

An immunological approach to the diagnosis of food sensitivity

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Summary

Nineteen patients with delayed onset food sensitivity were compared with fourteen patients with immediate reactions and twenty-one non-atopic subjects in terms of clinical symptoms, foods involved and IgE mediated immunological reactions. The immediate reactors were frequently positive to all tests used: skin tests (71%), allergen induced leucocyte histamine release (71%), radioimmunodiffusion (55%) and skin window (55%). Those with the delayed onset variety were seldom positive by skin testing (13%), or skin window (0%), while 39% were positive by leucocyte histamine release and 48% demonstrated specific IgE food antibodies. Control subjects had negative responses to immunological tests for IgE antibody except for leucocyte histamine release (24%). Reasons for the differences between immediate and delayed onset food sensitivity in clinical symptoms, foods involved and immunologic parameters are discussed. A careful history in conjunction with the elimination and challenge technique remains the most useful tool at present for the delayed onset group. *In vitro* methods for detecting specific IgE responses may also prove to be helpful.

Introduction

Food sensitivity, an important cause of multiple allergic symptoms in the atopic patient, remains a difficult and laborious diagnosis to make. Sensitivity is a preferable term to allergy or hypersensitivity when an immunological basis is uncertain, as is true in most delayed onset reactions to foods (Goldstein & Heiner, 1970). Symptoms occurring immediately after food ingestion can be diagnosed by history, and confirmed by immunological testing (Golbert, Patterson & Pruzansky, 1969; Bullock & Bodenbender, 1970) if necessary. However, the foods most commonly causing daily atopic symptoms may well be those ingested frequently, resulting in manifestations hours to days afterwards. It is this type of food sensitivity which has defied diagnosis by conventional allergy-immunological testing and indeed has raised the question of whether an immunological reaction is present at all.

In this study, patients with delayed onset food reactions are compared with those

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having the immediate onset variety by utilizing several immunological methods of demonstrating IgE mediated responses, particularly the leucocyte histamine release method. The leucocyte histamine release technique has been found to be a sensitive and accurate indicator of Type I hypersensitivity for both inhalant (Lichtenstein, 1964) and food allergens (Golbert *et al.*, 1969). It was hoped that an immunologic approach might not only provide possible useful diagnostic tests, but also clarify the pathogenesis of delayed onset food reactions.

Materials and methods

Subject categories

Fifty-nine subjects of both sexes aged from 2½ to 47 years were divided into groups based on their symptom pattern following food ingestion:

1. *Immediate reactions.* Symptoms occurring up to 1 hr following food ingestion are included here. Fifteen food allergens were tested in fourteen subjects.
2. *Delayed onset reactions.* Symptoms occurred 1 hr or more after ingestion and were usually difficult to relate to ingestion of a particular food. Elimination of test foods resulted in clinical improvement. One or more challenges resulted in exacerbation of symptoms. Twenty-seven food allergens were tested in nineteen subjects.
3. *Control subjects.* These subjects were asymptomatic and nonatopic. Forty-three foods were tested in twenty-one subjects.

Allergy evaluation

Allergy evaluation for each subject included a detailed history, physical examination and skin tests (usually scratch method) to the suspected foods. Specific foods were suspected as causative when there was a history of an allergic type reaction clearly related to food ingestion, or by striking improvement of clinical allergic status after food elimination, in conjunction with exacerbation of symptoms following food challenge particularly in the delayed onset type of food reaction. Those foods suspected on a clinical basis were then tested by immunological techniques.

Immunological techniques

Leucocyte histamine release technique (LHRT). The May *et al.* (1970) modification of the leucocyte histamine release technique (LHRT) was used. Whole dry food antigens (from Hollister-Stier) were prepared in Tris ACM buffer* as a 1:10 extract. Dilutions of the extract ranging from 10^{-1} to 10^{-4} were used for each experiment. To test for the presence of IgE on the cell membrane rabbit anti-IgE myeloma (P.S.)† absorbed with IgG myeloma protein (Ogawa *et al.*, 1969) diluted 1:1000 in Tris ACM buffer was used. Histamine release was expressed as a percentage of total cell histamine content after the appropriate corrections.

The skin window technique (Bullock & Bodenbender, 1970) for observing inflammatory exudation particularly allergen induced eosinophilia was performed in at least half of the subjects in each allergy category.

Ouchterlony radioimmunoassay technique (Morse & Heremans, 1962). Rabbit anti-human IgG myeloma or anti-human IgE myeloma serum was placed in a central well along with 10 µg of ^{131}I -labelled food allergens (chloramine T radio-labelling

* Tris ACM buffer contains Trizma buffer (pH 7.6), NaCl, KCl, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, albumin.

† Kindly supplied by Dr K. Ishizaka.

method used to label dialysed food allergen extract—McCohaney, *International Archives of Allergy*, **29**, 185, 1966). The peripheral wells contained 0.1 ml of patient's sera. After 48 hr incubation at 25°C, and extensive washing for 2 days, Kodak No-Screen X-ray film was placed over the dried slide, incubated for 48 hr and developed.

Positive results in the study include skin tests 2 plus or greater (erythema 10–15 cm with weal-pseudopod formation), skin window eosinophilia with three-fold increase of antigen window over control window (Bullock & Bodenbender, 1970) and histamine release 5% or greater than control values, since this amount is seldom due to spontaneous release (Lichtenstein, 1964).

Table 1. Immediate onset food reaction

Patient	Food	Symptoms	%H.R. (allergen)	%H.R. A-IgE	S.T.	S.W.
1 (C.L., 14, F)	Egg	AN°	53	N.T.	pos.	pos.
2 (A.E., 13, M)	Peanut	Urt., BA	8	0	pos.	pos.
3 (R.O., 6, M)	Peanut	AN°, BA	14	9	pos.	pos.
	Egg	AN°, BA	7	9	pos.	pos.
4 (J.O., 7, F)	Milk	AN°, BA	12	4	neg.	neg.
5 (R.E., 14, M)	Peanut	Throat itching	34	N.T.	pos.	pos.
6 (J.C., 35, M)	Walnut	Mouth itching	44	44	neg.	neg.
7 (M.E., 7, M)	Halibut	Urt., BA	13	33	pos.	pos.
8 (M.E., 5, F)	Halibut	Urt., BA	2	29	pos.	pos.
9 (R.H., 14, M)	Halibut	AN°, throat itching	27	0	pos.	N.T.
10 (D.D., 16, F)	Halibut	BA, skin itching (eczema)	36	0	pos.	N.T.
11 (J.P., 2½, M)	Egg	Urt.	0	13	pos.	N.T.
12 (D.G., 16, M)	Milk	BA	0	11	neg.	neg.
13 (W.G., 12, M)	Milk	BA	64	55	neg.	neg.
14 (M.G., 37, F)	Milk	Coughing, mucus	0	2	neg.	neg.

Key: S.T., skin test; S.W., skin window; H.R., Histamine release; N.T., not tested. BA, bronchial asthma; AN°, Angioneurotic oedema; URT, urticaria; A-IgE, anti-IgE antibodies.

Results

Group 1. Immediate onset reactions (Table 1, Fig. 1)

We found that immediate food reactions occurred after ingestion of fish, milk, egg, peanuts, and walnuts (Table 1, 3). Such immediate reactions were mainly of the anaphylactic type (urticaria, angioedema, itchy throat) in addition to bronchial asthma. Tests were frequently positive by all methods employed (Table 4, Fig. 1). Skin tests and histamine release were positive in approximately 70% of the subjects and allergens tested. In nine of ten cases histamine release was greater than 10%. Skin window responses were positive in approximately 58% of tests, the same incidence as occurred with anti-IgE histamine release reactions. Specific IgE antibodies were found to 46% of food allergens and in 55% of patients tested, while IgG antibodies were positive in 64% and 69%, respectively.

Table 2. Delayed onset food reaction

Patient	Food	Symptoms	%H.R. (allergen)	S.T.	S.W.	%H.R. A-IgE
1 (R.O., 6, M)	Milk	HA, SA, LA, fever, BA	21	pos.	neg.	*
2 (J.O., 7, F)	Milk	HA, SA, LA, BA	12	pos.	neg.	*
	Peanut	BA, AN ^o , hives	2	neg.	neg.	
3 (V.O., 5, F)	Choc.	Itchy throat, AR	8	neg.	N.T.	31
	Peanut	Itchy throat, AR	0	neg.	neg.	
4 (C.S., 6, M)	Choc.	BA, skin itching	2	neg.	N.T.	35
5 (D.D., 16, F)	Milk	BA	34	neg.	neg.	*
6 (J.P., 2½, M)	Milk	Diarrhoea, AR, chest congestion	1	pos.	N.T.	*
7 (K.P., 5½, F)	Milk	HA, SA, eczema	0	neg.	N.T.	41
	Corn	pallor	7	neg.	N.T.	
8 (E.Q., 12, M)	Milk	Coughing	0	neg.	neg.	0
9 (M.Q., 40, F)	Milk	HA, SA, LA, AR	5	neg.	neg.	10
10 (R.A., 11, F)	Milk	HA, LA, pallor, BA	0	neg.	N.T.	0
	Choc.	HA, LA, pallor, BA	0	neg.	N.T.	
11 (S.H., 14, F)	Milk	HA, LA, pallor, BA	0	neg.	N.T.	46
	Choc.	Eczema	0	neg.	N.T.	
12 (R.H., 17, M)	Milk	HA, LA, BA, eczema	2	neg.	N.T.	34
	Egg	HA, LA, BA, eczema	3	neg.	N.T.	
13 (E.C., 6, M)	Milk	BA, AR	0	neg.	N.T.	27
	Choc.	BA, AR	0	neg.	N.T.	
14 (R.A., 11, M)	Milk	AR	0	neg.	N.T.	4
15 (H.A., 17, M)	Milk	BA	20	neg.	N.T.	4
	Choc.	BA	3	neg.	N.T.	
16 (J.M., 12, M)	Milk	HA, SA, LA, CA, BA, AR	0	neg.	neg.	45
	Choc.	HA, SA, LA, CA, BA, AR	0	neg.	N.T.	
17 (P.N., 3, F)	Milk	Fatigue, pallor, irritability, BA	0	neg.	N.T.	35
	Choc.	Fatigue, pallor, irritability, BA	0	neg.	N.T.	35
18 (S.H., 14, M)	Milk	HA, pallor, night sweats, BA, AR	0	neg.	neg.	2
	Choc.	HA, pallor, night sweats, BA, AR	0	neg.	N.T.	
19 (J.B., 4, F)	Milk	Pallor, SA, LA, BA, AR	0	neg.	N.T.	48
20 (M.B., 12, M)	Milk	SA, vomiting, AR	0	neg.	N.T.	0
	Choc.	SA, vomiting, AR	0	neg.	N.T.	
21 (M.G., 4, F)	Wheat	Pallor, SA, fatigue	13	neg.	N.T.	90
22 (M.M., 33, F)	Milk	LA, SA, HA, AR	0	neg.	neg.	0
23 (M.Q., 41, F)	Milk	HA, SA, AR	5	neg.	neg.	0

Key: S.T., skin test; S.W., skin window; H.R., histamine release, N.T., not tested. BA, bronchial asthma; AR, allergic rhinitis; HA, headaches; LA, leg-aches; SA, stomach-aches.

* Included also in immediate reactions.

Table 3. Foods causing clinical allergic symptoms

	Immediate	Delayed onset
Egg	3	1
Peanut	3	2
Walnut	1	0
Fish	4	0
Milk	4	16
Chocolate	0	10
Corn	0	0
Wheat	0	0
Total	15	29

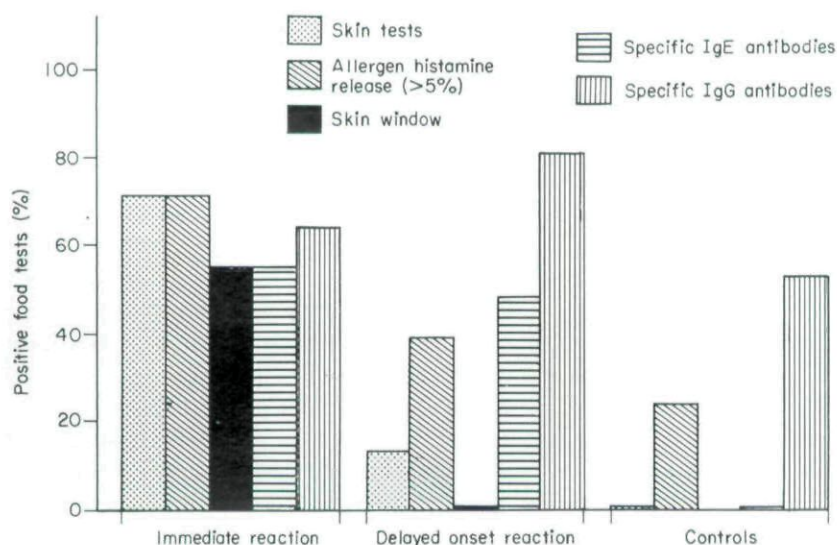
Group 2. Delayed onset reactions (Table 2, Fig. 1)

Reactions were usually secondary to milk and chocolate ingestion (Table 3). Symptoms were of the atopic variety, bronchial asthma and allergic rhinitis, as well as general symptoms of headache, stomach-ache, leg-aches, pallor, fatigue and irritability following ingestion of the offending food (Crook *et al.*, 1961).

The tests employed were generally less often positive than seen in Group 1 (Table 4, Fig. 1). Skin tests and skin window were usually negative, while the allergen induced histamine release was positive in approximately one-third of subjects (5/9 were greater than 10% release). Anti-IgE induced histamine release and specific IgE and IgG antibody responses were essentially the same as found in Group 1.

Group 3. Control category (Table 4, Fig. 1)

The control group reacted less frequently to allergen induced histamine release than Groups 1 and 2 (2/5 cases had greater than 10% release), but had a high incidence

**Fig. 1.** Incidence of positive food reactors.

(86%) of anti-IgE responses. All other responses were negative except for specific IgG antibody reactions to the food allergens which were similar to those seen in Groups 1 and 2.

Discussion

Immediate onset food sensitivity fulfils the criteria of a Type I (anaphylactic-type) hypersensitivity reaction (Coombs & Gell, 1968) and is relatively simple to diagnose from the patient's history. Therefore, tests are rarely required to detect the offending allergen, although skin tests are often used to confirm the patient's history. Our data and that of Haddad & Korotzer (1972) confirm the impression that skin tests and *in vitro* methods often detect an IgE type response to the allergen which is suggested by history alone in this type of food reaction. The rather low incidence of positive anti-IgE histamine responses is most likely due to the single dose of anti-IgE used, instead of several log doses, as was done with the allergen.

Table 4. Correlation of tests with food allergy

Test		Allergic responses		Controls
		Immediate	Delayed onset	
Skin test	Subjects	10/14 (71%)	3/23 (13%)	0/6 (0%)
	Allergens	11/15 (73%)	3/35 (9%)	0/12 (0%)
Histamine release ($\geq 5\%$) allergens	Subjects	10/14 (71%)	9/23 (39%)	5/21 (24%)
	Allergens	11/15 (73%)	9/35 (26%)	6/43 (14%)
Skin window	Subjects	6/11 (55%)	0/12 (0%)	N.T.*
	Allergens	7/12 (58%)	9/12 (0%)	
Histamine release anti-IgE ($\geq 5\%$)	Subjects	7/12 (58%)	11/19 (58%)	18/21 (86%)
Specific antibodies (radioimmunodiffusion)				
IgE	Subjects	6/11 (55%)	10/21 (48%)	0/15 (0%)
	Allergens	6/13 (46%)	10/35 (29%)	0/56 (0%)
IgG	Subjects	7/11 (64%)	17/21 (81%)	8/15 (53%)
	Allergens	9/13 (69%)	21/35 (60%)	13/56 (23%)

* N.T., Not tested with these controls, but all non-atopic subjects in our clinic population tested are negative.

Food sensitivity of the delayed onset produced many classical allergic symptoms, e.g. asthma, rhinitis and dermatitis, but may have many general or non-specific ones such as fatigue, irritability, headaches, stomach-aches and leg-aches (Crook *et al.*, 1961). The offending allergen can often be discovered by careful and rather tedious food elimination and challenge diets. A great deal of patient co-operation and patience are required. As confirmed by our data, skin tests are rarely helpful in this type of food sensitivity in contrast to the immediate type. It was in the hope that an objective laboratory test would confirm the role of the suspected allergen in delayed onset food sensitivity that this study was undertaken. We found that nine patients (9/24) with symptoms of delayed onset food sensitivity had significant histamine release, five of these greater than 10%. Although this is somewhat encouraging, this group must be compared to the control group category, where 5/21 subjects had positive release, two

greater than 10%, and those with immediate onset reactions who were positive in 10/14 cases, nine with histamine release greater than 10%. Dockhorn (1969) also found histamine release to be positive in 75% and skin tests in 50% of his food allergy patients, although he did not differentiate between immediate and delayed onset food allergy. Golbert *et al.* (1969) described a group of patients with systemic anaphylactic type reactions from ingestants and found a high percentage with positive histamine release confirmed by other Type I immunological tests. Bullock & Bodenbender (1970) found an excellent correlation between positive skin window responses, skin tests and immediate onset food reactions, while those patients with delayed onset symptoms were uniformly negative. These results were confirmed in the present study. We used 1 hr as cut off between immediate and delayed onset symptoms since immediate reactions usually occur seconds to minutes after antigen challenge and the reliability of history might be less accurate after 1 hr following food challenge. However, the fact that two patients (J.O. and V.O.) had anaphylactic symptoms (Table 2) more than 2 hr after food ingestion suggests that the 1 hr criterion might be too arbitrary.

Although a striking difference in leucocyte histamine release was present in immediate compared to delayed onset food reactions, specific IgE antibodies were seen with approximately the same frequency. However, we could find no correlation between serum IgE antibodies and leucocyte histamine release to the same suspected food allergen in delayed onset food sensitive cases, while in the immediate onset type five of six with IgE antibodies had significant histamine release to that food allergen. The reason for this difference is not clear, but could include differences in antibody tissue fixing avidity, or mediator involved as suggested below. No control subject had IgE antibodies to food allergens tested, while IgG antibodies were commonly seen in all groups. Kletter *et al.* (1971) using the radioimmunodiffusion technique (RID) recently found IgE milk antibodies in seven of twenty-six infants allergic to cow's milk. Although one with coeliac disease and two with recurrent aspiration demonstrated these antibodies, all nonatopic controls were negative. These investigators found a higher incidence of positive skin tests than serum IgE antibodies to the same food allergen, as we did in Group 1 patients. This probably reflects the greater sensitivity of the skin test (Ishizaka & Ishizaka, 1970) compared to the RID technique (Heiner & Rose, 1970). It is significant to note that of the twenty-three subjects in our delayed onset food category, nineteen had either significant histamine release (nine) or IgE antibodies (ten) to food allergens, while control subjects uniformly had no IgE antibodies and fewer histamine release reactions. Taken as a group the incidence of positive responses to these tests compares favourably with the immediate onset group. Thus, an IgE mediated reaction might be important in the pathogenesis of a significant number of delayed onset food cases.

However, it remains clear that immediate and delayed onset food sensitivity are different, particularly with respect to (a) speed of onset of symptoms, (b) clinical manifestation of anaphylaxis compared to atopic reactions, (c) frequent positive IgE immunologic tests in the immediate type and only selective positive responses in the delayed onset type, and (d) types of food involved. These differences might be explained on the following bases:

1. The antigen available for testing may not be in the antigenic form which triggers the allergic test response.
2. A non-IgE type of hypersensitivity response may be involved.

3. The pharmacological mediator may not be histamine exclusively and, therefore, not be detectable by skin test or leucocyte histamine release tests.
4. The symptoms may not have an immunological basis at all.

Selection of the appropriate antigen in immunological testing

In most studies whole food extracts are used for diagnostic testing. Failure to test with the appropriate food antigen could explain the generally unsatisfactory results in the diagnosis of food sensitivity. Two main approaches have been taken to elucidate the major allergenic component of foods.

It has been suggested for many years that the cause for the delayed onset of symptoms is the formation of important antigens in the digestive process (Cooke, 1947; Spies *et al.*, 1970; Ishizaka, Campbell & Ishizaka, 1960). Thus, a patient with allergic symptoms occurring 2 hr after milk challenge was skin tested and PK positive only when tested with the primary proteoses of cow's milk whey protein (Cooke, 1947). Bleumink (1970) and Berrens (1970) have investigated the biochemical characteristics of inhalants and food atopsens. They have postulated that all atopsens have in common an N-glycosidic protein-sugar linkage formed by non-enzymatic browning (Maillard) reactions. Thus, skin test reactivity to beta lactoglobulin could be increased by incorporating N-glycosidically-bound sugar into the protein molecule. Heating foods may accomplish the same phenomenon. In this respect, it is interesting to note that Küstner had anaphylactic reactions only after ingesting baked, fried or cooked fish, not fresh fish (Bleumink 1970, p. 505).

These examples illustrate the importance of determining the major active components of foods in testing for food allergy. Using whole food extracts alone is not adequate for diagnosis.

Non-IgE mediated hypersensitivity reactions

Parish (1970) has described recently an IgG heat stable, complement dependent, antibody to food allergens (milk and egg) in several patients with recurrent eczema and abdominal discomfort after ingestion of these foods. This antibody, unlike IgE, confers passive cutaneous anaphylaxis (PCA) sensitivity in the monkey for only 4 hr. It did not, however, give a PCA reaction in the guinea-pig.

Matthews & Soothill (1970) have suggested that certain allergic responses to milk may be due to a Type III hypersensitivity reaction or Arthus phenomenon requiring complement. This would involve IgG or IgM type antibodies and typically occurs 6-8 hr after antigen challenge, as compared to IgE mediated reactions, which occur seconds to minutes after such challenge. Heiner, Sears & Kniker (1962) and Wilson, Heiner & Lakey (1964) have described several clinical entities involving respiratory tract and gut, associated with high levels of serum precipitins to one or more milk proteins. Although the exact relationship of these precipitins to the clinical states is not known, perhaps these could represent a Type III hypersensitivity. Investigation here would entail an approach which Pepys (1969) has followed in elucidating the organic dust pneumonitides. We found that IgG food antibodies occurred with somewhat greater frequency in the delayed onset food cases compared to the immediate type and control subjects.

Cellular-Type IV hypersensitivity has not been fully explored in food sensitivity. Bicks (1967) using a DNCB model in animal experiments has shown immunological and morphological evidence of gastrointestinal delayed type hypersensitivity reactions

(Type IV) after oral challenge. This followed sensitization either by the oral or skin route with the same substance. Examples in man of this type of sensitivity include food preservatives, and additives such as flavouring agents, emulsifiers and dyes (Bleumink, 1970, p. 515). With the new tools currently being used to evaluate cellular immunity (Lawrence, 1969) a direct application to food sensitivity should be made.

Mediators

Histamine, the best known mediator of atopy and inflammation, causes immediate physiologic responses, while SRS-A and the kinins have a more delayed onset of action (Austen, 1965). Active kinins are released in local inflammatory processes of the gastrointestinal tract (Bleumink, 1970, p. 561). It has recently been observed that ingested atopens, incorporating the N-glycosidic groups, specifically inhibit the enzyme aminopeptidase (Bleumink, 1970, p. 561). This results in the accumulation of the decapeptide Kallidin II which could have profound inflammatory effects on the gut, or on any tissue where this reaction occurred. Kinin mediated reactions could fit the picture of the multiple organ involvement seen in delayed onset food reactions.

It has been found that the gut of the guinea-pig will both form and respond to SRS-A (Brocklehurst, 1968) so it, too, could be an important mediator in man. Finally the chromaffin cells in the upper intestinal tract contain considerable serotonin, which might be a mediator in this special tissue, although it is not involved in the usual atopic reactions in man.

Non-immunological mechanisms

It is possible that foods could cause allergic type symptoms on a toxic basis, similar to gliadin sensitive-coeliac disease (Gerrard & Lubos, 1967) or perhaps from an enzyme deficiency, e.g. disaccharidase deficiency (Davidson, 1967). However, the association of multiple system reactions to foods in the atopic patient who has similar symptoms from easily demonstrable immunological reactions to inhalants makes this possibility unlikely. This, however, must remain conjectural until immunological phenomena have been demonstrated or ruled out.

Conclusions

Several approaches to understanding the mechanism of delayed onset food reactions have been suggested. We have found a significant number of patients with delayed onset food sensitivity to have either positive leucocyte histamine release tests or serum IgE food antibodies, but not both to the same allergen. Although this suggests that an IgE mechanism may be important in a portion of these cases, other approaches to the understanding of the mechanism of delayed onset food sensitivity, as discussed, must be explored further. While these studies are in progress, the patient must be helped now. A careful history, in conjunction with the classic elimination diet and challenge remain the most useful tools, at present, for the diagnosis of this form of food sensitivity. *In vitro* tests for IgE responses to food allergens, however, may prove to be helpful adjuncts to the clinical evaluation of food sensitivity.

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