Diagnostic Tests for Food Allergy

Sami L. Bahna

Department of Allergy and Immunology, Cleveland Clinic Foundation A-72, 9500 Euclid Avenue, Cleveland, OH 44195

INTRODUCTION

Although in some patients the diagnosis of food allergy is obvious, in most patients it is a challenging task. One problem that clearly stands out is the identification of the offending food allergen. In many other diseases, the clinical manifestations and available laboratory tests are often characteristic of the causative agent or even pathognomonic. Such is not the case with food allergy, where the outcry for reliable diagnostic tests is great. The ideal test should have high reliability, be able to document that the patient's ailment is caused by certain food(s), and demonstrate that the offending food is causing the ailment through an immunologic mechanism. As to documentation, the medical history is usually the most reliable, although often verification by a supervised challenge test is needed. More often than not, however, the medical history does not provide any clue to a possible relationship between the symptoms and food. As to identifying a given food as the offending cause, one must be aware that the immunologic mechanisms of food allergy are multiple and more than one mechanism may be involved in the same patient. This multiplicity of mechanisms may occur even when there is a single clinical manifestation (I). It seems unlikely, therefore, that a single laboratory test would reflect the immunologic nature of all food hypersensitivity reactions. In fact, in many instances of obvious clinical hypersensitivity, the currently available laboratory techniques fail to clearly identify the underlying immunologic mechanism.
If and when the ideal test becomes available, a major breakthrough would occur and have many favorable repercussions. Today, the offending food may be suspected from the medical history or by a screening test (mainly skin testing and/or testing for serum specific IgE antibodies). The cause-and-effect relationship between a food and symptoms is best verified by the elimination-challenge test. The immunologic mechanism may be clinically apparent from the nature of symptoms or from the results of appropriate in vivo or in vitro tests. Table 1 outlines the various tests currently available or tried in research studies on food allergy diagnosis.

**Screening Tests for Food Allergy**

**Diagnostic Elimination Diets**

*Conventional*

Some physicians recommend trials with certain elimination diets when the patient's medical history suggests food allergy but does not point to any particular food and the symptoms are frequent or persistent. Such diets are devised to be devoid of a certain group or groups of highly allergenic foods. Rowe Elimination Diets (numbers 1, 2, 3, and 4) or their modifications are widely known examples (2,3). In some instances, the diet may need to be individualized or formulated to suit young children. In using these diets, one should be aware of their limitations, including the unpredictability of the results (4).

The selection of a diet to begin with may vary from one patient to another, and should be tried for 2 to 4 wk. If no satisfactory improvement is noted, other elimination diets may be tried. Once a satisfactory diet is obtained, i.e., the patient's symptoms definitely improve, the eliminated foods are reintroduced one at a time, first in an open way and then by a blind challenge as a means of verification. In this way the offending food(s) may be identified.

*Elemental Diet*

Patient's who are highly sensitive to numerous foods may need to be given a strict elimination diet, yet a nutritionally adequate
Table 1
Diagnostic Tests of Food Allergy

A Screening

1. Diagnostic elimination diets
   Conventional elimination diets
   Elemental diet
2. Food/symptom diary
3. Skin testing
   Epicutaneous (scratch, prick, puncture)
   Intracutaneous
4. Serum specific IgE antibody assays
   Radioimmunoassays
   Enzyme immunoassays
5. Less commonly used tests
   Serum specific IgG, IgM, or IgA antibodies
   Leucocyte histamine release
   Circulating immune complexes
   Lymphokine production
   Lymphoblast transformation
   Intestinal mucosal biopsy
   Organ culture (jejunal biopsy) challenge
6. Controversial and unproven tests
   Sublingual provocation
   Subcutaneous provocation
   Leucocyte cytotoxic test
   Serum specific IgG4 antibodies
   Neutrophil chemotactic assay

B Verification

1. Elimination-challenge test
   Open
   Single-blind
   Double-blind
2. Inhalation food challenge test
one. This objective can be attained by giving an elemental diet (e.g., Vivonex, Norwich Eaton Pharmaceuticals, or Vivasorb, Phrimmer A/S) that contains protein in the form of synthesized amino acids and is well balanced in fat, carbohydrate, vitamins, minerals, and trace elements. Although the diet can be used at any age, it is generally poorly accepted by older children and adults. Because of its high osmolality, the formula may need to be diluted when first given to patients having severe diarrhea.

As a diagnostic tool, the elemental formula is given alone until symptoms subside; then ordinary foods are gradually introduced, one at a time, and thus the offending foods may be identified. The usefulness of this approach has been shown by several studies (5–9).

**Food/Symptom Diary**

The keeping of a record, by date and time, of all foods eaten by the patient, and the appearance or exacerbation of symptoms, may provide a valuable tool for the physician in identifying the offending food(s). Recording this information in a tabular form enhances its interpretation. The food/symptom diary would be most effective when the symptoms occur intermittently, shortly after exposure, with the offending foods eaten infrequently. In delayed-onset food reactions, identifying the offending food by this method is often difficult. The diary would be of little help if the record is incomplete or the offending food is included among frequently eaten foods. In many instances, the patient may not be aware of a "hidden" food allergen in a commercially prepared food, either because it is not listed on the label or it is listed under a name that is unfamiliar to the public, such as whey, casein, ovalbumin, and ovomucoid.

Ideally, diary recordings should be complete and entered daily, and for a duration that encompasses at least a few recurrences of symptoms. In some instances, additional events should be recorded, such as places visited, emotional events, physical exercise, and social activities. Such factors may contribute to the patient’s symptoms or potentiate the effect of a food allergen.

Unless the patient is well motivated, compliance in recording is often poor, particularly when the symptoms occur infrequently. In such instances, the physician may recommend the keeping of a "modified" food/symptom diary, where the recording is done only
when symptoms occur and includes the date, time, and description of symptoms, and the foods eaten and events occurring during the preceding 24 h or so.

**Skin Testing**

The most common diagnostic procedure in allergy in general, and probably in food allergy as well, is skin testing. It is expected to be positive in the immediate-type hypersensitivity reactions (Gell and Coombs type I). The test may be applied epicutaneously or intracutaneously.

The *epicutaneous* method is the most commonly used. The test extract is applied to the mast cells in the superficial part of the epidermis on the back or on the flexor aspect of the forearm by scratch, prick, or puncture technique. A variety of scarifiers, needles, and lancets have been in use, and each allergist seems to develop skill in use of one instrument or another.

Commercially available food extracts for epicutaneous testing are provided in an optimal concentration, usually 1:20 or 1:10 w/v (50% glycerinated). Fresh food, however, may be superior to the commercial extracts (10–12). Recently, a skin pricker with its tip preloaded with allergen (Phazet-Pharmacia Diagnostics) has become available in Sweden for inhalant allergens (13) and might be available to test for food allergens in the future.

*Intracutaneous* testing is done by intradermal injection (in the flexor aspect of the forearm or lateral aspect of the upper arm) of 0.02 mL of an aqueous extract, usually 1:1000 w/v. It may be resorted to when the food was suspected by virtue of the patient's history but the epicutaneous test for that food was equivocal or negative. Using epicutaneous testing first minimizes the risk of systemic reaction or large local reactions from intradermal administration of an allergen that the patient might be highly sensitive to.

The skin test reaction is read 15–20 min after epicutaneous application of the allergen and 10–15 min after intradermal administration. The reaction is scored 0–4+, depending on the size of the wheal-and-flare response, as compared with the patient's responses to the diluent as a negative control and to histamine (1mg/mL for epicutaneous and 0.1 mg/mL for intradermal). Although the size of wheal is important to note, one should not ignore a substantial erythema that is not associated with a "significant" wheal.
Table 2 presents a scoring system used by this author. A reaction of 2+ or greater is considered positive, though not necessarily clinically relevant. Reports on the reliability of skin testing in the diagnosis of food allergy showed inconsistent results (14–17).

We (18) recently compared the results of skin testing with the results of double-blind food challenge testing in allergic patients whose symptoms were compatible with type I hypersensitivity and in whom food allergy was strongly suspected. In the positive challenge group, the skin test was positive (2+ or greater) to the offending food in 58% of instances, with a wide variation from one food to another, being lowest for tomato (33%) and highest for fish (83%). In the negative challenge group, the skin test was positive in 35% of instances, being lowest for orange (11%) and highest for peanut (43%). The overall positive predictive accuracy was 48%, being highest for fish (83%) and lowest for crab (33%). The overall negative predictive accuracy was 74%, highest for egg white (89%) and lowest for cow's milk (44%).

The corollary is that the reliability of skin testing, as currently done, varies widely from one food to another. Caution, however, should be exercised in interpreting the result of a properly conducted skin test when it is not in concordance with the result of a challenge test. The skin test result is not necessarily a "false" one. Any of several factors might explain such a discrepancy, e.g., technical errors, non-IgE mediated reactions, reagins predominantly located in the shock organ, or latent hypersensitivity.

The suboptimal degree of reliability of skin testing in food allergy diagnosis should caution one against depending solely on it in decision-making—but neither should it deter one from using it as a screening tool. In a study of 102 adults with "idiopathic" anaphylaxis, skin testing with food extracts correctly identified certain foods as the cause of anaphylaxis in 7 subjects (19). An excellent correlation between positive skin test results and challenge test results was noted by using purified allergen extracts, such as cod fish (15) and peanut (20). Purification and standardization of inhalant allergen extracts have already begun, and food extracts are expected to follow.
Table 2
Scoring System for Allergy Skin Testing

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Similar to the control (diluent)</td>
</tr>
<tr>
<td>1+</td>
<td>Slightly larger than control (by 25–50%)</td>
</tr>
<tr>
<td>2+</td>
<td>Definitely larger than control (&gt;50%) but smaller than histamine</td>
</tr>
<tr>
<td>3+</td>
<td>Similar to histamine (75–125%) and the wheal has no pseudopods</td>
</tr>
<tr>
<td>4+</td>
<td>Larger than histamine (&gt;125%) or the wheal has pseudopods</td>
</tr>
</tbody>
</table>

*aHistamine 1 mg/mL for epicutaneous and 0.1 mg/mL for I.D. testing.

Serum Specific IgE Antibody Assays

Allergen-specific IgE antibody assay is the in vitro counterpart of skin testing in the sense that both reflect type I hypersensitivity reactions. Skin testing, however, does not only reflect the presence of antibody but also the releasability of mediators from mast cells.

The original assay for measuring specific IgE antibodies, a solid-phase radioimmunoassay developed by Wide et al. in 1967, was called the radioallergosorbent test (RAST) (21). A cellulose paper disk coupled with the allergen is incubated with the serum to allow the binding of specific antibodies to the relevant allergen. After washing, 125I-labeled anti-IgE is added to the disk for another incubation and washing, and then the radioactivity bound to the disc is counted. The IgE antibody level or a score is then obtained from a reference curve. Several technical factors should be considered in doing the test (22). An enzymeimmunoassay (EIA) analogous to RIA was developed later, using an enzyme-labeled—instead of a radio-labeled—anti-immunoglobulin. In EIA a substrate is incubated to produce a color, the intensity of which is measured by photometry. Several modifications of both the RIA and EIA have
become available. Using the principle of autoradiography, MAST Immunosystems developed a RIA method for measuring IgE antibodies specific to each of multiple allergens coupled on cellulose threads (23). Recently, the method has been replaced by an enzymatic version (MAST-CLA) that utilizes the principle of chemiluminescence and autoimmunography (24). Information on commonly available assays is presented in Table 3. The assays differ mainly in the solid phase used to fix the allergen, the tracer attached to anti-IgE, and the instruments required. The efficacy of these assays in the diagnosis of food allergy needs to be adequately investigated and the results compared.

We compared Phadebas RAST and RIA-MAST methods in measuring serum IgE antibodies to five common allergenic foods (cow's milk, eggwhite, peanut, crab, and shrimp) in patients subjected to double-blind oral challenges (25). The correlation coefficient between MAST and RAST scores for various foods tested ranged from 0.7 to 0.8. The scores of the two methods did not differ in 65% of samples and differed by one class in 24%, by two classes in 9%, and by three classes in 2%. MAST tended to be less sensitive than RAST in egg white but was more sensitive in the other four foods studied.

Variability may also occur between laboratories, even when they use the same method. Two foods (cow's milk and wheat) were included in the proficiency survey by the Centers for Disease Control of specific IgE determination (26); positive and negative test sera were blindly tested by 33 laboratories. For the negative sera, a positive result to milk antibody was reported in 10% and to wheat antibody in 17%. In the positive sera, a negative result to milk was reported in 7% and to wheat in 0%. The scores reported by different laboratories varied widely; results on negative sera differed by up to two classes, and results on positive sera differed by up to three classes (Table 4).

Most recently, a multiple-antigen disk has been used to measure IgE antibodies directed to a whole group of allergens (27, 28). Cellulose disks coupled with a selected number of inhalant allergens have been manufactured by Pharmacia Diagnostics (Phadiatop) and by Ventrex Laboratories (Seasonal Disc) for screening purposes. Similar disks may be developed in the future to screen patients for multiple food allergens, and whenever the test is positive, discrete RAST tests would identify the individual allergen(s).
### Table 3
Comparison of Commonly Available Assays for Serum Level of Allergen-Specific IgE Antibodies

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Phadebas RAST&lt;sup&gt;o&lt;/sup&gt;</th>
<th>Phadezym RAST&lt;sup&gt;o&lt;/sup&gt;</th>
<th>VAST&lt;sup&gt;o&lt;/sup&gt;</th>
<th>IP-SYSTEM&lt;sup&gt;o&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer</td>
<td>Pharmacista</td>
<td>Pharmacibia</td>
<td>Ventrex</td>
<td>Ventrex DRT</td>
</tr>
<tr>
<td>Methodology</td>
<td>Paper disk; RIA&lt;sup&gt;o&lt;/sup&gt;, polyclonal</td>
<td>Paper disk; EIA&lt;sup&gt;o&lt;/sup&gt;, polyclonal</td>
<td>Paper disk; RIA; polyclonal</td>
<td>Microteter; EIA; polyclonal</td>
</tr>
<tr>
<td>Kit size</td>
<td>Enzyme 60 determinations; ref unit 4–7 runs; allergen disks 10, 12, 25, 30/vial or cassette</td>
<td>Enzyme 60 determinations; ref unit 4–7 runs; allergen disks 10, 12, 25, 30/vial or cassette</td>
<td>Isotope 4 mo; ref unit 6 mo; allergens 18–24 mo</td>
<td>Isotope 4 mo; ref unit 7mo; allergens?</td>
</tr>
<tr>
<td>Shelf life</td>
<td>Isotope 4 mo; ref unit 6 mo; allergens 18–24 mo</td>
<td>Enzyme 60 determinations; ref unit 6 mo; allergens 18–24 mo</td>
<td>Isotope 4 mo; ref unit 6 mo; allergens 18–24 mo</td>
<td>According to expiration date</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.35 pru or 0.1 if E pt is used</td>
<td>0.35 pru or 0.1 if E pt is used</td>
<td>0.35 pru or 0.1 if E pt is used</td>
<td>Not stated</td>
</tr>
<tr>
<td>Incubation time</td>
<td>3 h + overnight or O/N + O/N + 60 min 37°C</td>
<td>3 h + O/N or O/N + O/N + 60 min 37°C</td>
<td>3 h + O/N or O/N + 60 min 37°C</td>
<td>3 h (or O/N 2–8°C) 1 h + 1/2 h in dark</td>
</tr>
<tr>
<td>Pipetting steps</td>
<td>Addition of disks, 2 pipetting steps</td>
<td>Addition of disks, 4 pipetting steps</td>
<td>Addition of disks, 2 pipetting steps</td>
<td>4 pipetting steps</td>
</tr>
<tr>
<td>Sample volume</td>
<td>50 or 100 μL</td>
<td>50 or 100 μL</td>
<td>100 μL</td>
<td>200 μL</td>
</tr>
<tr>
<td>Standard curve range</td>
<td>A, B, C, D, Birch 17.5, 3.0, 0.7, 0.35 pru</td>
<td>A, B, C, D, Birch 17.5, 3.5, 0.7, 0.35 pru</td>
<td>A, B, C, D, Alder 17.5, 3.5, 0.7, 0.35 pru</td>
<td>A, B, C, D, June grass, Alder 17.5, 12.5, 3.12, 0.78, 0.29 IU of IgE</td>
</tr>
<tr>
<td>Standards</td>
<td>4 + extrapolated E (0.1 pru)</td>
<td>4 + extrapolated E (0.1 pru)</td>
<td>4 + extrapolated E (0.1 pru)</td>
<td>4</td>
</tr>
<tr>
<td>Instrument</td>
<td>Gamma counter</td>
<td>Photometer 420 nm</td>
<td>Gamma counter</td>
<td>Micro Eliza 492 nm Reader</td>
</tr>
</tbody>
</table>

(Continued)
Table 3 (Continued)
Comparison of Commonly Available Assays for Serum Level of Allergen-Specific IgE Antibodies

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Allercoast RAST*</th>
<th>Allercoast EAST*</th>
<th>IgE-FAST*</th>
<th>MAST**-CLA†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer</td>
<td>Kallestad</td>
<td>Kallestad</td>
<td>Diagnostic</td>
<td>Immuno-</td>
</tr>
<tr>
<td>Methodology</td>
<td>Paper disk; RIA; polyclonal</td>
<td>Paper disk; EIA; polyclonal</td>
<td>Microtiter; EIA; monoclonal</td>
<td>Cellulose threads; EIA; polyclonal</td>
</tr>
<tr>
<td>Kit size</td>
<td>Isotope 100 determinations; ref unit 7 runs; allergens 25/vial</td>
<td>Enzyme 100 determinations; ref kit; allergens 25/vial</td>
<td>96 determinations + 1 S.C.; ref kit 4 runs; allergens 24/package</td>
<td>Patient profiles/kit; 12 profiles available; 35 allergens/pette; includes total IgE</td>
</tr>
<tr>
<td>Shelf life</td>
<td>Isotope 4 mo; ref unit 12 mo; allergens?</td>
<td>Enzyme 12 mo; ref unit 12 mo; allergens?</td>
<td>1 Y</td>
<td>Reagents up to 6 mo</td>
</tr>
<tr>
<td>Sensitivity Incubation time</td>
<td>Not stated</td>
<td>Not stated</td>
<td>0.01 IU/mL</td>
<td>Not stated</td>
</tr>
<tr>
<td></td>
<td>3–18 h + O/N</td>
<td>0/N + O/N + 1 1/2 h, or 3h + O/N + 2 h 37°C</td>
<td>2 h + 2 h + 45–60 min</td>
<td>O/N + 4 h + 1/2 h exposure to film</td>
</tr>
<tr>
<td>Pipeting steps</td>
<td>Addition of disks, 2 pipeting steps</td>
<td>Addition of disks, 4 pipeting steps</td>
<td>3 pipeting steps</td>
<td>Equivalent to 3 pipeting steps</td>
</tr>
<tr>
<td>Sample volume</td>
<td>50 or 100 µL</td>
<td>50 or 100 µL</td>
<td>100 µL</td>
<td>1.3 mL</td>
</tr>
<tr>
<td>Standard curve range</td>
<td>A, B, C, D, Peren rye-grass 17.5, 3.5, 0.7, 0.35</td>
<td>A, B, C, D, Peren rye-grass 17.5, 3.5, 0.7, 0.35</td>
<td>A, B, C, D, Peren rye-grass 20, 4, 0.84, 0.1 IU</td>
<td>No standard curve necessary</td>
</tr>
<tr>
<td>Standards</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>No standards needed; only pos and neg controls</td>
</tr>
<tr>
<td>Instrument</td>
<td>Gamma counter</td>
<td>Spectrophotometer</td>
<td>Fluorometer</td>
<td>Photocassette and densitometer</td>
</tr>
</tbody>
</table>

*Assembled from information in the manufacturer's package insert.
*RAST, radioallergosorbent test.
*VAST, Ventrex allergosorbent test.
*IP-System, immunoperoxidase system.
*FAST, fluorescentallergosorbent test.
**MAST, multiple-thread allergosorbent test.
†CLA, chemiluminescent assay.
‡EIA, enzymeimmunoassay.
Table 4
Serum IgE Antibody Results to Cow’s Milk and Wheat as Reported by 33 Laboratories in a Survey Conducted by Centers for Disease Control\textsuperscript{a}

<table>
<thead>
<tr>
<th>Test sera and allergen</th>
<th>Reported class distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>Negative sample</td>
<td></td>
</tr>
<tr>
<td>Milk</td>
<td>14</td>
</tr>
<tr>
<td>Wheat</td>
<td>15</td>
</tr>
<tr>
<td>Positive sample</td>
<td></td>
</tr>
<tr>
<td>Milk</td>
<td>25</td>
</tr>
<tr>
<td>Wheat</td>
<td>26</td>
</tr>
</tbody>
</table>

\textsuperscript{a}(Adapted from Przybyszewski and Taylor, 1983) (26).

My group investigated the usefulness of the paper-disk RIA RAST (Phadebas RAST, Pharmacia Diagnostics) in identifying the food allergen(s) in patients whose symptoms were compatible with type I hypersensitivity and their histories were suggestive of food allergy (29). We compared the RAST result with that of double-blind oral challenge testing. In the positive challenge group, RAST was positive (1+ or greater) to the offending food in 58% of instances, which was not statistically different from the 47% in the negative challenge group. A wide variation was noted, however, between individual foods. For example, RAST was positive in 100% of positive challenges with fish but in only 33% of positive challenges with egg, and in 71% of negative challenges with peanuts but in 33% of negative challenges with cow’s milk. The overall positive predictive accuracy of RAST was 44%; highest for fish and cow’s milk (71%) and lowest for egg white (14%). The overall negative predictive accuracy was 67%, being highest for crab (80%), and lowest for fish (0%).

As was the case with skin testing, RAST reliability varied widely from one food to another. The same was observed by other workers (15,30–32). Again, a RAST result that is discordant with the chal-
lenge result should not necessarily be considered to be a "false" one; other explanations should also be considered. One should remember that IgE antibodies to certain pollen may crossreact with certain food proteins. This is particularly noticeable between grass pollens and cereals, as well as birch pollen crossreacts with apple, carrot, potato, or celery (10,33).

An area not yet adequately investigated is the nature of allergen used in skin testing or RAST (or other assays) compared with the allergen actually causing the clinical reaction. Some investigators have noted that certain milk-sensitive patients had skin test and/or RAST results negative to the native protein but positive to the enzymatic digest of beta-lactoglobulin (34,35). Another study group found that this difference could not be verified, however (36). Physicians using RAST (or similar assays) should be aware of the advantages and disadvantages of the procedure (37,38).

Less Commonly Used Tests

Several tests, with varying degrees of reliability, have been used in research studies on food allergy, albeit on a limited number of food allergens. In most of these tests, both performance and interpretation of the result require one to have extensive experience. The tests are not available in routine laboratories, and none has a degree of reliability sufficient to endorse it for use in clinical practice. Only a summary of selected tests will be mentioned here.

Serum Specific IgG, IgM, and IgA Antibodies

Hypersensitivity reactions of Gell and Coombs type II (cytotoxic or complement activation) or type III (immune complex or Arthus type) are mediated by antibodies belonging to the IgG, IgM, or IgA class. Antibodies of these isotypes can be detected by a variety of methods, such as precipitation, hemagglutination, complement fixation, enzyme-linked immunoassay, and florescent immuno-sorbent test (38,39). Limited quantities of these antibodies are often detected in normal subjects, and high titers may be present in a variety of conditions other than food allergy.

The precipitation test is usually carried out by the double-diffusion in agar technique originally developed by Ouchterlony (40) or by its more sensitive modification (micro Ouchterlony) using
Plexiglas reservoirs (41,42). Antibodies of the IgG and IgA classes can be detected in concentrations as low as 50 µg/mL, and of the IgM class in concentrations as low as 200 µg/mL. The antigen is usually put in the central well and the sera to be tested are put in the peripheral wells, but various arrangements can be designed for certain purposes. The antigen and its specific antibody diffuse into the agar and they form precipitate line at the site of reaction. Controls of normal sera and known positive sera should be included for comparison. Interpretation of the results should be based on the antigen and serum dilutions, and on the precipitate line thickness, intensity, and location between the wells. The precipitation test is most useful in evaluating the conditions of infants suspected of having the syndrome of milk-induced pulmonary disease (Heiner syndrome) or milk-induced gastrointestinal bleeding (38).

The hemagglutination test is usually performed by the indirect (passive) technique using antigen-coated erythrocytes of rabbit, sheep, or human group O (43). Sera to be tested should first be absorbed with washed, uncoated erythrocytes to remove heterophilic antibodies that would otherwise nonspecifically agglutinate the cells. IgM antibody is about 750 times as efficient as IgG in agglutination. Hemagglutinins to food antigens are common in normal persons and are of low reliability in food allergy diagnosis (38,44,45).

The complement fixation test is based on the measurement of reduced complement activity in the presence of antigen-antibody complexes. The food antigen and the serum to be tested are incubated with a certain amount of complement, then the remaining complement activity is measured by adding a suspension of sensitized sheep erythrocytes and observing for hemolysis (46). The test's sensitivity is greater than that of the precipitation test, but it is similar to that of the hemagglutination test (1 µg/dL). The complement fixation test is rarely used in food allergy diagnosis (47).

The enzyme-linked immunosorbent assay (ELISA) is a method for measuring specific antibodies of a certain immunoglobulin class using enzyme-labeled rather than radiolabeled anti-immunoglobulin (48,49). As currently used, ELISA tends to be less sensitive than the RIA technique.

IgG-RAST, analogous to IgE-RAST, has been available to test for specific IgG antibodies. It is commonly used for monitoring the pro-
Table 5
Food IgG-RAST vs Double-Blind Oral Challenge

<table>
<thead>
<tr>
<th>Challenge result</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Positive</td>
<td>4</td>
<td>36.4</td>
</tr>
<tr>
<td>Negative</td>
<td>7</td>
<td>63.6</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>100</td>
</tr>
</tbody>
</table>

\( \chi^2 = 0.13; \text{ N. S.} \)

Protective effect of hymenoptera venom immunotherapy. It has been promoted by certain laboratories for diagnosing food allergy. In a pilot study on patients suspected of having immediate-type food hypersensitivity, we noted that the test did not differentiate between the challenge-positive and the challenge-negative groups (Table 5).

Fluorescent immunosorbent test for IgG, IgA, and IgM antibodies to cow's milk proteins has been described (50). The patient's serum is incubated with antigen-coated polyacrylamide agarose beads, then washed, and rabbit antihuman immunoglobulin is added. After another incubation and washing, the adding of fluorescein-labeled goat anti-rabbit immunoglobulin will show the presence of antibodies of the corresponding immunoglobulin isotype. One should be aware, however, that the test has shown positive results in patients having gastrointestinal disorders other than milk allergy (49).

Leukocyte Histamine Release

Sensitized peripheral blood basophils release histamine in vitro in the presence of the offending food antigen (51,52). The antigen is presumed to react with its specific IgE antibodies fixed on the cell surface, whereby the release of histamine reflects a type I reaction. In at least one study, however, basophils of food-sensitive patients released high quantities of histamine spontaneously in vitro (14).
Table 6
Limitations of Leukocyte Histamine Release Assays

Technical complexity and high cost.
Blood samples should be tested within 24 h.
Certain drugs reduce the reactivity of basophils, e.g., ephedrine and aminophylline.
Certain drugs interfere with the assay, e.g., antihistamines and beta-adrenergic agonists.
Basophils of food-allergic patients may release increased quantities of histamine spontaneously.
Certain foods contain histamine, e.g., sauerkraut, yeast, and tuna.
Certain foods may release histamine directly without the presence of specific antibodies, e.g., shellfish, strawberries, egg white, and vegetable lectins.
Histamine release diminishes by too high amounts of allergen.
Not highly specific.
Not practical for routine allergy diagnosis.

In this test peripheral leucocytes (basophils) are mixed in vitro with the suspected food allergen, and the amount of histamine released is expressed as a percentage of the total histamine in the leukocyte. The reaction is dose-dependent in that too low or too high amounts of allergen will produce a diminished histamine release (53). Each allergen should be tested at various concentrations, therefore, usually in three 10-fold dilutions. The percentage released, by the optimal allergen concentration, must be compared with that released spontaneously in vitro from cells not exposed to any antigen. Measurement of histamine may be done by spectrofluorometry (54) or by an enzymic-isotopic assay (55). The latter method is more specific and sensitive, enabling one to detect quantities of histamine as low as 0.1 ng.

The leukocyte histamine release test has several limitations (Table 6) and is not practical for diagnosis of food allergy in clinical practice (53,56,57). In a study of egg-sensitive patients (52), the test had a concordance (i.e., both positive or both negative) of 83%
with prick test, and of 71% with RAST. Quantitatively, however, the three tests did not agree well. It should be realized that basophils differ structurally and functionally from tissue mast cells.

**Immune Complexes**

Circulating immune complexes may be detected by a variety of methods having different properties and that can detect complexes of various sizes (43,58). In addition to quantitation of the complexes, the specific antigen and the isotype of the incorporated immunoglobulin should be determined (59,60). Several studies have shown the test has low reliability in differentiating food-sensitive patients from normal subjects (61–64). The concentration of food immune complexes in the circulation is greatly influenced by the degree of the gastrointestinal permeability. Increased concentrations are common in subjects with selective IgA deficiency (65).

**Lymphokine Production**

When lymphocytes from atopic patients are stimulated in vitro with the offending allergen, their lymphokine production may increase several-fold. One such lymphokine tested for the diagnosis of milk allergy is leucocyte migration inhibition factor (LIF) (66). When lymphocytes from milk-sensitive patients were stimulated with cow's milk protein, their production of LIF increased remarkably. Interestingly, although lymphokine production reflects T-cell function, the symptoms in that series of milk-sensitive patients were mostly compatible with type I hypersensitivity reaction. The test is possibly more useful in the diagnosis of delayed-onset food allergy (67).

**Lymphoblast Transformation**

Antigen-induced lymphoblast transformation, a function of T cells, has been investigated in recent years as a diagnostic test for food allergy. Cultures of the patient's peripheral blood lymphocytes are incubated with the food proteins to be tested. The degree of lymphocyte proliferation is reflected in the radioactivity count of the incorporated tritiated thymidine.

A high reliability for the test to certain food antigens has been reported by some workers (68–70), but not by others (56). A significant nonspecific mitogenic effect may be induced by certain food
proteins, particularly lectins and, to some extent, beta-lactoglobulin (71,72). More studies are needed to ascertain this method’s reliability.

**Intestinal Biopsy**

Morphologic gastrointestinal mucosal changes are commonly noted in association with food allergy, particularly when the symptoms involve the gastrointestinal tract. Such changes have been noted in some patients with eczema resulting from food allergy (73). Examination of intestinal tissue taken by biopsy both while the patient is off the suspected food and after oral food challenge has been proposed by some workers as a diagnostic test for food allergy, particularly when the challenge does not induce clinical symptoms (74–76). The histologic changes are nonspecific and inconsistent, however (77,78). Also, routinely using intestinal biopsy to verify each suspected food in a particular patient would be far from being practical or acceptable by the patient.

On the other hand, valuable research information may be obtained from immunofluorescence studies of the intestinal tissue. The mucosa often shows a variety of immunologic changes, particularly an increase in the number of immunoglobulin-bearing or complement-bearing immunocytes (74,79,80).

**Organ Culture Challenge**

Iyngkaran et al. (81) demonstrated that jejunal biopsy specimens can be maintained in organ culture and repeatedly challenged with the test food. Changes in alkaline phosphatase were found to correlate with clinical sensitivity. In vitro challenges of the specimens can be done conveniently and repeatedly without the need for frequently subjecting the patient to biopsy. The technique appears promising but not practical, and its reliability needs to be ascertained.

**Controversial and Unproven Tests**

Several diagnostic procedures have been introduced into the allergy practice throughout the years without any evidence of their reliability. The *sublingual provocation, subcutaneous provocation,*
and *leukocyte cytotoxic tests* are probably the most commonly advocated. The promotion of such tests by certain clinicians has raised much concern by several investigators and among members of allergy-immunology societies, notably the American College of Allergy and Immunology and the American Academy of Allergy and Immunology. This issue has been the subject of many publications (3,82–93).

In recent years, investigation of the role of *IgG4 antibody* in allergy has gained substantial interest. An early study suggested that IgG4 antibody determination might have diagnostic value in certain patients (94). From my experience as well as from data of many other studies, however, using RIA and ELISA techniques, the serum level of food-specific IgG4 antibodies could not differentiate food-sensitive patients from control subjects (95–101). In a small series of food-sensitive patients, we did not notice a certain level of serum IgG4 antibodies that could differentiate between the challenge-positive and the challenge-negative groups (Table 7).

The *chemotactic activity of neutrophils* of subjects having food allergy was investigated in only few studies. In infants with gastrointestinal intolerance to cow’s milk and/or soy protein, Butler et al. (102) reported that active symptoms are associated with reduced neutrophil chemotaxis and increased random migration. On
the other hand, Papageorgiou and Lee (103) reported increased neutrophil chemotactic activity in three subjects with asthma from cow's milk or soybean. More studies are needed to clarify this controversy.

**VERIFICATION OF FOOD ALLERGY**

**Elimination-Challenge Test**

Today, the elimination-challenge test is the only procedure for verification of a cause-and-effect relationship between a particular food and the occurrence of certain symptoms. This technique should be used to test every food suspected by the history, skin testing, and/or in vitro test to be the cause of the patient's symptoms.

The first phase of the test aims at documenting that avoidance of the suspected food results in definite improvement in the symptoms. All suspected foods should be completely eliminated from the diet for a duration sufficient to allow improvement in symptoms. If the symptoms do not improve significantly within one to two weeks of complete avoidance of the food(s) in question, either that food is not the offending one or other offending agents were not avoided. Patients whose symptoms improve should take no or minimal medications to confirm that eliminating the offending food(s) by itself is beneficial. With the avoidance of the agent from the diet for a time longer than the symptom-free intervals the patient had had, he or she should be subjected to the second phase of the test, i.e., oral challenge.

The second phase of the test aims at affirming that exposure to the offending food results in recurrence of the symptoms. This step is essential for verification of the cause-and-effect relationship. If the food being tested had been suspected of causing systemic anaphylaxis in the past, however, the challenge test should be avoided. Generally, the challenge test should be done under supervision and in a place where facilities are available for the management of possible severe reactions. This precaution is particularly important when the patient has a history of severe reactions. We, as well as others (104, 105), witnessed the occurrence of severe reactions, including systemic anaphylaxis, from oral challenge tests in patients who had had less severe reactions previously.
The challenge test should be done while the patient has no or minimal symptoms, shows no symptoms of intercurrent illness, and is taking no or minimal allergy medications. The choice of food for the first challenge dose should depend on the severity of symptoms the patient gets and on the quantities he or she used to eat of that particular food while symptomatic. Subsequent doses (in the same or in increasing quantities) may be administered every 20 to 30 min, as long as no reaction develops, until a quantity is reached similar to that the patient used to eat. In a clinic setting, the patient is usually observed for a few hours then sent home with instructions to maintain the elimination diet. If no symptoms occur by the next day, then more of that food can be eaten during the subsequent days. If the patient remains asymptomatic while eating the food for a few days, the challenge test result is considered negative. In some instances, the offending food may need to be eaten for several days before symptoms occur. In certain instances, other factors may be needed in addition to the offending food for a clinical reaction to appear, e.g., exercise after or before ingesting the food (106,107). Whenever symptoms occur, the physician should document the clinical symptoms and signs and, whenever possible, record any changes in relevant laboratory findings, such as pulmonary function, rhinomanometry, eosinophilia in the blood or local secretion from the shock organ, serum antibody titer, or fecal blood loss.

Definite subsidence of symptoms after each of two elimination periods and recurrence of symptoms after one challenge would be enough evidence for a positive elimination-challenge test. If the findings are equivocal, however, the challenge should be repeated with larger doses. In some instances, no symptoms appear because the challenge test is conducted under controlled conditions that differ from the ordinary circumstances of the patient. A subclinical reaction in the gastrointestinal tract may be documented by observing histological or immunological changes in a biopsy from the intestinal mucosa (75,76). Similarly, a subclinical reaction in the bronchial tree may be documented by testing the airway reactivity, e.g., to histamine (108).

The challenge tests may be conducted in an open, single-blind, or double-blind manner. The open challenge procedure would be acceptable in infants and young children whose allergy manifestations can be evaluated objectively. The procedure may be used also
as a preliminary screening step, then only those foods that give a positive open challenge test would be single- or double-blind tested. This approach would save a lot of time since a negative challenge test occurs in more than two-thirds of patients suspected of being food-sensitive (109–111).

Blind challenges should be considered in older children and adults, particularly when a psychologic component is suspected or when the anticipated reaction results in subjective symptoms such as headache, dizziness, tinnitus, depression, abdominal pain, myalgia, arthralgia, or numbness. The food may be given as a freeze-dried powder in opaque capsules or hidden in an elemental formula (Vivonex) or certain foods (e.g., apricot juice or lentil soup). The purpose is to hide the food's identity, particularly as to taste, odor, and color. The patient is tested randomly with the disguised suspected food or a placebo, preferably with each taken more than once to minimize the chance of guessing by the patient. The single-blind test (the observer knows but the patient does not) is relatively easy to do and would be acceptable for clinical practice. In the double-blind challenge test (neither the observer nor the patient knows the testing material), the food is disguised and coded by a third person. Double-blind testing is often essential in research studies and sometimes in clinical practice. It would be ideal but is not always feasible, particularly in patients whose symptoms occur only to large quantities of food that cannot be easily disguised. Furthermore, providing a placebo that matches the suspected food in consistency, color, odor, taste, and quantity is not always possible.

**Inhalation Food Challenge**

In certain patients, food allergy reactions, often respiratory, may occur from inhalation rather than ingestion of the offending food allergen, such as flour in baker’s asthma (112,113), egg (114), crab (115), garlic (116,117), and coriander (118). Appropriate bronchial inhalation challenges with the specific allergen and placebo would verify the diagnosis in these patients.

**Conclusion**

Currently, the diagnosis of food allergy is based predominantly on clinical grounds. The medical history often provides valuable
clues and occasionally definitive information on the offending food. Additional screening tests applicable to clinical practice include trials of elimination diets, a food/symptom diary, skin testing, and serum IgE antibody determination. Certain other tests may be impractical, highly unreliable, or available only in specialized laboratories and used mostly for research. A highly reliable diagnostic laboratory test for food allergy is yet to be developed.

All foods suspected by any of the aforementioned methods of causing the patient's symptoms should be subjected to verification by a supervised elimination-challenge test. Ideally, the latter would be done without the patient knowing whether or not he or she is ingesting the suspected food, giving the suspected food in a disguised form randomly matched with an appropriate placebo. This method is particularly important whenever a psychologic component might be involved or the symptoms are subjective. In addition to watching for the appearance of symptoms and clinical signs, the clinician should use relevant laboratory tests whenever applicable and appropriate. When the elimination-challenge test gives an equivocal result, it should be repeated. The test should be avoided, however, if the anticipated reaction might cause a systemic anaphylaxis.

REFERENCES


*Clinical Reviews in Allergy* Volume 6, 1988