunther *et al.*, 1960, 1962; Peterson & Good, 1963; Kletter *et al.*, 1971; Parish, 1969, 1971).

However, we found that the mean amounts of serum antibodies to milk proteins in children who were 6 to 15 years old and not hypersensitive to food were considerably less than in infants (Table 2). Therefore it was feasible for us to compare the findings in this age group with results in children who were hypersensitive to some foods. The mean amounts of serum antibodies to all but one of the five milk proteins we tested were significantly greater in children hypersensitive to food than those not hypersensitive (Table 3); the incidences of detectable serum antibodies to the milk proteins were also greater in the hypersensitive group, but not strikingly so (Table 4). We cannot ascertain whether our finding is actually contrary to the finding of Rothberg & Farr (1965), because their data on the amounts of serum antibodies are not provided separately for infants and for children aged 6 years and over.

The age period at which serum antibodies to milk proteins decline in normal individuals may not be crucial. A more important consideration may be whether persons allergic only to inhalants and those hypersensitive to foods behave similarly with respect to antibody production to ingested food proteins. The limited data in Table 8 may be taken to indicate that as early as 11 to 12 years of age, children hypersensitive only in inhalants do not exhibit greater amounts of serum antibodies to milk than normal children who are not hypersensitive to food. In our study, the level of antibodies to milk proteins in sera from children hypersensitive to food is clearly greater than for those not hypersensitive to food (Table 3).

Thus we propose that the greater amounts of serum antibodies to ingestants like milk proteins and ovalbumin we observed in older allergic children may be a feature associated predominantly, if not exclusively, with hypersensitivity to some food (not

necessarily milk or egg), rather than with other categories of the allergic state; for example, hypersensitivity to inhalants unaccompanied by hypersensitivity to food.

Persistence of antibodies in the serum may reflect local hypersensitivity or antibody production in the gut. Modulation of antibody production through interrelations among various types of T lymphocytes may be involved.

The idea has been commonly proffered that persistence of elevated levels of serum antibodies to milk or other foods beyond infancy into adulthood may reflect a corresponding extension of a greater degree of permeability that the gut may possess in infancy. Or the persistence could be a consequence of subclinical injury to the gut by immunological reactions in asymptomatic hypersensitivity to food (immediate-type sensitization by IgE or reaginic antibody demonstrated by positive skin test). Unremitting stimulation of production of serum antibodies by antigenic material from food passing through the intestinal mucosa would be conceivable in either case. Increased permeability of the gut in allergic adults was not supported by experiments of Farr and associates (Korenblatt *et al.*, 1968). Instead, they obtained data indicating that an immunological unresponsiveness to BSA had developed in three non-allergic adults. This conclusion was based on lack of detectable antibody to BSA in the serum after subcutaneous injection of up to 3 mg of BSA, which may not have been a definitive test of responsiveness. Further pursuit of this appealing idea is indicated, e.g. to determine whether unresponsiveness also develops toward food antigens in persons hypersensitive only to inhalants.

The continued presence of antibodies to food proteins in the circulation affords an opportunity for combination with antigen absorbed from the gut to form immune complexes. It would not be surprising if conditions sometimes occurred, albeit infrequently, that could give rise to immune complex disorders. From our findings and the literature it is clear that persistently elevated serum antibodies to a food protein would not necessarily be accompanied by clinical or laboratory evidence of immediate-type hypersensitivity to the corresponding food.

Substantial amounts of antibody to food proteins in the serum at any age should not be lightly considered as natural and without potentially pathogenic significance. The incidence of detectable serum antibody to a food antigen may be similar in health and disease, but the amount or other characteristics may influence the pathogenetic potential. This was the position taken by Heiner, Sears & Kniker (1962) and Holland *et al.* (1962) some years ago, and recent reports lend support to this idea (Anderson *et al.*, 1974; Boat *et al.*, 1975).

Acknowledgements

Supported by Grants AI-11623 and AI-10872, National Institute of Allergy and Infectious Diseases, U.S. Public Health Service.

References

- ANDERSON, J.A., WEIS, L., REBUCK, J.W., CABAL, L.A. & SWEET, L.C. (1974) Hyperreactivity to cow's milk in an infant with LE and tart cell phenomenon. *Journal of Pediatrics*, **84**, 59.
- BARRICK, R.H. & FARR, R.S. (1965) The increased incidence of circulating anti-beef albumin in the sera of allergic persons and some comments regarding the possible significance of this occurrence. *Journal of Allergy*, **36**, 374.
- BOAT, T.F., POLMAR, S.H., WHITMAN, V., KLEINERMAN, J.I. STERN, R.C., & DOERSHUCK, C.F. (1975) Hyperreactivity to cow milk in young children with pulmonary hemosiderosis and cor pulmonale secondary to nasopharyngeal obstruction. *Journal of Pediatrics*, **87**, 23.

- BOCK, S.A., BUCKLEY, J., HOLST, A. & MAY, C.D. (1977) Proper use of skin tests with food extracts in diagnosis of hypersensitivity to food in children. *Clinical Allergy*, **7**, 375.
- BOLTON, A.E. & HUNTER, W.M. (1973) The labelling of proteins to high specific radioactivities by conjugation to a ^{125}I -containing acylating agent. *Biochemical Journal*, **133**, 529.
- FARR, R.S. (1971) Determination of antigen-binding capacity. *Methods in Immunology and Immunochemistry* (ed. C. A. Curtis and M. W. Chase), Vol. 3, p. 66. Academic Press, New York.
- GUNTHER, A., ASCHAFFENBURG, R., MATTHEWS, R.H., PARISH, W.E. & COOMBS, R.R.A. (1960) The level of antibodies to the proteins of cow's milk in the serum of normal human infants. *Immunology*, **3**, 296.
- GUNTHER, M., CHEEK, E., MATTHEWS, R.H. & COOMBS, R.R.A. (1962) Immune responses in infants to cow's milk proteins taken by mouth. *International Archives of Allergy*, **21**, 257.
- HEINER, D.C., SEARS, J.W. & KNIKER, W.T. (1962) Multiple precipitins to cow's milk in chronic respiratory disease. *American Journal of Diseases of Children*, **103**, 634.
- HOLLAND, N.H., HONG, R., DAVIS, N.D. & WEST, C.D. (1962) Significance of precipitating antibodies to milk proteins in the serum of infants and children. *Journal of Pediatrics*, **61**, 181.
- KLETTER, B., GERY, S., FREIER, S. & DAVIES, A.M. (1971) Immune responses of normal infants to cow milk. *International Archives of Allergy*, **40**, 656.
- KORENBLATT, P.E., ROTHBERG, R.M., MINDEN, P. & FARR, R.S. (1968) Immune response of human adults after oral and parenteral exposure to bovine serum albumin. *Journal of Allergy*, **41**, 226.
- MARCHALONIS, J.J. (1969) Enzymatic method for the trace iodination of immunoglobulins and other proteins. *Biochemical Journal*, **113**, 299.
- MAY, C.D. (1976a) Objective clinical and laboratory studies of immediate hypersensitivity reactions to foods in asthmatic children. *Journal of Allergy and Clinical Immunology*, **58**, 500.
- MAY, C.D. (1976b) High spontaneous release of histamine *in vitro* from leukocytes of persons hypersensitive to food. *Journal Allergy and Clinical Immunology*, **58**, 432.
- MCCONAHEY, P. & DIXON, F.J. (1966) A method of trace iodination of proteins for immunologic studies. *International Archives of Allergy*, **29**, 185.
- MINDEN, P. & FARR, R.S. (1973) The ammonium sulphate method to measure cow's milk in chronic respiratory disease. *American Journal of Diseases of Children*, **103**, 634.
- PARISH, W.E. (1969) Detection of reagins, IgG, IgA and IgM antibodies in human sera. *International Archives of Allergy*, **36**, 245.
- PARISH, W.E. (1971) Detection of reaginic and short-term sensitizing anaphylactic or anaphylactoid antibodies to milk in sera of allergic and normal persons. *Clinical Allergy*, **1**, 369.
- PETERSON, R.D.A. & GOOD, R.A. (1963) Antibodies to cow's milk proteins—their presence and significance. *Pediatrics*, **31**, 209.
- ROTHBERG, R.M. & FARR, R.S. (1965) Antibovine serum albumin and anti-alpha lactalbumin in the serum of children and adults. *Pediatrics*, **35**, 571.

Copyright of Clinical Allergy: Journal of the British Allergy Society is the property of Blackwell Publishing Limited and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.