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Allergenicity of proteins

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The Annual Congress of the British Toxicology Society (BTS) was held in Warwick from 24–26 March 1997. As part of that Congress the Immunotoxicology and Allergy and Biotechnology Specialty Sections of the BTS organized a joint scientific meeting titled 'The Immunotoxicology of Biotechnology Products'. One important aspect of the safety assessment of proteins is consideration of their ability to induce allergic sensitization. The purpose of this commentary is to address some of the relevant issues and to identify the challenges that protein allergenicity poses.

The ability of proteins to cause allergic disease is of course well established. A number of proteins, deriving from both plants and animals, is known to induce sensitization of the respiratory tract and the symptoms of rhinitis and asthma. Moreover, foods contain an enormous range of proteins and it is clear that some of these have the potential to induce allergic responses in certain individuals. Among the better characterized natural allergens in common foods are those deriving from cows milk (including β -lactoglobulin), fish and crustacea, legumes (including peanut allergens Arah2 and peanut lectin), tree nuts, eggs (including ovalbumin), cereal grains and fruits. The common symptoms of food allergy are nausea and vomiting, abdominal pain and diarrhoea. However, systemic anaphylactic (shock) reactions are not uncommon with some food allergens.

Protein allergy is most usually associated with IgE antibody-dependent mechanisms. Specific IgE antibody provoked by exposure of the susceptible individual to the inducing allergen associates with mast cells. Following subsequent exposure of the sensitized subject to the same allergen mast cells are stimulated to degranulate and to release both preformed and newly synthesized mediators (including vasoactive amines and leukotrienes) that initiate the acute inflammatory responses which characterize allergic reactions in the respiratory and gastrointestinal tracts. Only a small proportion of those exposed to a protein allergen will become

sensitized (less than 2% of the population having allergy to food proteins for instance); susceptibility being determined both by genetic predisposition and environmental factors.

The toxicology of protein allergy has assumed increased significance with the expanded use of enzymes in industrial applications and consumer products and a considerable and growing investment in the development of transgenic crop plants that express novel proteins introduced from other sources. The central issue is one of defining and characterizing protein allergenicity. Not all proteins display allergenic potential, despite being immunogenic, or at least proteins differ very markedly in their ability to induce IgE antibody-mediated allergic sensitization. The molecular and immunobiological bases for these differences are unclear, but there is nevertheless a need to determine whether a protein with a potential industrial application or a candidate protein for genetic introduction into a crop plant has the ability to induce allergic sensitization and to estimate likely potency.

The issue of allergenicity of foods produced by genetic modification has been addressed most thoroughly by the International Food Biotechnology Council in collaboration with the International Life Sciences Institute. The report produced sought to provide a rational basis for assessment of the allergenic potential of the products of genes introduced into food plants.¹ A critical first step in the hierarchical approach proposed is consideration of the source of the introduced gene and whether it derives from an allergenic food. In such instances it is recommended that the protein is evaluated *in vitro* with respect to reactivity with immune sera from subjects known to be sensitive to the food source. The existence within such immune sera of IgE antibodies specific for the protein of interest is determined using solid-phase immunoassays such as the radioallergosorbent test (RAST). Sera from a minimum of 14 subjects with known clinical sensitivity to the relevant food are examined. If all fail to react it is concluded that there is a very high probability that the protein does not represent an important food allergen. If negative or

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equivocal results are obtained then it is proposed that confirmation is obtained by skin prick tests, again using a minimum of 14 subjects. If necessary, further confirmation for lack of allergenic activity may be sought from conduct of double-blind placebo controlled food challenges (DBPCFC). If the protein derives from a less commonly allergenic food then sera from the required number of sensitized subjects may be unavailable. In such instances, where negative results in RAST or related assays have been obtained with less than five sera, the recommendation is that analysis of protein stability is considered.

A different approach is taken if the protein derives from a source considered not to be associated with allergy in humans. In this case it is proposed that the amino acid sequence of the protein is compared with sequence data available for major allergens. Based upon consideration of the minimum peptide length that acts as a T cell epitope (IgE antibody responses being T cell-dependent) a match between the protein of interest and known allergens of eight or more contiguous amino acid sequences is sought. If this degree of homology is not found it may be concluded that there does not exist a shared linear epitope with a known allergen. Alternatively, if some degree of identity is found then the approach again would be to examine reactivity with the candidate protein of immune sera from individuals sensitized to the relevant allergen. The limitations of this approach are apparent and in the absence of sequence homology it is recommended that information is sought regarding the stability of the protein to digestion and/or to food processing. With respect to the former Astwood and colleagues² have described recently a systematic evaluation *in vitro* of the stability of proteins in a simulated gastric fluid. They found that common food allergens were stable under these conditions for up to 60 min, whereas food proteins considered not to possess allergenic potential were digested very rapidly (within 15 s).

In those instances where a protein from a non-allergenic source is found to lack significant sequence homology with a known allergen and in addition is shown to be unstable in a relevant biological matrix some comfort can be taken regarding the absence of important allergenic potential. However, consideration of sequence homology and digestibility are rather empirical approaches and the availability of a relevant animal model would clearly be of value, not only in the context of prospective identification of sensitizing potential, but also in providing a means of investigating the mechanistic basis for allergenic activity. It has been concluded previously that although appropriate animal models would be of value, the difficulty of extrapolating experimental responses to human allergic disease suggest it will

be difficult to develop a reliable method that will be predictive for human allergenicity.¹ While this is true, insofar as it is not possible to accommodate within animal models human polymorphism and the considerable variability in susceptibility of humans to individual protein allergens, it is nevertheless possible to consider the use of experimental systems for evaluation of the inherent allergenic potential of proteins in the context of hazard identification and possibly estimation of relative allergenic potency. Examination of the considerable number of animal models that have been developed for the investigation of protein allergy is beyond the scope of this commentary. However, some recent examples of toxicological approaches to the evaluation of allergenicity will serve to illustrate the point.

Methods for measuring the relative allergenic potential of new detergent enzymes have been proposed. These include the intratracheal administration of the test enzyme to guinea pigs or mice with subsequent measurement of antibody production and comparison with responses induced by a reference allergenic detergent enzyme such as Alcalase (protease subtilisin Carlsberg).³ More recently a similar approach in mice based instead upon intranasal exposure to the test protein has been described also.⁴ An alternative method is the analysis in mice of the immunogenicity and allergenicity of proteins following intraperitoneal administration. It is clear from studies to date that proteins which in mice are of comparable immunogenicity display considerable variation in their ability to mount allergic responses characterized by the production of specific IgE antibody.⁵ It will be necessary of course to confirm that those proteins defined as allergenic or non-allergenic in methods such as those described above reflect what is known of relative sensitizing activity in humans. Notwithstanding that, it may prove possible to develop robust methods that will at least identify proteins which have a clear potential to provoke the quality of immune response necessary for the stimulation of IgE antibody production and the development of allergic responses. The availability of reliable models of that kind would also facilitate investigation of perhaps the most interesting question relevant to this issue; what characteristics confer on some proteins the ability to induce allergy.

In conclusion, it is apparent that the accurate assessment of protein allergenicity, particularly in the context of genetically modified food, provides interesting and exciting new challenges for immunotoxicologists. Significant progress has been made in providing a rational approach to the assessment of allergenic activity. However, needs remain for the development of appropriate animal models and for a greater understanding of the characteristics which distinguish protein allergens.

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