

SPECIAL GUEST EDITOR SECTION**Methodological Aspects of Food Allergens**

The availability of accurate and sensitive detection methods for food allergens is an important issue for consumers, health care professionals, the food industry, and government regulatory agencies. The increased prevalence of hypersensitive responses to food allergens over the last 10 years (1) has been paralleled by increased numbers of allergen-related recall actions taken by regulatory agencies (2; Ben Rejeb et al., this issue). A combination of factors may be contributing to these statistics. Changes in population demographics, changes in dietary patterns, consumption of a wider variety of exotic or imported foods, and the introduction of novel proteins into the food supply as a result of agricultural biotechnology can all lead to increased exposure of a population to food allergens. Because an avoidance diet is the only preventive measure available to sensitized individuals, accurate and unambiguous labeling of commercial products is desirable. Unfortunately, unintended exposures are still common when food products containing undeclared allergens are ingested, or by accidental consumption at food service establishments. Not only are allergen detection methods critical to retrospective analyses following the reporting of an adverse event, they also contribute to the prevention of unintentional contamination during the manufacturing process. The papers contained in this Special Section are intended to describe food allergen detection methods that are currently available or under development, properties of food allergens that can impact detection methods, effects of various manufacturing processes on allergenic proteins, methods for the identification of potentially allergenic proteins, and regulatory issues faced by governmental agencies.

*Kristina M. Williams***Identification of Food Allergens**

Critical to the development of any detection procedure is the identification of a target analyte. In the case of food allergens, over 180 food allergenic proteins have been identified (3) and, for many, their DNA and amino acid sequences have been determined. All allergenic foods contain one or more allergenic proteins and numerous nonallergenic proteins and other components, complicating the purification and characterization of proteins or DNA that may be useful for diagnostic purposes and methods development. Of the many known allergenic foods, greater than 90% of severe food hypersensitivity responses in the United States are caused by 8 foods or food groups—milk, eggs, fish, shellfish, tree nuts, peanuts, wheat, and soybeans. Three additional food allergens—sesame seed, mustard, and celery—have been identified by the Commission of the European Union as having emerging international importance. The development of detection methods, as well as regulatory labeling guidelines, has focused on these major food allergens. Three papers in this issue (Koppelman et al.; Poms et al.; Goodwin) describe in detail the development of assays for some of these food allergens. Because of geographic variation in the incidence of specific food allergies, other countries have targeted food allergens and developed labeling standards that are specific to the needs of their allergic populations. The paper by Ben Rejeb et al. describes this process as it applies to Canadian regulatory activities.

In addition to the identification of naturally occurring food allergens, the marketing of food produced through agricultural biotechnology has necessitated the development of methods for predicting potential allergenicity and for detecting any expressed protein products of the introduced gene. Recommendations for evaluating potential allergenicity include evaluation of the source of the gene, structural analysis, stability of protein as demonstrated by its resistance to enzymatic and chemical degradation, and induction of an IgE response in a validated animal model. A bioinformatics approach would include a comparison of primary amino acid sequence with that of known allergens and identification of shared amino acid sequences with known linear IgE-binding epitopes. Also, predicted 3-dimensional structural characteristics and surface IgE-binding sites of known allergenic proteins could be compared to that predicted for a novel protein. Computational studies use known databases, some with tools for sequence and epitope comparison. A review of these databases is presented in this issue by Gendel. In cases where the gene is from a known food allergen, or when the expressed protein has structural similarity to a known allergen, testing with antigen-specific IgE from sensitized individuals would be appropriate. The use of human IgE to evaluate potential allergenicity of bioengineered crops is reviewed in the paper by Goodman and Leach.

A number of rodent and nonrodent animal models have been developed for the assessment of allergic potential of natural foods and foods derived from biotechnology. The immune response in the animal must be representative of the response in humans in that the potential allergen induces a preferential type 2 T-helper (CD4+) cell cytokine secretion profile and IgE antibody response directed against the protein(s) of interest. A review of murine models for the assessment of potential

allergenicity is presented by Westphal and Raybourne in this issue. An appropriate animal model could also be used for basic research into mechanisms involved in the development of food allergy, for the development of novel therapeutic strategies for the management of severe allergic responses, or to evaluate hypoallergenic variant foods. The use of animal models in the development of immunotherapeutic strategies is presented by Helm and Burks.

Sampling and Sample Preparation

Allergens can be detected in raw ingredients, processed foods or wash solutions from manufacturing equipment (Stephan et al. in this issue). In addition to being used for government inspection and regulatory actions, routine sampling and testing procedures should become a part of the food industry's Good Laboratory Practice (GLP) and Hazard Analysis and Critical Control Point (HACCP) plans. Limiting factors with any analytical method are the availability of adequate sample and the choice of sample processing or extraction procedures to ensure compatibility with the subsequent analytical method. The availability and condition of the sample may restrict the appropriateness of a particular assay method, since manufacturing processes can affect the structure and/or antigenicity of proteins and the integrity of nucleic acids. Because foods are complex systems, whole food extracts are difficult to characterize because they include not only allergenic proteins, but also nonallergenic proteins and numerous other known and unknown components. The sample material used to develop food extracts, as well as the extraction procedure, can lead to considerable laboratory-to-laboratory variability in extract composition, and assay results. This point is illustrated in the paper by Westphal et al.

Manufactured food may undergo a wide variety of processing procedures, ranging from milling processes, steeping techniques, chemical or enzymatic modifications, and exposure to high temperatures or irradiation. The effects of different processing procedures on food allergens are reviewed in this issue by Poms and Anklam. Although such procedures may not necessarily destroy the ability of an allergen to induce an IgE response in a sensitized individual, they may destroy linear or conformational epitopes that are recognized by antibodies in an immunoassay-based detection method. This would lead to an underestimation of the presence of an allergen in that particular sample, or possibly a false-negative interpretation. Similarly, manufacturing conditions may physically shear, chemically modify, or partially degrade amplifiable DNA, again leading to false-negative assay results when DNA-based methods are used. On the other hand, processing of foods could degrade the allergenic protein itself, leaving the DNA relatively intact. In this case, a positive DNA analysis could lead to unnecessary labeling or recall action. The best approach is to use DNA-based methods in situations where the detection of DNA correlates with the presence of a stable allergenic protein.

Conversely, process-dependent modification of some allergenic proteins can lead to increased allergenicity, resulting in an enhanced hypersensitivity response in sensitized individuals, or increased antigenicity, resulting in an overestimation of true allergen concentration with antibody-based detection methods. Processing effects can alter 3-dimensional structure of proteins, which could render a potential allergen more stable to digestive secretions, or cause the exposure of more allergen-specific epitopes. The creation of "neoantigens" through biochemical reactions between the food components themselves, or with components of the sample matrix, is another possible effect. Maleki and Hurlburt, in this issue, review the processing effects on 2 major peanut allergens, Ara h 1 and Ara h 2, resulting in their biochemical modification and, possibly, enhanced allergenicity.

Sample Analysis

Current technologies for food allergen detection generally rely on the quantitative or qualitative assessment of proteins or DNA in a complex food matrix. Immunoassays rely on the use of antibodies for the detection of specific allergenic proteins and/or protein(s) which serve as a marker for the allergenic food. Immunoassays have had wide application for the detection of food allergens because of their sensitivity, specificity, and stability, and because they require little pre-assay sample purification or enrichment of the crude sample extract. DNA-based methods do not analyze protein directly, but detect instead the gene which encodes for that protein. DNA-based methods for food allergens have been slower to enter the marketplace, probably because of the need to identify and sequence appropriate target genes, to select appropriate DNA primer pairs, and to optimize procedures for the extraction of amplifiable DNA. Immunochemical and DNA-based methods for food allergen detection are reviewed in this issue by Poms et al. and Goodwin.

There are several considerations to be made when choosing an immunoassay method for the detection of food allergens. Because there are no standardized food allergen protein solutions or extracts available for methods development, individual laboratories use in-house extract preparations for production of antibodies, for use as control samples, and to develop serial calibrators for quantitative assays. Because the specificity of these antibodies and the composition of extract solutions can vary between laboratories, comparisons of assays are not easily made, even when the assay formats are identical. Polyclonal antisera derived from whole food extracts may have specificity toward allergenic proteins, nonallergenic proteins, and/or other nonprotein components of that extract. When used in a detection method, a positive result with these antisera is not necessarily an indicator of the presence of the allergenic protein(s) *per se*. It does indicate the presence of one or more components of that

allergenic food, increasing the likelihood that allergenic protein(s) are present. The ability of commercial peanut assay kits to detect peanut allergenic proteins is the subject of the paper by Nogueira et al. in this issue. The specificity of an immunochemical assay can be improved through the use of affinity purified antibodies or monoclonal antibodies, or by using purified allergenic proteins for antibody production. Tests for peanut allergen Ara h 1 (4), the egg allergen ovalbumin (5), milk allergens B-lactoglobulin and casein (6, 7), Brazil nut 2S protein (8), and shrimp allergen tropomyosin (9) are examples of immunoassays that are specific for allergenic proteins. In this issue, Schmitt et al. describe the development of competitive immunoassays that are specific for the peanut allergens Ara h 1 and Ara h 2.

Polymerase chain reaction (PCR) is the most commonly used DNA-based method for the detection of food allergens. Because PCR allows the selective amplification of a specific DNA sequence, it is often assumed that PCR methods are inherently more specific than most immunoassays methods. However, the specificity of PCR also relies on choosing reaction conditions that limit hybridization to the exact target sequence. Repeated cycles of replication amplify the product of the PCR in an exponential fashion, explaining the sensitivity of the technique. As with protein-based methods, there are practical considerations associated with the use of PCR. These include the elements of primer design, the theoretical size of the resulting PCR amplicon, the food matrixes to be examined, the sample size, and the quality of the DNA template. The quantity and condition of the sample to be analyzed, matrix considerations, and processing effects are all factors that will influence the choice between a DNA- or protein-based method.

In addition to enzyme-linked immunoassay (ELISA) and PCR methods, other analytical methods for food allergies are being developed and implemented. Rapid immunochemical methods, such as dipstick and lateral flow, are variations of the conventional microplate ELISA that use nitrocellulose or polyester cloth as a solid support. Results obtained with these devices are generally qualitative or semiquantitative, but these tests have the advantages of low cost, portability, and ease of use, making them suitable for screening purposes or point-of-sale applications (Goodwin, this issue). Another technology that is being applied to the detection of food allergens is PCR-ELISA. PCR-ELISA is similar to traditional PCR in that specific DNA sequences are amplified using pairs of primers and DNA polymerase. However, amplicons are detected by ELISA by using a complementary DNA probe that is subsequently detected by using an enzyme-labeled antibody. The advantages of this assay are that it is quantitative as well as sensitive and specific, and several samples can be tested simultaneously. A new technology being evaluated for its application to food allergen detection is the array biosensor. The design of the array biosensor and its application to food allergen detection is reviewed in the paper by Shriver-Lake et al. in this issue. Advantages of biosensor methods are that they are quantitative, they monitor the sample in real-time, and they have a high potential for automation.

It is the purpose of this Special Section to introduce the reader to the issues associated with food allergen identification and detection. The ultimate goal is to protect the consumer from becoming sensitized to a food allergen by developing a better understanding of the characteristics of allergenic proteins and the human immune response to them. Once sensitized, the second line of defense for the consumer is the correct identification and labeling of potentially allergenic food or food ingredients. The availability of rapid, sensitive, and specific detection methods is critical to prevention of unexpected food allergic responses and to investigate these responses when they occur.

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