

News and Views

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The introduction of novel genes into food plants has prompted considerable debate about the most appropriate approaches to safety assessment. Attention has focused particularly on the need to determine whether, and to what extent, the products of novel genes have the potential to induce allergic responses and allergic reactions among consumers. This poses a challenge for toxicologists (Kimber et al., 1999). In contrast to chemical allergens, where although our understanding is far from complete there is some appreciation of the structural requirements for sensitisation, little is known of the characteristics that confer on proteins the ability to induce allergic responses and allergic reactions. Important characteristics might include molecular mass and structure, enzymatic activity (particularly proteolytic activity) or lack of it, inherent stability and glycosylation status and there is clearly a strategic need to define the influence of these and other factors on sensitising activity (Huby et al., 2000). In advance of that, however, there remains a pragmatic requirement to identify those proteins that have the potential to cause food allergy in susceptible individuals from those that do not. At a simplistic level the need is to be able to distinguish proteins that are frankly allergenic from those which are antigenically foreign, and therefore potentially immunogenic, but which fail to cause sensitisation.

Before examining the immunobiological bases for this distinction, and what approaches are and

may be available to identify allergenic potential, it is necessary to consider the toxicological context in which evaluation of sensitisation potential must be made. The objective is to assess the likelihood that exposure to a novel protein, in the context of a food matrix, will induce allergic sensitisation. This apparently straightforward objective is compounded by a number of important issues such as:

- the opportunities for sensitisation to a novel protein that might result from routes of exposure other than food intake (inhalation or dermal absorption).
- the frequency of sensitisation to a novel protein, if any, that is acceptable among an exposed population, and in this context are there any proteins that are known never to induce allergy despite widespread opportunities for exposure.
- the fact that some protein allergens, such as for instance those associated with peanut allergy, usually cause a persistent and severe sensitisation, whereas others (including for instance some milk proteins) are characterised by a less profound and more transient sensitisation.

While acknowledging that these are important issues that must be addressed, the immediate need is to assess accurately the overall potential of a protein to cause sensitisation. To date, the only systematic consideration of this issue has been conducted by the International Food Biotechnology Council (IFBC) in collaboration with the International Life Sciences Institute (ILSI). The

resulting report sought to provide a rational and structured framework for assessment of the allergenic potential of products of genes introduced into food plants (Metcalf et al., 1996). The principles of the approaches recommended have been summarised elsewhere (Kimber et al., 1997).

An important first step in the hierarchical approach described in Metcalfe et al. (1996) is to consider the provenance of the introduced gene and whether it derives from a plant or other source that is known to be associated with allergic disease in humans. If this is the case then clearly there is a need to confirm or otherwise the immunological identity and cross-reactivity of the novel gene product with known allergens, and there are relatively well-defined strategies for achieving this (Metcalf et al., 1996; Kimber et al., 1997).

If, however, the gene of interest derives from a source that has not previously been associated with human allergic disease then the approach taken is somewhat different. The first step is to examine whether there exists sequence homology between the protein of interest and previously identified human allergens; eight or more contiguous amino acid residues being considered to be an alert for sensitisation potential which would trigger further investigations. This is facilitated by the availability of appropriate sequence databases and while it provides a useful point of departure the approach is fallible and the failure to detect homology does not provide evidence for lack of potential sensitising activity. Such *in silico* investigations are supplemented by examination of the relative stability of the protein in a simulated gastric fluid (SGF); the assumption being that those proteins which are highly labile under such conditions are unlikely to be available for sufficient time in the gastrointestinal tract to allow their recognition by the immune system (Astwood et al., 1996). There is no doubt that some correlation exists between allergenicity of proteins and the ability to survive intact in a hostile biological matrix such as SGF. However, such correlations are not absolute and it is known, for instance, that some fruit allergens which are associated with the oral allergy syndrome in humans are actually very labile. Moreover, it is apparent that stability

per se does not confer on proteins the ability to induce allergic responses (Kenna and Evans, 2000).

Despite the undoubtedly useful information that derives from investigation of stability and of sequence homology with known allergens, there is a real need for methods that will permit the direct assessment of the ability of novel proteins to provoke allergic responses. The view previously has been that no appropriate animal models are available (Metcalf et al., 1996). However, since then some progress has been made and it is timely now to consider the options that are available, or which may become available in the future. In reviewing these options it is instructive to return to the apparent distinction between allergenic proteins and immunogenic proteins discussed above. There is no doubt that in most instances food allergy is associated with, and is effected by, specific IgE antibody. It is possible therefore to speculate that an important, and probably pivotal, difference between immunogens and allergens is the ability of the latter to induce an IgE antibody of sufficient vigour to cause sensitisation. It is likely that this, in turn, reflects the ability of protein allergens to induce the quality of immune response necessary to support the production of IgE. Qualitative differences in immune responses resulting from the selective development of discrete functional subpopulations of CD4 and CD8 T lymphocytes are known to be of central importance in determining the characteristics of allergic reactions to sensitising chemicals (Kimber and Dearman, 1997). Although such preferential T lymphocyte responses have not yet been characterised systematically in the context of the toxicological evaluation of protein allergenicity, there are indications of their importance (Dearman et al., 2000), and a general acknowledgement that human atopy in general, and food allergy specifically, are associated in humans with selective type 2 immune responses which favour the production of IgE antibody.

On this basis attempts to develop experimental methods for the prospective identification of food allergens have focused largely upon the measurement of induced IgE antibody responses. In general terms the view is that the ability of a protein

to induce in an experimental animal a vigorous IgE antibody response is indicative of its inherent potential to cause food allergy in susceptible humans. This assumption invariably generates some debate. One argument is that human allergic disease is so multi-factorial and the human population so diverse that it will not be possible to define sensitisation hazards in rodents. In addressing this issue it is necessary to distinguish carefully between hazard identification (evaluation of the inherent potential of a protein to elicit an allergic response) and hazard and risk assessment (the likelihood that under certain conditions of exposure this inherent potential to induce allergic responses will translate into a real risk of sensitisation and allergic disease in the human population). The diversity and complexity of human allergic disease, compounded sometimes with uncertainties regarding exposure, does indeed make hazard and risk assessment of protein allergenicity a significant challenge. Nevertheless, it is relevant to invest considerable energy into determination of inherent sensitisation potential, if for no other reason than the clear demonstration that a protein lacks any innate potential to induce sensitisation reduces the likelihood of allergy and hence mitigates the risk assessment. In this context it is relevant also to make the point that the potential of chemicals to cause skin sensitisation and allergic contact dermatitis in humans can be modelled with some accuracy using appropriate mouse and guinea pig methods (Basketter et al., 1999). There is some reason to suppose, and some evidence to suggest, that the ability of discrete proteins to induce sensitisation in humans will be reflected by their immunological activity in rodents.

Several strategies for the experimental investigation of the sensitisation potential of food proteins have been proposed. The purpose here is not to provide an exhaustive survey of the available methods, but is rather to highlight selected approaches to illustrate the current position and to address some of the issues raised. Among the methods being examined are those in which immune responses to proteins are characterised in Brown Norway (BN) rats; a strain known to favour the elicitation of type 2 immune responses

and IgE antibody production. Using BN rats two main strategies have been described. The first of these seeks to assess allergenic potential as a function of IgE production following the repeated exposure of rats by gavage to the protein of interest (Knippels et al., 1998). An apparent advantage of this approach is that the test protein is administered by the relevant route, although it should be borne in mind that no IgE responses were detectable when the allergen ovalbumin was given *ad libitum* in the drinking water rather than by gavage (Knippels et al., 1998). The second approach relies on systemic exposure (by intraperitoneal injection) of BN rats, in this case the protein being co-administered with the adjuvant carrageenan (Miller et al., 1999) and again antibody (IgG and IgE) responses are measured. Arguably the weakness of this second approach is that the use of an adjuvant will modify or skew the nature of immune responses induced by heterologous proteins such as to confer the potential to provoke IgE responses on otherwise non-allergenic proteins.

One approach that we currently favour in this Laboratory is the measurement of IgE antibody production induced by proteins following systemic (intraperitoneal or intradermal) immunisation of BALB/c mice; a strain of mouse that in common with the BN rat favours type 2 immune responses (Hilton et al., 1997; Dearman et al. 2000).

Whatever the relative strengths and weaknesses of these different approaches, an interesting issue is the importance of the route of exposure. As indicated above, an apparently attractive feature of gavage studies is that encounter with the test protein is via oral administration. However, such protocols are lengthy and expensive and it is by no means clear as yet that they have the sensitivity or selectivity necessary to provide an accurate identification of allergenic proteins. In this context it is relevant to note that oral administration would not normally be chosen for immunisation, this route of exposure being frequently associated with immunological tolerance. The other strategy is to expose animals systemically (preferably in the absence of adjuvant) by a route that is known to favour immune responsiveness. A case can be

made for this being the preferred option for identifying the inherent potential of proteins to provoke IgE responses. It must be acknowledged of course that the ability of a protein to induce specific IgE antibody following, for instance intraperitoneal injection, may not be maintained when exposure is via the gastrointestinal tract. This being the case then a hierarchical approach could be proposed in which the first tier investigates the inherent sensitising potential of a protein as a function of its ability to elicit IgE antibody following systemic administration. In cases where the protein provokes an immune response (IgG antibody production), but fails to elicit IgE antibody, the conclusion could be drawn that the protein lacks the potential to cause sensitisation. If, however, the protein was found instead to have induced an immune response that included IgE antibody, then the strategy might be to move to the next tier of investigation and question whether similar immunological activity is observed following oral administration. Although this strategy appears workable, it is important to emphasise that as our understanding of hazard identification grows then it may prove that other approaches emerge as preferred options.

All the methods described above clearly need to be interrogated more fully with a wider range of allergenic proteins of varying potency and with non-allergenic proteins. However, therein lies one difficulty; it is not easy to identify with certainty proteins that are truly non-allergenic and which can serve as negative controls for challenging the selectivity of novel predictive test methods. Notwithstanding these and other practical difficulties, the test methods summarised above (and no doubt other approaches) are beginning to show some promise and are encouraging a productive scientific debate regarding the requirements for hazard and risk assessment. No validated test methods are available, but progress to date suggests that the judicious interpretation of results obtained from appropriate animal models, together with other sources of information,

will contribute importantly to the safety assessment of novel proteins and novel foods.

Ian Kimber, Keith T. Atherton, J. Gerald Kenna,
Rebecca J. Dearman
*Zeneca Central Toxicology Laboratory,
Alderley Park,
Macclesfield, Cheshire SK10 4TJ, UK*

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