Chemistry and Detection of Food Allergens

Progress is being made in the identification and characterization of food allergens, and immunoassays are being developed to test for the presence of allergens in foods

Steve L. Taylor

□ FOOD ALLERGENS can be defined as those substances in foods that initiate and provoke the immunological reactions of food allergy. The primary immunological mechanism involved in allergic reactions to foods is probably the immediate hypersensitivity reaction mediated by allergen-specific immunoglobulin E (IgE) (Taylor, 1987). In IgE-mediated food allergy, the allergens are usually naturally occurring proteins found in the food. The sole exception is a transfer RNA in shrimp that apparently serves as an allergen (Nagpal et al., 1987).

Foods contain an enormous number of different proteins. Most of these proteins are not known to trigger IgE-mediated food allergies. Very little is known, with a few notable exceptions, about the characteristics of those food proteins which can function as allergens. These proteins seem to be found predominantly in foods of plant and marine origin; proteins of animal origin, with the notable exception of cows' milk proteins and egg proteins, even though present in the diet at comparatively high concentrations are rarely implicated in food allergies.

General Characteristics

By definition, food allergens must be capable of stimulating IgE production. In theory, any food protein has the potential for stimulating IgE production and sensitizing an individual to become allergic to that protein. But, in practice, only a few categories of foods are associated with the vast majority of food allergies. The properties peculiar to the allergenic proteins in these foods that are responsible for their ability to stimulate IgE production are not well understood.

The immunogenicity of a protein is related, in general, to its amino acid sequence and its three-dimensional structure. It is also related to its perceived degree of foreignness to the host (Crumpton, 1974). However, this concept of foreignness is not well understood, especially as related to IgE production. Food proteins, and other proteins for that matter, can stimulate a variety of immunological responses, both cellular and humoral. These proteins can stimulate the production of several classes of antibodies: IgG, IgM, IgA, IgD, and IgE. Immunogenic proteins are more likely to stimulate IgG production than IgE production. Also, many allergenic food proteins induce the formation of IgG, IgM, and IgA in addition to IgE.

While the molecular factors associated with these antibody responses have not been elucidated, it is very important to note that the allergenicity of a food protein may not always correlate with its overall immunogenicity. A food protein that induces an IgG response in animal models, even humans, will not necessarily induce an IgE response. The allergenic determinants on a protein that stimulate IgE production may be quite different from the determinants that stimulate IgG production. Also, it must be stressed that only a few individuals will develop allergen-specific IgE and an allergic response after exposure to a potentially allergenic food protein (Taylor et al., 1987a). Only those individuals who have inherited the genetic predisposition to produce IgE antibodies in response to allergen stimulation are likely to display such responses, and then only if they are exposed under suitable conditions (Aas, 1978).

The mechanism of IgE-mediated allergic reactions is portrayed in Fig. 1 (Taylor et al., 1989). Basically, the sensitization process involves the stimulation of allergen-specific IgE resulting from exposure to the allergen. Once sensitized, subsequent exposure to the allergen results in interactions between the allergen and the allergen-specific IgE on the surface of sensitized mast cells and basophils. This interaction results in the release of histamine and several dozen other mediators of the allergic response.

The binding between the allergen and the allergen-specific IgE on the surface of the mast cell imposes certain other structural requirements on the allergen. The allergen must be able to bridge between two IgE antibody molecules on the surface of the mast cell membrane. Consequently, the allergens must be the appropriate size to allow such bridging and must possess more than one IgE-binding site (Aas, 1978). The ideal size (molecular weight) for allergens appears to range from 10,000 to 70,000 daltons (Taylor et al., 1987a).

The ideal size of a food allergen would seem to be dictated by at least three factors: the immunogenicity of the protein, the intestinal permeability of the protein, and the bridging requirement. Small proteins are less immunogenic than large proteins (Crumpton, 1974). A molecular weight of 10,000 may represent the lower limit for an immunogenic response, although small peptides and other molecules can bind to larger proteins and induce a haptenic response (Taylor et al., 1987a).

The upper limit on the molecular weight of an allergen may be dictated by the constraints of intestinal permeability. Proteins larger than a molecular weight of 70,000 are less likely to be efficiently absorbed through the intestinal mucosal membranes and to obtain access to the IgE-producing cells of the body (Taylor et al., 1987a).

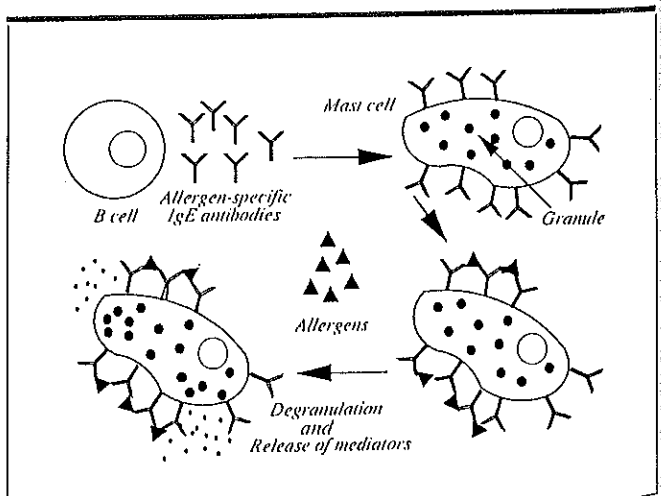

Fig. 1—**Mechanism** *of the immediate hypersensitivity (IgE-mediated) allergic reaction*

The author, a Professional Member of IFT, is Head, Dept. of Food Science and Technology, University of Nebraska, Lincoln NE 68583-0919.

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Apparently, the typical size range for food allergens is also suitable to allow the bridging of adjacent IgE antibody molecules on the surface of the mast cell membrane to occur. The requirement that allergens must possess more than one IgE-binding site to trigger the release of histamine and other mediators from the mast cells and basophils may be one factor determining whether a protein has the potential to be an allergen, although its importance cannot be determined at this time. Not only must the allergen possess two or more allergenic determinants (IgE-binding sites), but these determinants must be separated at distances appropriate to allow the bridging of the IgE antibody molecules on the mast cell membranes (Aas, 1978). However, multiple allergenic determinants of an allergen do not need to be identical (Aas, 1978); this could theoretically allow many proteins to function as allergens.

Allergenic food proteins must retain their allergenicity through various food processing treatments (Taylor, 1980, 1986). In general, food allergens are comparatively stable to heat and acid treatments. Food allergens must also survive the digestive process (Taylor, 1986; Taylor et al., 1987a). These proteins must be resistant to peptic-tryptic digestion and be stable in acid if they are to reach the intestinal mucosa in an immunogenic form.

Only a few of the many proteins in foods are likely to cause allergic sensitization and then only on rare occasions in susceptible individuals. Table 1 lists the most common allergenic foods. Somewhat surprisingly, many of these foods appear to contain multiple allergens (Taylor et al. 1987a, 1989). However, in most cases, one or two of these allergens serve as major allergens. Major allergens can be defined as those allergens affecting 50% or more of the patients with that allergy.

The existence of multiple allergens remains a puzzle. Obviously, different individuals may mount allergic responses to different proteins. But why do specific patients develop IgE to more than one protein in a given food? It is tempting to speculate that these multiple allergenic determinants might contain identical or cross-reacting allergenic determinants (Taylor et al., 1987a), but limited evidence exists at this time to support this hypothesis. Matsuda et al. (1991) demonstrated that a monoclonal antibody specific for a purified rice allergen has the ability to bind to several rice proteins. This finding strongly suggests that cross-reacting allergenic determinants or epitopes exist on these multiple rice allergens, which are all found in the albumin fraction (Matsuda et al., 1991). Pedersen and Djurtoft (1989) showed that several subunits of the soy protein, glycinin, could react with soy-specific human IgE, suggesting that multiple epitopes occur within this large protein. Similar evidence of cross-reacting determinants must be sought in other foods containing multiple allergens before any generalization can be made about the frequency of such occurrences.

The molecular characteristics which are most important in determining whether a particular food protein will be an allergen are unknown, although a variety of possible factors were discussed above. Obviously, the prevalence of the particular protein in the food may also be an important factor. Major proteins may be more likely to be major allergens. However, such correlations cannot yet be made, as a result of our overall lack of knowledge about food allergens. Some major food proteins such as those found in beef, pork, and chicken are rarely involved in allergic reactions, indicating that the prevalence of the protein in the diet is but one factor in its likelihood to be a major food allergen.

Known Food Allergens

Although many foods are known to be allergenic, very little information exists regarding the nature of the allergens in most of these foods. As noted previously, all food allergens are proteins, with the sole exception of the transfer RNA from shrimp (Nagpal et al., 1987), although shrimp also contain proteinaceous allergens (Hoffman et al., 1981; Lehrer et al., 1990; Nagpal et al., 1989). Although much is known about the proteins in some of the allergenic foods such as cows' milk, eggs, and soybeans, the identity and nature of the allergens are not precisely known.

Table 1—**Common Allergenic Foods**

Legumes, especially peanuts and soybeans
Crustacea: Shrimp, crab, lobster, crayfish
Milk, including cows' milk and goats' milk
Eggs from all avian species
Tree nuts: Almonds, walnuts, Brazil nuts, hazelnuts, etc.
Fish: Cod, haddock, salmon, trout, etc.
Molluscs: Clams, oysters, scallops, etc.
Wheat

Table 2—**Known Food Allergens**

Source	Allergen
Cows' milk	β-lactoglobulin, α-lactalbumin, caseins
Egg white	Ovomucoid (*Gal d* I), ovalbumin (*gal d* II), conalbumin (*Gal d* III)
Egg yolk	Lipoprotein, livetin, apovitellenin I, apovitellenin VI
Peanuts	Peanut I, concanavalin A-reactive glycoprotein
Soybeans	Kunitz trypsin inhibitor, β-conglycinin, glycinin, unidentified protein (20,000 daltons)
Green peas	Albumin protein (1,800 daltons)
Potato	Unidentified proteins (16,000-30,000 daltons)
Peach	Unidentified protein (30,000 daltons)
Papaya	Papain
Rice	Glutelin fraction, albumin proteins (14,000-16,000 daltons)
Buckwheat	Trypsin inhibitor
Wheat	Albumins and globulins
Codfish	Allergen M (*Gad c* I), a parvalbumin
Shrimp	Antigen I (9,000-20,000 daltons), antigen II (31,000-34,000 daltons), transfer ribonucleic acid

Table 2 lists the food allergens which have been isolated and at least partially characterized. Some of these known allergens are major allergens, while others are minor allergens. Many of the allergenic foods contain multiple allergens Frequently, serum IgE from an allergic individual will bind more to more than one protein. And different binding profiles are often noted with the serum IgE from different patients with the same food allergy. Thus, the issue of allergen identification and characterization is complex. The reasons for these differences are not known.

Progress in the isolation and identification of food allergens has occurred primarily in the past decade. This progress has been possible through the combination of good protein separation techniques, particularly SDS-polyacrylamide gel electrophoresis, and immunoblotting using the serum from specific food-allergic patients and radioactively labeled anti-human IgE to detect those separated proteins capable of binding to IgE (Tovey and Baldo, 1987). The binding of proteins to human IgE can be measured very sensitively with radioactively labeled anti-human IgE. Therefore, the isolation and characterization of food allergens is further complicated by the need for scrupulously purified and/or separated proteins and the ability to detect rather low degrees of binding to minor allergens.

• **Milk.** Cows' milk is the most common allergenic food among young infants (Taylor, 1986). It contains multiple allergens, including all of the major proteins. β-lactoglobulin, casein, and α-lactalbumin seem to be major allergens (Baldo, 1984; Lebenthal, 1975; Taylor, 1986; Taylor et al., 1987a). The bovine immunoglobulins and bovine serum albumin are minor allergens (Baldo, 1984; Taylor et al., 1987a). IgE antibodies to several other minor milk proteins—including lactoferrin, lactoperoxidase, alkaline phosphatase, and catalase—have been identified in a few patients (Baldo, 1984), but the significance of these proteins as allergens remains to be identified. However, it is quite clear that both the casein and whey fractions contain major allergens. Milk from other animals is also likely to be allergenic to individuals with cows' milk allergy; the allergenicity of goats' milk is well known (Darnton-Hill et al., 1987).

• **Eggs.** Eggs are probably the second most prominent allergenic food in the pediatric population. For many years, egg white was considered responsible for most egg allergies, and ovomucoid was thought to be responsible for the vast majority of allergic reactions (Bleumink and Young, 1971). More recent studies have identified at least three major allergens in egg whites: ovomucoid, ovalbumin, and ovotransferrin, also known as conalbumin (Hoffman, 1983; Langeland, 1982, 1983a). The allergenic determinants in ovalbumin have been characterized (Elsayed et al., 1986, 1991). The major allergenic epitope of ovalbumin is thought to encompass residues 323–339, while the N-terminal decapetide, residues 1–10, is a monovalent epitope capable of blocking but not triggering the allergic reaction (Elsyaed et al., 1991). In addition, lysozyme and ovomucin appear to be minor allergens in egg whites (Holen and Elsayed, 1990; Walsh et al., 1988).

Egg yolks are now known to be much more important in allergic reactions than previously thought. Multiple allergens have also been detected in egg yolks. Anet et al. (1985) found IgE-binding activity in three egg yolk fractions: lipoproteins, livetin, and granules. Further investigation resulted in the identification of two lipoproteins, apovitellenin I and apovitellenin VI, as allergens in egg yolks (Walsh et al., 1988). Phosvitin, one of the major proteins of egg yolk, also appears to have IgE-binding activity (Walsh et al., 1988). Further work will be necessary to determine which of the egg yolk proteins are major allergens. Eggs from all avian species appear to contain allergenic proteins (Langeland, 1983b).

• **Legumes.** Peanuts are the major allergenic food among adults in the United States and are also one of the leading allergenic foods among children. Peanuts contain multiple allergens (Barnett et al., 1983), but most of these allergens remain unidentified. Both of the major protein fractions of the peanut, arachin and conarachin, possess allergenic activity (Barnett et al., 1983). Sachs et al. (1981) purified a major peanut allergen that they called Peanut I; it was an acidic glycoprotein and was likely a subfraction of either arachin or conarachin. A lectin-reactive glycoprotein which was isolated independently appears to be a major allergen (Barnett and Howden, 1986; Kemp et al., 1985). It is unclear whether Peanut I is related in any way to the lectin-reactive glycoprotein.

Soybeans also contain multiple allergens (Herian et al., 1990; Shibasaki et al., 1980). Although both peanuts and soybeans are legumes, few individuals are allergic to both materials. Herian et al. (1990) found several allergens with subunit molecular weights of 50,000–60,000 daltons, using the sera of adult subjects with histories of adverse reactions to both peanuts and soybeans. A major allergen with a molecular weight of approximately 20,000 was identified with the sera of patients who were sensitive only to soybeans (Herian et al., 1990). Earlier studies in Japan had indicated that the major soy protein fractions, the 2S, 7S, and 11S fractions, possessed allergenic activity (Shibasaki et al., 1980). Burks et al. (1988), using the sera of infants with soy-induced atopic dermatitis, identified the 7S fraction as the most allergenic. Other investigators have focused on the glycinin fraction and partially elucidated the nature of the epitopes for this protein fraction (Pedersen and Djurtoft, 1989). The Kunitz trypsin inhibitor was implicated as a significant allergen in one patient (Moroz and Yang, 1980), but this protein appears to be a relatively minor allergen.

The allergens in one additional legume, green peas, have also been characterized to some extent. The albumin fraction possessed allergenic activity, while the legumin and vicilin fractions did not (Malley et al., 1975). In further attempts to purify the allergen in this fraction, Malley et al. (1976) isolated a peptide with a molecular weight of 1,800 that contained 30% carbohydrate and suggested that it was a fragment of a larger protein from the pea albumin fraction.

Peanuts and, to a lesser extent, soybeans appear to account for most of the allergic reactions to legumes. The sera of peanut-allergic individuals often bind to proteins from a wide variety of legumes (Barnett et al., 1987). However, despite the evidence of IgE-binding, these individuals can often consume the other legumes with no ill effects (Bernhisel-Broadbent and Sampson, 1989; Bernhisel-Broadbent et al., 1989). Thus, allergic reactions to multiple legumes seem to be the exception rather than the rule.

• **Seafood.** Allergen M from codfish is the most extensively characterized food allergen. It is a sarcoplasmic protein belonging to a group of proteins known as parvalbumins (Elsayed et al., 1991). It contains 113 amino acid residues and one glucose moiety, has a molecular weight of 12,328, and has an isoelectric point of 4.75 (Elsayed and Bennich, 1975). Allergen M contains at least three IgE-binding sites distributed along the polypeptide chain (Elsayed and Apold, 1983). A hexadecapeptide corresponding to residues 49–64 in allergen M is one of the IgE-binding sites, and it is the only epitope from a food allergen that has been sequenced (Elsayed et al., 1980). A second peptide representing residues 13–32 also possesses allergenic activity (Elsayed et al., 1991). Both of these sites have the divalent determinants necessary to elicit an allergic response (Elsayed et al., 1991). The third IgE-binding site, located at residues 88–103, is a monovalent binding site that can block but not trigger the allergic reaction (Elsayed et al., 1991). Much additional information is known regarding allergen M (Taylor et al., 1987a, b).

Most other fish allergens remain to be studied. Most fish

have parvalbumins, so proteins similar to allergen M may occur in other fish. Protamine sulfate is also a known allergen and may be responsible for a few allergic reactions to fish (Knape et al., 1981).

Allergic reactions to crustaceans such as shrimp, crab, lobster, and crayfish are one of the most common forms of food allergy among adults in the U.S. Several attempts have been made to purify and characterize allergens from shrimp. Hoffman et al. (1981) isolated two major allergens which were called antigens I and II. Antigen I was heat labile and had a molecular weight of approximately 20,500. Antigen II was heat stable and had a molecular weight of approximately 38,300. Subsequently, Lehrer et al. (1985) identified at least seven allergens in shrimp, including three major allergens. The major allergens were also present in other crustacea: lobster, crab, and crayfish.

Recently, an allergen similar to antigen II has been identified, along with several other allergens, in the cook water after boiling of shrimp (Lehrer et al., 1990). Independently, Nagpal et al. (1989) purified two heat-stable allergens from shrimp. One was somewhat similar to antigen II, having a molecular weight of 34,000. The other was much smaller, having a molecular weight of 8,200. The same investigators also isolated a transfer RNA which was capable of binding IgE (Nagpal et al., 1987). While this observation is novel and noteworthy, the presence of some protein contamination cannot be totally discounted in that study.

Although many patients react adversely to all crustacea (Lehrer et al., 1985), some patients react only to certain species of shrimp (Morgan et al., 1989). The allergens responsible for these shrimp-specific reactions have not been studied.

• **Grains.** Cereal grains are not particularly common causes of allergic reactions. Hence, cereal grain allergens have not been extensively studied. Celiac disease, which may be a form of delayed food allergy, is the exception but will not be reviewed here (see Taylor et al., 1989). Several studies have documented IgE binding by the albumin and globulin fractions of wheat (Donovan and Baldo, 1990; Hoffman, 1975; Sutton et al., 1982). However, the interpretation of these studies is complicated by the lack of overt symptoms to wheat ingestion in many of the patients included in the studies. The binding of wheat proteins to IgE may occur in many individuals with allergies to grass pollens (Donovan and Baldo, 1990). In one isolated case report, a patient with exquisite wheat allergy was shown to react to the gliadin fraction (Goldstein et al., 1969).

Three IgE-binding proteins in the molecular weight range of 8,000–9,000 have been isolated from buckwheat seeds (Yano et al., 1989). One is a trypsin inhibitor, and all three are quite heat stable.

Shibasaki et al. (1979) determined that two of the major protein fractions of rice (glutelin and globulin) were able to bind IgE from the sera of sensitive patients. Subsequently, Matsuda et al. (1988) purified an allergen with a molecular weight of 16,000 from the albumin fraction of rice. After preparing a monoclonal antibody against this purified allergen, a group of related allergens in the molecular weight range of 14,000–16,000 were isolated from the albumin fraction of rice.

• **Fruits and Vegetables.** The allergens present in fruits and vegetables (other than legumes) have received little attention. Some limited investigations were conducted on the allergens of tomatoes, which appeared to be glycoproteins (Bleumink et al., 1966, 1967). Wadee et al. (1990) isolated from peaches a 30,000-dalton protein that bound to IgE from the serum of a peach-allergic patient. This protein or a similar one was also present in guavas, bananas, mandarins, and strawberries (Wadee et al., 1990). Papain, the proteolytic enzyme from *Carica papaya* which is widely used as a meat tenderizer, has been identified as an allergen in a few rare cases (Mansfield and Bowers, 1983). Potatoes contain multiple allergens which have yet to be well characterized (Wahl et al., 1990).

Detection of Food Allergens

Food-allergic individuals can react to mere traces of the offending food allergens (Taylor et al., 1986; Taylor, 1989, 1990). Specific avoidance diets are the major means of prevention of these adverse reactions (Taylor et al., 1986; Taylor, 1989). If a person is allergic to peanuts, he or she must avoid peanuts in the diet to prevent adverse reactions. However, since trace levels of the offending allergen can elicit an adverse reaction, total avoidance can be extremely difficult. The patient must rely on food labels to determine if the offending food is present. The source of some food ingredients, such as starch and hydrolyzed vegetable protein, is often unclear. Processing errors and oversights can also result in the unlabeled presence of small quantities of the offending allergen in other foods. Examples include the failure to adequately clean common equipment, the use of re-work (a practice common in certain segments of the industry), and the inadvertent (or sometimes intentional) addition of an ingredient that is not supposed to be present in the formulation. Of course, certain foods are unlabeled because of standards of identity and bulk distribution. Restaurants do not typically label the ingredients of their foods. Adverse reactions have also been reported from the sharing of utensils in food preparation or consumption, from kissing the lips of a person who has recently eaten the offending food, from the opening of food packages, and from inhalation of vapors from the cooking food.

Exposure to food allergens by allergic individuals can have devastating consequences. Yunginger et al. (1988) reported several deaths associated with the inadvertent ingestion of the offending foods by food-allergic individuals. Many of these unfortunate incidents occurred in restaurants or other food-service settings. Sampson et al. (1991a) reported similar life-threatening or fatal reactions among young children exposed to the offending food. Gern et al. (1991) reported several cases of adverse reactions to foods that occurred from the unlabeled use of milk-based ingredients in foods. Yunginger et al. (1983) investigated several adverse reactions that were related to the inadequate cleaning of equipment. Many other suspicious cases have been reported but not sufficiently well investigated to allow definitive establishment of a cause-and-effect relationship.

Thus, food processors should attempt to avoid the processing errors and oversights that cause the inadvertent contamination of one food with another and result in such adverse reactions. To establish a suitable quality assurance program, food processors must have access to reliable methods for the detection of trace quantities of food allergens in food matrices. Immunoassay procedures offer some hope in this regard, but few such assays are currently commercially available. The sera of food-allergic patients have been widely used by clinical investigators to detect the presence of food allergens in various food materials (Keating et al., 1990; Porras et al., 1985; Gern et al., 1991; Yunginger et al., 1983). While immunoassays using human serum IgE are very sensitive and specific for the food allergen, these assays may never be available on a widespread basis because of the need for human sera.

The need exists to develop highly specific immunoassays for particular foods based on animal antisera and/or monoclonal antibodies. It can be assumed that, if the food is present, the allergen(s) from that food will also be present. Such an immunoassay has recently been developed for the detection of trace quantities of gluten for the benefit of celiac patients (Skerritt and Hill, 1991). Clearly, more efforts of this type will be needed to develop the battery of immunoassays that will be necessary to implement the appropriate quality assurance procedures.

Food-specific immunoassays could have other uses besides detecting trace quantities of allergenic foods in other foods. In the implementation of effective avoidance diets, it is frequently assumed that all food products derived from the specific offending food will be allergenic. In fact, some of these products may not contain the allergens, or the allergens may be sufficiently degraded to be inactive. As noted previously, however, most food allergens are comparatively stable to heat and resistant to proteolysis.

Immunoassays with human serum IgE have been used to evaluate the allergenicity of products made from peanuts (Nordlee et al., 1981). Similar assays have also been used to evaluate the allergenicity of casein and whey protein hydrolysates (Asselin et al., 1988, 1989; Pahud et al., 1985; Sampson

et al., 1991b) and rice protein hydrolysates (Watanabe et al., 1990).

It may not be possible to develop alternatives to the immunoassays involving human serum IgE for the determination of the allergenicity of a product, because the IgE-binding activity of the proteins must be determined and is not likely to be identical to the IgG-binding activity of the same proteins. Also, the interpretation of the significance of any residual IgE-binding activity must be made with caution, because allergic reactions can be caused by such residual activity. Partially hydrolyzed casein and whey proteins serve as good examples of the potential allergenicity of partially degraded allergens (Ellis et al., 1991; Gern et al., 1991).

Direct food challenges with allergic individuals are clearly the best method for determining if a specific food product contains active allergens. Double-blind challenge testing has been conducted to determine that edible oils from peanuts, soybeans, and sunflower seeds do not cause allergic reactions in patients sensitive to these foods (Bush et al., 1985; Halsey et al., 1986; Taylor et al., 1981). Similar challenge tests were conducted by Sampson et al. (1991b) to demonstrate that a new casein hydrolysate formula was safe to infants allergic to cows' milk. Great care must be taken in the evaluation of the allergenicity of food products derived from allergenic source foods. The term hypoallergenic should be reserved for foods such as the edible oils that have no demonstrated effect upon direct challenges with allergic individuals.

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