

Immunotoxicological Aspects of Food Allergy

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INTRODUCTION

Food allergy is an important health problem and an issue of growing relevance for toxicology. The characteristics of food allergy have been described elsewhere in this volume, and in other recent review articles (1, 2). Nevertheless, it is appropriate here to place the relevant toxicological imperatives in the context of clinical and immunological aspects of food allergy.

An important consideration is one of classification. By definition, allergic diseases are driven by specific immune responses. This distinguishes food allergy from other forms of food intolerance. One working classification is to regard food allergy as one type of food intolerance; the definition of the latter being a reproducible adverse reaction to a specific food or food component that results from an allergic response (food allergy), or is due to other mechanisms including pharmacological effects and enzyme deficiencies (3). In most instances, food allergy is associated with specific IgE antibody responses, although it in some cases other immunological mechanisms may play a predominant role. The best example is gluten-sensitive enteropathy, or celiac disease, that is characterized by a cell-mediated immune response (3). In addition, food allergic reactions are sometimes manifest in the absence of detectable IgE antibody, one interpretation being that in such instances other immune mechanisms are causing the symptoms. However, some care is needed in drawing this conclusion. Confirmation that suspected food allergy is mediated by IgE antibody derives from measurement of specific IgE in serum and/or skin prick test reactivity; both of which assessments require that detectable levels of IgE antibody are available systemically. However, the results of recent investigations suggest that localized IgE responses (in the gastrointestinal [GI] tract) also may elicit allergic reactions. Patients with

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confirmed food allergy, but who lacked measurable serum IgE antibody and who were skin prick test negative, were investigated. Examination of duodenal biopsies revealed a significant correlation between the appearance of symptoms and the presence of IgE-bearing cells (and activated eosinophils and T-lymphocytes) in the duodenal mucosa. The interpretation is that even in patients who lack detectable levels of systemic IgE as measured by routine methods, localized IgE responses nevertheless may play a decisive role in the elicitation of GI tract hypersensitivity reactions (4). The conclusion drawn is that IgE antibody is the major effector of food allergy, and it is on considerations of IgE sensitization to food proteins that this article focuses.

FOOD ALLERGY: GENERAL CONSIDERATIONS

The prevalence of true food allergy among adults is between 1% and 2%, and in children approximately 5% (2). It is clear now, at least in Western societies, the prevalence of atopic allergy is increasing (5–7), and although definitive evidence is lacking, it is likely that the prevalence of food allergy is increasing also. In the United States and Western Europe most cases of food allergy are associated currently with a relatively limited range of produce; the most commonly implicated sources being peanuts, tree nuts, hens' eggs, cows' milk, wheat, soybeans, fish, and shellfish (8–12). It must be recognized, however, that the importance of different foods varies both geographically, as a function of regional dietary practices, and with time, due to the introduction of new food products (13). For instance, there has been an increasing frequency in allergy to sesame associated with more widespread use of the seed and its oil in food preparation (14). Geographic and temporal changes in the pattern of specific food allergies emphasize the importance of the opportunities for exposure in the acquisition of sensitization.

The most common symptoms of food allergy include nausea and vomiting, abdominal pain, distension, flatulence, and diarrhoea. However, other organ systems may be involved, and in particular the skin (acute urticaria and angioedema and atopic dermatitis) and the respiratory tract (allergic rhinitis and asthma) (1). Severe systemic (anaphylactic) reactions occasionally may be precipitated (sometimes only when ingestion of the relevant food is accompanied by subsequent exercise) and these may prove fatal (1, 15, 16). Currently, no effective therapeutic interventions for food allergy exist, and the disease is managed by avoidance of the relevant allergen(s) (17).

IMMUNOLOGICAL ASPECTS OF FOOD ALLERGY

As indicated above, IgE antibody plays a central role in the acquisition of sensitization to food proteins and the subsequent elicitation of allergic reactions. Specific IgE antibody production is induced when a susceptible subject encounters protein allergen in sufficient quantity and under

appropriate conditions of exposure, and as a result mounts an immune response of the necessary vigor and quality. The antibody distributes systemically and associates with tissue mast cells. If the sensitized subject is exposed subsequently to the inducing allergen, then a reaction will be provoked. Antigen will associate with, and cross-link, IgE antibody bound to mast cells causing cellular degranulation and the release of both newly synthesized and pre-formed inflammatory mediators (including histamine, serotonin, prostaglandins, and leukotrienes) that act in concert to initiate a reaction locally (at the site of exposure) or systemically (as in anaphylaxis).

The generation of IgE antibody responses is tightly regulated and depends upon the induction of an immune response of the necessary quality. The qualitative characteristics of adaptive immune responses are governed largely by the preferential development of discrete functional subpopulations of T lymphocytes. Thus, for example, the most differentiated subsets of T helper (Th) cells (Th1 and Th2 cells) develop during the evolution of immune responses and display phenotypes of selective cytokine secretion. Th2 cells produce cytokines (such as interleukins 4, 5, 10, and 13; IL-4, IL-5, IL-10, and IL-13) that are required for the development of IgE antibody responses and that facilitate the elicitation of IgE-mediated allergic reactions. In contrast, products of Th1 cells (and importantly interferon γ ; IFN- γ) antagonize IgE production and allergic hypersensitivity. The second major population of T lymphocytes (T cytotoxic cells; Tc) also has functional subsets that contribute via cytokine production to the regulation of IgE responses (18–22).

A detailed consideration of the very complex cellular and molecular events that orchestrate the selective development and function of Th and Tc subpopulations is not necessary here. Sufficient for the purposes of this article is recognition of the fact that the effective acquisition of allergic sensitization to food proteins requires conditions wherein the selective generation of Th2-type immune responses is favored. This issue is addressed later.

TOXICOLOGICAL PERSPECTIVE

An increasing interest in the development of novel foods, and in particular in those derived from genetically modified (GM) crops, has generated some considerable debate regarding the likelihood of adverse health effects and how best the safety of new foods can be assured (23–29). Inevitably a major focus of attention has been allergenicity, the main questions being whether the product of a novel gene expressed in a crop plant will have the potential to induce *de novo* sensitization or to elicit a hypersensitivity reaction in those who are already sensitized. The requirements for allergenicity safety assessments of novel foods, and how these might best be addressed, have been the subject of much debate and of detailed reviews (30–38).

The first structured approach to allergenicity safety assessment was suggested by the International Life Sciences Institute (ILSI), Allergy and Immunology Institute, in association with the International Food Biotechnology Council (IFBC). A hierarchical (decision tree) approach was recommended in which the route taken in assessing potential allergenicity was determined by whether or not the gene product of interest derived from a source associated with human allergic disease (30). Thereafter, the following three evaluations were proposed:

1. Consideration of the serological identity of the protein of interest with proven human allergens.
2. Assessment of the structural similarity to, or amino acid sequence homology with, allergenic proteins.
3. Measurement of the resistance of novel proteins to digestion in a simulated gastric fluid (30).

Further details of some aspects of these approaches are available elsewhere in this volume. There is no doubt that these recommendations provided the first conceptual framework for the conduct of safety assessments. However, these approaches neither individually, nor collectively, necessarily provide a definitive evaluation of inherent sensitizing potential. Although it is true that the original decision tree provides a useful approach to the identification of proteins that are likely to have sensitizing activity based upon their antigenic, molecular, or structural similarities with known allergens, the absence of serological or structural homology with already characterized protein allergens does not preclude inherent sensitizing potential. Moreover, although (as described elsewhere in this volume) a correlation exists between the relative resistance of a protein to digestion by pepsin in a simulated gastric fluid and allergenic activity (39), this relationship is not absolute.

In light of these considerations, methods are needed that provide a more holistic and more definitive assessment of allergenic potential and that can identify novel proteins with inherent sensitizing potential that lack structural homology or serological cross-reactivity with known allergens. To this end there has been an increasing interest in the development of suitable animal models, including methods based on the use of mice (40–46), rats (47–51), dogs (52), and swine (53). Descriptions of approaches employing rodents appear elsewhere in this volume and a detailed survey of the methods proposed are outside the scope of this article. Nevertheless, it is important to emphasize that although none of these approaches has yet been validated (or even evaluated thoroughly), the judicious use of an accurate and robust animal model, in tandem with the other approaches summarized above, would be of great utility in safety assessment. This was recognized by the consultation panel convened in 2001 by the Food and Agriculture Organization and World Health Organization where one conclusion reached was that animal models may contribute valuable information regarding the likely allergenicity of foods derived from GM crops.

In summary, therefore, considerable progress clearly has been made in providing a framework for the identification of proteins that have the potential to cause allergic sensitization. The individual approaches embraced within the original decision tree, and subsequent revisions of it, are not perfect, however; there is currently considerable interest in achieving further refinement and in defining how animal models may best be integrated into safety assessment paradigms. It is against this background of progress in addressing the present imperatives for ensuring the safety of novel proteins that we consider here more broadly some of the generic issues relating to toxicologic evaluation of allergenicity. Thus, this article addresses first intrinsic hazard and second, the factors that determine whether that hazard is likely to translate into a risk of allergic disease in humans. An alternative way of viewing this is in the context of two specific questions: why are some proteins allergenic, and what is the basis for individual susceptibility to allergic sensitization?

PROTEIN ALLERGENICITY

The key consideration here is definition of the characteristics that confer on proteins the ability to induce allergic sensitization. One could argue, of course, that in theory all proteins are potential allergens if the levels of exposure were high enough and the conditions of exposure appropriate. In practice, however, this appears not to be the case because only a small proportion of the food proteins to which individuals are regularly exposed in their diet is associated with allergic disease. From a teleological point of view, of course, the fact that proteins differ in terms of the type of immune response they preferentially elicit makes sense when viewed from the perspective of adaptive immune function. In terms of effective host defence against infectious disease, the quality of response required to resist infection from, for instance, a multicellular parasite is very different from immune responses that confer resistance to viral infection.

In effect, therefore, the question is what distinguishes protein allergens from other proteins that despite being potentially immunogenic fail to cause sensitization. Clearly, the answer to this question is not only of academic interest; determination of the properties of proteins that are essential for, or associated with, allergenic potential will undoubtedly inform the development and refinement of methods for hazard identification and characterization. At present, however, there is no clear answer. It is possible to identify several characteristics that are known or suspected to influence the sensitizing activity of proteins; (39,54–57). These include:

1. Size of the protein.
2. Resistance to proteolytic digestion.
3. Glycosylation status.
4. Biological function [enzymatic activity, storage protein, etc.].

5. Overall immunogenicity.
6. The way in which the protein is recognized, internalized, and processed for presentation to the immune system.

It is relevant to make two points regarding the attributes of protein allergens. First, no single characteristic appears to be absolute or definitive and, as a consequence, it is not possible to draw general conclusions about, for example, the association between glycosylation status *per se* and sensitizing activity. Second, and related to the first, many of these features of proteins are interrelated in terms of how they might impact on the acquisition of sensitization. For example, glycosylation is known to affect not only physicochemical characteristics, but also the recognition and internalization of proteins by dendritic cells and other antigen-presenting cells (55). In addition, relative resistance to proteolytic digestion inevitably has an impact on the processing of internalized proteins by antigen-presenting cells. This, in turn, may influence the characteristics of peptide presentation to responsive T lymphocytes and the vigor and quality of induced immune responses (55). Indeed, evidence shows that the inherent stability of proteins, and the characteristics of peptides displayed by antigen-presenting cells, are able to affect in various ways the polarization of immune responses (58, 59).

A unifying hypothesis might be that collectively these relevant properties of proteins act in concert, in variable ways and according to circumstance, to shape the quality of induced immune responses; the implication being that protein allergens have the spectrum of characteristics that favor the emergence of selective Th2-type T lymphocytes. Certainly, the results of preliminary experiments conducted in this laboratory suggest that exposure of BALB/c strain mice to peanut lectin (peanut agglutinin, a known peanut allergen) is associated with a selective type-2 response based on cytokine secretion profiles. When draining lymph node cells from peanut lectin-

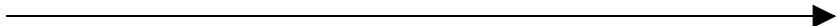
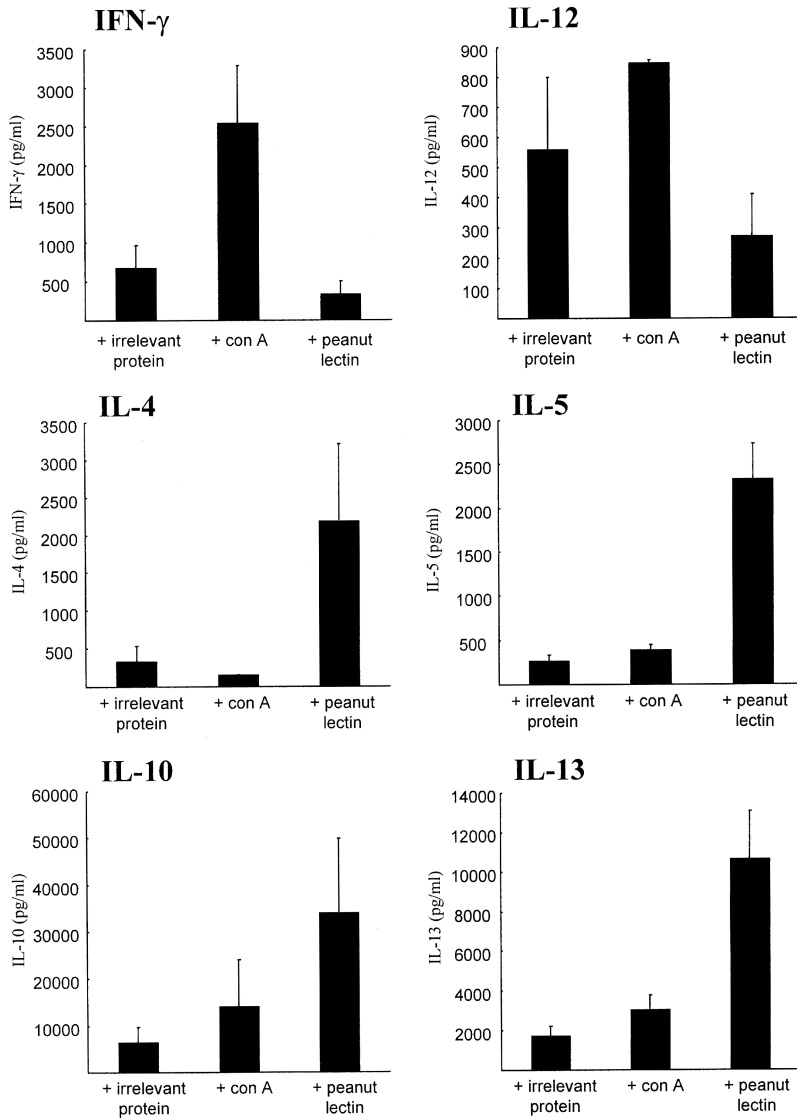


FIGURE 1 Cytokine secretion profile induced by exposure to peanut allergen. BALB/c strain mice ($n = 10$) received 30 μ l of 0.2% peanut (*Arachis hypogea*) lectin (peanut agglutinin) in phosphate buffered saline by intradermal injection in the dorsum of both ears on days 0 and 7. Fourteen days after the initiation of exposure, draining auricular lymph nodes were excised, and a single cell suspension of lymph node cells (LNC) prepared by mechanical disaggregation. LNC were cultured at 10^7 cells/ml in the presence of the T-cell mitogen concanavalin A (con A; 2 μ g/ml), purified peanut lectin (200 μ g/ml), or in the presence of irrelevant protein (potato [*Solanum tuberosum*] lectin; 200 μ g/ml), for 120 hr. Supernatants were harvested and the concentrations of interferon γ (IFN- γ), interleukin 12 (IL-12), interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 10 (IL-10), and interleukin 13 (IL-13) measured by cytokine-specific enzyme-linked immunosorbent assays. Results are expressed as mean and SE of a minimum of three independent experiments.



sensitized mice were cultured *in vitro* with the same protein, there were, compared with cells cultured with an irrelevant protein or with the T cell mitogen concanavalin A, relatively high levels of secreted IL-4, IL-5, IL-10, and IL-13, but lower levels of IFN- γ and IL-12 (the latter being another cytokine associated with type 1 responses) (Figure 1).

The conclusion drawn is that proteins vary with respect to their inherent potential to induce allergic sensitization. Although the relationships between protein structure and function and sensitizing activity are far from clear, the current view is that some key characteristics of protein allergens favor the selective development of type-2 immune responses and IgE antibody production. The question now is what variables determine whether such intrinsic potential to cause sensitization translates into a risk for human health.

INDIVIDUAL SUSCEPTIBILITY: PREDISPOSITION AND EXPOSURE

We make the case here that for an inherently allergenic protein to induce sensitization, there is a requirement for both a heritable or acquired predisposition, linked with the appropriate conditions of exposure. With regard to predisposition, probably the most important factor is inheritance of an atopic phenotype, with possibly maternal inheritance carrying the greater risk (60, 61). An interesting aspect is that although a genetic predisposition to mount vigorous IgE responses *per se* (atopic phenotype) is heritable, the specific proteins against which such responses will be directed (and to which sensitization will be acquired) appear not be programmed genetically. This illustrates the importance of exposure parameters in determining in an inherently susceptible (atopic) subject which particular proteins will cause sensitization.

Other acquired factors also may affect on individual susceptibility. This is evidenced by the fact that in Western societies an increase in the prevalence of atopic disease has been too rapid to be accounted for by changes in the gene pool (62). A theory that has gained support is that some aspects of modern lifestyle may be associated with a predisposition to atopic allergy (63). One specific suggestion is that improved standards of hygiene have reduced opportunities for microbial challenges to orientate the immune system of infants to a phenotype in which type 1 responses predominate (the hygiene hypothesis). Irrespective of the validity of this particular hypothesis, there is reason to believe certain environmental factors influence the overall likelihood of developing sensitization to dietary proteins.

Irrespective of the possession or otherwise of a susceptible phenotype, the vigor and magnitude of immune responses stimulated by protein antigens depends upon the conditions of exposure. One important aspect is the quantity of protein encountered. There have been considerations of the

amounts of certain foods or food proteins required to elicit reactions following a food challenge of sensitized subjects, but this is not always easy to predict and establishing thresholds remains problematic (64–67). Even less is known about the requirements for the initial acquisition of sensitization, although (by analogy with other forms of allergy) one might speculate that effective induction of sensitization would demand higher levels of exposure than would provocation of a reaction in an already sensitized subject. While this may be true, it must be acknowledged that confounding factors may exist that have an impact on dose-response relationships at both the induction and elicitation phases of food allergy. One such is immunological tolerance.

It is necessary first to consider what is understood by the term “immunological tolerance”, and in the context of this article, oral tolerance. It is not uncommonly supposed that dietary exposure to food proteins results in one of two outcomes: either immunological priming resulting in sensitization and subsequently allergic disease, or immunological unresponsiveness or tolerance. The implication is that allergic sensitization to food proteins results from an immune aberration characterized by a breakdown of tolerance. This rather simplistic interpretation is attractive inasmuch as it reflects the clinical picture of food allergy; some individuals develop allergic sensitization to food proteins that are tolerated in the diet by the majority without ill effect. However, in practice the characteristics of immune responses to food components are rather more complex.

Oral tolerance as an immunological phenomenon has a long history and has been investigated extensively in rodents (68, 69). The mechanisms through which tolerance is induced and maintained are complex and have been considered in detail elsewhere (68–74). A similar review is unnecessary here but it is relevant to note that in experimental systems a variety of factors (including age, genetic background, and conditions of exposure) have been found to influence the effectiveness with which tolerance is established (69, 74). The important point is that oral tolerance should not be regarded as an absolute phenomenon characterized by the ablation of all immune responsiveness to a specific antigen. The more appropriate description is immunological hyporesponsiveness (rather than unresponsiveness) associated with a reduction in some, but not all, aspects of specific immunity.

The evidence is that in general terms tolerance of T cells and a reduction in cell-mediated immunity are achieved more readily than B-cell tolerance and compromised humoral immune function (although IgE antibody responses appear an exception to this rule). Specific hyporesponsiveness appears not to be an experimental artefact restricted exclusively to rodents, as there is evidence that oral exposure of humans (to chemical or protein antigens) may be associated with partial tolerance (75–77). It is therefore reasonable to speculate that compromised tolerance to dietary antigens, particularly with respect to T lymphocyte responses and IgE antibody production, is an important factor in the pathogenesis of food allergy and that the extent to which hyporesponsiveness is induced or maintained will be

dependent not only upon genetic background, but also the conditions and timing of exposure.

That tolerance is rarely complete is illustrated by the fact that IgG antibodies specific for dietary proteins are found in subjects with no history of food allergy (78–82). Indeed, the elaboration of IgG antibody responses might be the normal consequence of regular dietary exposure to food proteins, the benefit being a mechanism for eliminating proteins or peptides that have been inadvertently absorbed from the GI tract (80, 83–85). An interesting speculation that has received little attention is whether and to what extent the presence of IgG antibodies specific for a protein allergen influences the clinical picture of food allergy. In circumstances where a robust IgG response exists, it is reasonable to suppose that there may be competition with IgE antibodies specific for the same or different epitopes on the allergenic protein. If this is the case, then the interplay between allergen-specific antibodies of different isotypes may explain in part observations that some subjects with detectable IgE remain asymptomatic, and why differences exist between sensitized individuals with respect to the amount of food necessary to provoke a reaction that appear not to correlate with specific IgE antibody levels. The effects of IgG antibody on the clinical characteristics of food allergy have yet to be clarified.

Two important considerations are the routes through which antigens are encountered and the age of first exposure.

Although there is no doubt that dietary intake is the most important and most common route of exposure for sensitization to food proteins, there is reason to believe that exposure by other routes to food proteins, or proteins immunologically cross-reactive with food proteins, may be relevant in some instances. It is possible that dermal exposure to certain food proteins may induce the quality and quantity of immune responses necessary for the acquisition of systemic sensitization and the subsequent elicitation of GI hypersensitivity reactions following dietary exposure to the same antigen. With respect to cross-reactivity, the oral allergy syndrome provides one example (86, 87). This syndrome is characterized by IgE-mediated allergic reactions in the oropharyngeal mucosa provoked by foods (frequently fruits and vegetables) that contain proteins that are immunologically cross-reactive with pollen proteins to which symptomatic subjects are sensitized (presumably from prior inhalation exposure).

The relationship between the age at which exposure to allergen first occurs and the pathogenesis of food allergy is a fascinating, but as yet unresolved, issue (88). Of particular interest and relevance is whether sensitization may be acquired as the result of exposure *in utero* or during lactation, or alternatively whether such early exposure may in fact be protective. Other important questions relate to the immune status of the mother during pregnancy and lactation (and possibly the availability of IgG antibodies that would serve to reduce allergen availability), and the age at which infants are first introduced to solid foods (3).

Taken together, the evidence suggests that against a background of differing congenital and acquired predisposition, the level and route of exposure to antigen and the age at which that exposure first occurs are important determinants of the extent to which sensitization to dietary proteins will be induced.

CONCLUSIONS

Sensitization to dietary proteins and food allergy present immunotoxicologists with some important and intriguing challenges. There is a pressing need to characterize in far greater detail the characteristics that confer on proteins the ability to cause allergic sensitization, and, in addition, the immunobiological events that influence the relative potency of allergic responses. Progress in addressing these issues undoubtedly will facilitate the development and refinement of strategies for evaluating the sensitizing potential and potency of novel proteins in the context of food safety assessments. There is a requirement also for a more detailed appreciation of the factors that govern interindividual differences in susceptibility to food allergy and in particular the relevance of prenatal and neonatal exposure to protein allergens.

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