

A mouse model for food allergy using intraperitoneal sensitization

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Abstract

Food allergy is an important health issue. With the increasing interest in novel foods derived from transgenic crop plants, there is a growing need for the development of approaches for the characterization of the allergenic potential of proteins. Although most foreign proteins are immunogenic (able to induce IgG antibody responses), relatively few are important food allergens with the capacity to provoke IgE antibody production. There is currently no validated animal model for the determination of allergenic potential of food proteins. One approach that appears to show some promise is outlined in the current chapter. BALB/c strain mice are immunized by intraperitoneal injection and the potential to cause allergenicity assessed as a function of the induction of specific IgE antibody, measured by homologous passive cutaneous anaphylaxis. Progress to date with this method is summarized, and comparisons are made with other experimental models, including considerations of route of exposure, use of adjuvants and selection of appropriate end points.

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1. Introduction

The prevalence of food allergy has been estimated recently at 3.5–4% of adults in the USA and approximately 6–8% of young children and infants [1,2]. With increased interest in the development of novel foods, including foods and food products derived from genetically modified crops, there is a need for the provision of appropriate safety assessment strategies. One important issue is whether the products of novel genes introduced into crop plants have the potential to induce allergic sensitization [3–5]. This concern is not simply academic. Given that the introduction into the diet of conventionally produced novel foods, such as that of kiwi fruit in the UK, has resulted in the appearance, and a steady increase in the number, of cases of food allergy to this product [6]. However, there is good reason to suppose that not all proteins are equally allergenic. Only a small proportion of the food proteins consumed regularly is associated with allergic disease. Indeed, most cases of food allergy in the

USA and Western Europe are associated with a relatively limited range of produce; most commonly peanuts, tree nuts, hens' egg, cows' milk, wheat, soybeans, fish and shellfish [2,7–9]. These observations suggest that there is indeed a spectrum of allergenicity among food proteins. Food allergy is a complex disease, with genetic predisposition, environmental factors and exposure conditions all contributing to inter-individual differences in susceptibility [10]. It is therefore very unlikely that a single method using experimental animals will be developed that is capable of accurately predicting all aspects of the likely prevalence, persistence and severity of food allergy among human populations exposed to a novel allergen in the diet. Despite this, however, the first step of any safety assessment process is to identify accurately intrinsic hazard, or lack of it, and it is for this purpose that current approaches to the development of animal models of food allergy are directed. Once intrinsic hazard has been identified, the next steps in the risk assessment process will be to determine the characteristics of that hazard and to define the likely conditions and extent of exposure, and on those bases assess likely risks to human health.

In 1996, a collaboration between the International Food Biotechnology Council (IFBC) and the International Life

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Sciences Institute (ILSI), Allergy and Immunology Institute outlined the first systematic approach for such allergenicity testing [11]. A hierarchical strategy was suggested that incorporated consideration of the serological identity of the novel protein with proteins known to be allergenic in humans, examination of amino acid homology with, and/or structural similarity to, allergenic proteins and measurement of resistance to proteolytic digestion in a simulated gastric fluid [11,12]. However, it must be recognized that these approaches (individually or collectively) may not provide a definitive evaluation of inherent sensitizing potential. The IFBC/ILSI 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. However, the absence of serological or structural homology with already characterized protein allergens does not automatically preclude inherent sensitizing potential. Furthermore, although there exists a correlation between the relative resistance of a protein to digestion by pepsin in a simulated gastric fluid and allergenic activity, this relationship is not absolute, with reports of both pepsin resistant non-allergens [13], and pepsin sensitive allergens [14].

In the light of these considerations there is a need for methods that will provide a more holistic and more definitive assessment of allergenic potential, and that will have the ability to identify novel proteins with inherent sensitizing potential that lack structural homology or serological cross-reactivity with known allergens. As a consequence there has been a growing interest in the design and development of appropriate animal models and their potential integration into safety assessment paradigms [10,15]. Progress in this area was acknowledged in a re-evaluation of the 1996 ILSI/IFBC decision tree reported by the joint Food and Agricultural Organization of the United Nations and the World Health Organization (FAO/WHO) Expert Consultative Committee on the Allergenicity of Foods Derived from Biotechnology [16]. One of the conclusions reached in this report was that sufficient evidence has now accumulated to suggest that some animal models may provide valuable information regarding the potential allergenicity of foods derived from biotechnology [16]. Investigators have explored the use of various species for the assessment of allergenic potential, including rats, dogs and swine [17–19]. An alternative approach is the development of mouse models of sensitization to food proteins [20–24]. The mouse has a number of advantages compared with other animal models, particularly with respect to the availability of inbred high IgE responder strains and of various immunological and molecular reagents.

It is important to emphasize that currently none of these approaches has been validated (or even evaluated thoroughly) for the purposes of hazard identification in the context of a safety assessment. However, the available evidence suggests that the judicious use of an accurate and robust animal model, in tandem with the other approaches summarized above, would be of considerable utility in safety

assessment. In this chapter, we describe one method that is being developed currently; in which inherent allergenic potential is evaluated as a function of the ability of proteins to induce the production of IgE; antibody of this isotype being the major effector of immediate-type allergic reactions, including food allergic reactions [25,26]. In this regimen, test protein is administered systemically (by intraperitoneal injection) to BALB/c strain mice in the absence of adjuvant, allowing the intrinsic allergenic potential of each protein to be assessed [20–22,27]. The BALB/c strain mouse was selected as such mice are high IgE responders, equivalent to an atopic phenotype. Specific IgG antibody production is measured by enzyme-linked immunosorbent assay (ELISA) and specific IgE antibody responses assessed by homologous passive cutaneous anaphylaxis (PCA) assay. The strategy is to identify as potential allergens those proteins that have the ability to induce IgE antibody responses. These can be distinguished from non-allergenic proteins that despite being antigenic, and therefore able to provoke IgG antibody production, either fail to elicit IgE, or stimulate only low titre IgE antibody. Although only a relatively limited number of proteins has been examined to date, marked and very significant differences in the ability of allergens and presumed non-allergens to stimulate IgE responses have been demonstrated over a wide range of doses, and under conditions where the same proteins are of equivalent overall immunogenicity in terms of IgG antibody production [20–22,27]. Experience to date suggests that the measurement of antibody (IgE) responses in BALB/c mice serves to identify food allergens accurately, and to distinguish them from those materials that apparently lack significant allergenic potential.

This method using BALB/c strain mice is described, progress to date is summarized, and comparisons are made with other experimental models, including considerations of route of exposure, use of adjuvants and selection of appropriate end points.

2. Description of methods

2.1. Animals

Young adult (8–16 weeks old) female BALB/c strain mice (Harlan Seralab, Oxfordshire, UK) are used.

2.2. Animal husbandry

Animals are maintained under hygienic barriered conditions with free access to food and water. The composition of the diet should be monitored and where possible, proteins from the same source as the test protein avoided. Pelleted Special Diet Services Rat and Mouse No 1 Maintenance Diet comprising primarily cereal products (Special Diets Services, Witham, Essex) may be suitable. The ambient temperature is maintained between 20 and 24°C and relative humidity is maintained between 40 and 70% with a 12 h light/dark cycle.

2.3. Test materials

Test proteins are characterized with respect to purity, the method is currently suitable for the assessment of allergenic potential of purified proteins and is not appropriate currently for the assessment of the allergenic activity of proteins administered within a complex food matrix. Test proteins are suspended in phosphate-buffered saline (PBS). Up to three doses of proteins are tested. To optimize confidence in a negative result (failure to detect IgE antibody), then proteins should be tested at relatively high concentrations (up to 10% w/v). One important issue is contamination of the protein preparations with bacterial endotoxins as there is evidence that low doses of endotoxin can enhance IgE antibody responses [28]. Endotoxin content is assessed chromatographically by quantitative limulus amoebocyte (LAL) assay according to the manufacturer's instructions in a microtitre 96 flat-bottomed well plate format (Cambrex BioSciences, Wokingham, UK). Serial dilutions of proteins are generated using endotoxin-free water (Sigma) in endotoxin free glass test tubes (Sigma), and all solutions are vortexed for 1 min prior to diluting or plating. Protein solution (100 µl) or endotoxin standard (*Escherichia coli* 055:B5 endotoxin; range 0.005–50 EU/ml; Sigma) is incubated (10 min at 37°C in an incubating plate reader) prior to addition of the Kinetic-QCL™ reagent (a co-lyophilized mixture of lysate prepared from the circulating amoebocytes of the horse-shoe crab *Limulus polyphemus* and chromogenic substrate; 100 µl per well [Cambrex]). Samples are monitored automatically over time in an incubating plate reader for the appearance of a yellow colour. The reaction time is inversely proportional to the amount of endotoxin present; that is, the higher the concentration of endotoxin, the shorter the reaction time. Samples are tested in quadruplicate, with two of these containing the test protein alone and two of these containing test protein which is spiked with 0.5 EU endotoxin, as a quality control to check the recovery of endotoxin by the assay. Endotoxin standards are tested in duplicate. In our experience, levels of endogenous endotoxin vary from 1 to 2000 EU/mg of protein; in practice, levels of endotoxin of 100 EU/mg of protein appear to be without marked effect on IgE anti-protein responses. If unacceptably high levels of endotoxin are observed, these can be depleted using polymyxin B (PMB) columns (Pierce, Perbio Science, UK). Columns are regenerated using 3 column volumes (CV) of 1% sodium doxycholate (Sigma) and washed with 3 CV of distilled water followed by 3 CV of PBS. Protein is loaded onto the column and incubated for at least 1 h (at room temperature). Protein is eluted with 4 CV of PBS and the procedure repeated with more protein. The eluted protein is concentrated to the required concentration (2500 rpm at 30 min) using Centriplus spin tubes (molecular weight cut off 30 kDa [Millipore, UK]).

2.4. Sensitization procedure

Groups of mice ($n = 5$) receive 0.25 ml of protein in PBS by intraperitoneal injection. Seven days later treatment is

repeated. To minimize adverse effects, it is advisable to perform sighting studies using 1 or 2 mice per group. It is particularly important to monitor for anaphylactic responses following the second intraperitoneal injection.

2.5. Serum isolation

Mice are exsanguinated 14 days after the initiation of exposure. Blood is collected and allowed to clot overnight at 4°C. Individual serum samples are prepared following centrifugation 13000 rpm for 10 min. Serum samples are pooled on a treatment group basis; equal volumes of serum from each individual animal contribute to the pool. Samples are stored at –20 °C until analysis.

2.6. Anti-protein IgG antibody analysis

Protein-specific IgG antibodies are detected using enzyme-linked immunosorbent assay (ELISA). Plastic microtitre plates (Nunc, Copenhagen, Denmark) are coated with 100 µg/ml of protein in PBS by overnight incubation at 4°C. The plates are blocked by incubation for a further 30 min at 37 ° with 2% BSA (Sigma Chemical Co., St. Louis, MO) in PBS. Doubling dilutions of mouse serum samples (derived from individual animals; starting dilution 1:32) diluted in 1% BSA in PBS are added to consecutive wells and incubated for 3 h at 4°C. There follows a further incubation for 2 h at 4°C with peroxidase-labelled sheep anti-mouse IgG (Serotec, Kidlington, UK) diluted 1:4000 diluted 1 in 1000 with 1% BSA in PBS. Enzyme substrate (*o*-phenylenediamine and urea hydrogen peroxide) is added and the reaction stopped after 15 min by the addition of 0.5 M citric acid. Between each incubation, the plates are washed with PBS containing 0.05% Tween 20. Substrate conversion is measured as optical density at 450 nm using an automated reader (Flow Laboratories, Irvine, UK). Antibody titres are calculated as the highest dilution at which substrate conversion ($OD_{450\text{ nm}}$) was greater than 0.5. Control samples include serum derived from naïve (untreated) animals. It may be necessary to raise positive control sera; animals can be immunized intraperitoneally with protein in the presence of an adjuvant such as aluminium hydroxide. Data are expressed as mean and SE of IgG reciprocal titre ($\log_2$) for each treatment group. $OD_{450\text{ nm}}$ readings for naïve control serum never exceed 0.5 even at the maximum concentration tested.

2.7. Measurement of anti-protein IgE antibody

The presence in mouse serum of IgE antibodies is detected by homologous passive cutaneous anaphylaxis (PCA) assay.

2.8. Number of responder animals

To determine the number of responder animals, individual serum samples were analyzed. Individual serum samples

(neat) are injected (30 μ l) into the dermis of the ears of naïve recipient mice ($n=2$); each serum sample is administered to two separate mice to control for inter-animal variations. There is insufficient serum obtained to allow for increased group size for these determinations. Mice are restrained with light anaesthesia and oxygen to administer the intradermal injection. The injection must be administered slowly without causing substantial trauma. Concurrent negative control serum samples (derived from naïve animals) are incorporated into every PCA assay; these are of particular importance as the technique of intradermal injection is technically demanding and it is necessary to rule out false positives due to the trauma of an imperfectly performed intradermal injection. It is also important when dealing with new proteins to rule out the possibility of false positives due to inherent mast cell degranulating properties of proteins. Two days later, 0.25 mg of relevant protein, together with Evans Blue dye (1.25 mg), in 0.25 ml of physiological saline are injected intravenously. Care must be taken with the injection to ensure that no air bubbles are introduced. The solution is filtered through 0.45 μ m filters to ensure there is no particulate matter in the preparation. Thirty minutes following challenge, mice are terminated and the diameter of cutaneous reactions measured. A serum sample is identified as an IgE responder if the challenge resulted in a blue lesion in the skin with a mean diameter of 3 mm or greater.

2.9. IgE titre determination

Due to limitations on the amount of serum available, IgE titres are determined using serum samples pooled on a treatment group basis that in some treatment groups may comprise both IgE negative and positive individual serum samples. Pooled serum samples diluted to various extents with physiological saline are injected (30 μ l) into the dermis of the ears of naïve recipient mice ($n=4$); dilution series is neat; 1 in 2, 1 in 4, 1 in 8, 1 in 16, 1 in 32, 1 in 64, 1 in 128, 1 in 256, 1 in 512, 1 in 1024, 1 in 2048, 1 in 4096. It is advisable to first analyse the number of responder animals in each treatment group as described above. The number of responder animals and the vigour of the response will be informative regarding starting dilutions in the dilution series outlined above. Mice are restrained with light anaesthesia and oxygen to administer the intradermal injection. The injection must be administered slowly without causing substantial trauma. Concurrent negative control serum samples (derived from naïve animals) are incorporated into every PCA assay; these are of particular importance as the technique of intradermal injection is technically demanding and it is necessary to rule out false positives due to the trauma of an imperfectly performed intradermal injection. It is also important when dealing with new proteins to rule out the possibility of false positives due to inherent mast cell degranulating properties of proteins. Two days later, 0.25 mg of relevant protein, together with Evans Blue dye (1.25 mg), in 0.25 ml of physiological saline are injected

intravenously. Care must be taken with the injection to ensure that no air bubbles are introduced. The solution is filtered through 0.45 μ m filters to ensure there is no particulate matter in the preparation. Thirty minutes following challenge, mice are terminated and the diameter of cutaneous reactions measured. A positive (IgE) response is recorded at a given serum dilution if the challenge results in a 3-mm or greater blue lesion in the skin of 50% or more of recipient animals, with antibody titre recorded as the highest dilution of serum resulting in a positive PCA reaction.

3. Data interpretation and troubleshooting

Antibody responses induced by two proteins, peanut agglutinin and potato agglutinin, are used as examples of proteins that stimulate vigorous IgE production or little detectable IgE, respectively. The former is a constituent of peanuts to which 20–50% of peanut allergic individuals have detectable serum IgE antibody, the latter being a material that is considered to lack significant allergenic activity, a purified potato protein, potato agglutinin [29,30]. It has been demonstrated that neither the peanut nor the potato agglutinin are mitogenic for mouse lymphoid cells. Culture of naïve lymph node cells in the presence of either agglutinin failed to induce significant proliferation or cytokine expression [31]. Of some interest also is the observation that both materials have been shown to be stable to digestion in simulated gastric fluid [27], a property that is often associated with, but not exclusive to, allergens [11–14]. Mice ($n=5$) were exposed to either 0.1% peanut agglutinin or 5% potato agglutinin; as described previously for potato agglutinin, which is expected to be negative for IgE, relatively high doses are used (5%) to generate a secure negative IgE that is not simply due to administration of insufficient amounts of protein to stimulate an immune response.

Consistent with previous observations, systemic exposure to either 0.1% peanut agglutinin or to 5% potato agglutinin in each case resulted in the stimulation of marked specific IgG antibody responses as measured by ELISA [20,21,27,32]. IgG antibody titres achieved following treatment with peanut agglutinin ranged from 1 in 256 to 1 in 8192 whereas those derived following exposure to potato agglutinin varied from 1 in 256 to 1 in 1024 (Fig. 1; individual animals). Despite peanut agglutinin inducing somewhat more vigorous IgG antibody responses compared with similar treatment with potato agglutinin, both materials are clearly immunogenic under these conditions of exposure. Against this background of immunogenicity (IgG antibody responses), specific IgE antibody responses are measured by homologous PCA. Initially, the number of IgE responder mice per treatment group is assessed by monitoring the frequency of positive cutaneous reactions following exposure of recipient mice ($n=2$) to undiluted (neat) serum prepared on an individual animal basis. Results displayed in Fig. 2 demonstrate that for serum derived from peanut agglutinin immunized mice, the majority

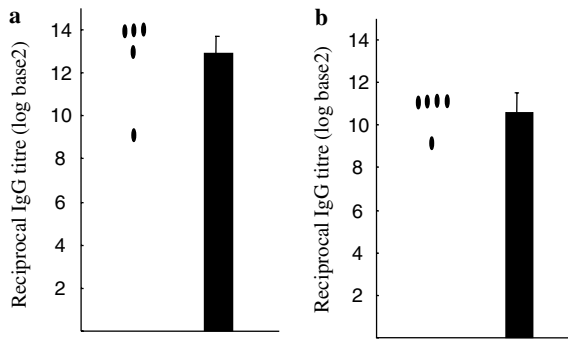


Fig. 1. IgG antibody responses following systemic exposure to 0.1% peanut agglutinin or 5% potato agglutinin : individual serum samples. Groups of mice ($n = 5$) received 0.25 ml of (a) 0.1% peanut agglutinin or (b) 5% potato agglutinin by intraperitoneal injection on days 0 and 7. Seven days later animals were exsanguinated and individual serum samples tested for the presence of specific IgG antibody by enzyme-linked immunosorbent assay (ELISA). Negative control serum samples (from naïve animals) were tested concurrently and were routinely negative (data not shown). Antibody titres for each individual serum sample are displayed (●) and mean and SE of IgG (■) reciprocal titre (log₂) for both proteins.

of cutaneous reactions elicited in recipient mice (8/10) are maximal responses (10 mm; the approximate diameter of a mouse ear and thus the maximum size of skin response achievable) and that very similar sized responses are generally induced in each of the two recipient sites. Serum derived from one peanut agglutinin immunized animal (serum number 5; Fig. 2) showed rather more variation, with values of 10 and 2 mm recorded. The less vigorous response of 2 mm cutaneous reaction could be due to inter-animal variation in responsiveness to mast cell degranulation among recipient animals but is more likely to be due to variations in the intradermal injection. Nevertheless, serum derived from all five mice immunized with peanut agglutinin caused average skin reactions of 3 mm or greater; thus

5/5 animals are classified as IgE responders. The negative control performed with naïve (untreated) mouse serum demonstrates that the technician performing the intradermal injections is proficient (no significant trauma) and that the challenge protein is without inherent mast cell degranulating properties, with cutaneous reactions of <3 mm recorded (0 and 1 mm). A different profile is observed for serum isolated from potato agglutinin immunized mice (Fig. 2). A maximal response (10 mm) is not achieved with any of the serum samples in any of the recipient mice. Three of the potato agglutinin immunized mice are IgE responders, with skin reactions varying from 3 to 8 mm, with reactions of similar diameters recorded in each duplicate reading (animal numbers 1, 2 and 5). The remaining two mice (animal numbers 3 and 4) are non-IgE responders, with cutaneous reactions of <3 mm recorded (0 mm or 1 mm) in all analyses. Intradermal administration of naïve (untreated) mouse serum failed to induce a positive cutaneous response (values of 0 mm), confirming that the assay has been performed competently and that potato agglutinin is without inherent mast cell degranulating activity.

Due to constraints on the amount of serum available, IgE titres are analyzed using serum pooled on a treatment group basis; such serum pools may well contain IgE responders and IgE non-responders as is the case for serum derived from animals exposed to potato agglutinin (Fig. 2). The PCA data from the individual serum samples are used to inform starting dilutions for the determination of IgE titre in the pooled serum samples. The percentage of IgE responders (100%) and the vigour of the cutaneous reactions (maximal responses for the majority of samples) for serum derived from mice immunized with peanut agglutinin are indicative of a relatively high titre IgE response. High concentrations of serum (neat and 1 in 2 dilution) will almost certainly be strongly positive. The dilution series selected to determine IgE titre for the pooled sample from

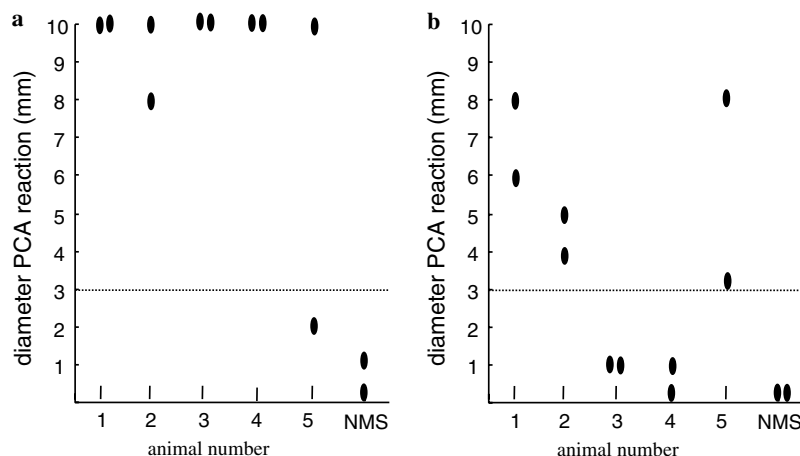


Fig. 2. IgE antibody responses following systemic exposure to 0.1% peanut agglutinin or 5% potato agglutinin : individual serum samples. Groups of mice ($n = 5$) received 0.25 ml of (a) 0.1% peanut agglutinin or (b) 5% potato agglutinin by intraperitoneal injection on days 0 and 7. Seven days later animals were exsanguinated and individual serum samples (undiluted; neat) tested for the presence of specific IgE by homologous passive cutaneous anaphylaxis assay. Negative control serum samples (from naïve animals; NMS) were tested concurrently. Each serum sample was tested in two individual animals. Data are displayed as diameter of cutaneous reaction (mm) for each recipient mouse (●). Horizontal line denotes cut off for positivity at 3 mm.

the peanut agglutinin sensitized mice comprises serial doubling dilutions of 1 in 4 to 1 in 64 (Fig. 3). A dose response is observed. As predicted, positive cutaneous reactions (4/4 recipient mice) are achieved at serum dilutions of 1 in 4, 1 in 8 and 1 in 16. At a serum dilution of 1 in 32, 3 of 4 recipients are IgE positive and the vigour of the reaction is decreased whereas at the next titration (1 in 64) all skin reactions are below the threshold for positivity (3 mm). The IgE titre for 0.1% peanut agglutinin immunized mice is therefore 1 in 32, this being similar to values reported previously [20,21,27,31]. A different strategy is employed for the serum samples derived from mice sensitized to 5% potato agglutinin. Here, only 3 of 5 individual serum samples tested positive for IgE and these responses were not maximal. The expectation is therefore that low titre antibody only will be detected in the pooled sample (which comprises both the positive and negative serum samples) which is initially tested undiluted (neat) (Fig. 3). Skin reactions recorded in the 4 recipient mice are all below 3 mm (the threshold for positivity). The IgE titre for mice immunized with 5% potato agglutinin is therefore <1 and no further testing of diluted sera is required. This is consistent with previous observations in which titres of <1 or 1 (positive with neat serum only) were achieved [20,21,27,31].

These examples serve to illustrate several important points. As with any technique it is necessary to incorporate appropriate controls. This is particularly critical for the PCA assay, a technique that is technically extremely demanding and for which false positive results (due to trauma of the intradermal injection or mast cell degranulating properties of the test protein) or false negative results (due to inability to administer the full volume of serum intradermally) may be generated. Another important point is that the failure to generate an IgE antibody response (such as is the case for potato agglutinin) should only be interpreted as a lack of potential allergenicity if the absence of specific IgE is observed against a background of immu-

nogenicity (IgG antibody responses). Such ensures that the test animals have been exposed to sufficient test protein to induce an immune response, but demonstrates that the quality of immune response is not appropriate for the stimulation of IgE (allergic) responses. Failure to generate an IgG antibody response may be a result of the lack of immunogenicity of the protein in the mouse strain of choice, or may relate to prior dietary exposure to a cross-reactive protein. In some circumstances it might be necessary to generate positive control sera (for both IgG and IgE analyses) by immunizing mice with protein in the presence of a known adjuvant such as alum.

Although as described above, there are no validated tests available currently for the prospective identification of potential food allergens, the approach described herein represents one possible method. If this method is to gain wider acceptance in the future then it will be necessary to demonstrate the robustness of the approach and the ability to transfer effectively the method between laboratories. There are two aspects to be considered when performing inter-laboratory comparisons for this particular endpoint, the induction of anti-protein IgE responses. First, whether identical responses to the different proteins can be provoked in both laboratories, and second, whether the measurement of IgE by homologous PCA (an experimental technique that is not trivial to master) yields equivalent results in both laboratories. The first of these two issues has been addressed in an inter-laboratory collaboration. In each of two laboratories, two independent experiments were performed in which BALB/c strain mice were exposed by intraperitoneal injection to peanut agglutinin, ovalbumin (OVA; a major allergen in hens' egg) or potato agglutinin [20]. All serological assessments were conducted in one of the laboratories. Each of the proteins induced vigorous IgG and IgG1 antibody responses, with no statistically significant differences in titres recorded between laboratories. Furthermore, OVA and potato agglutinin induced

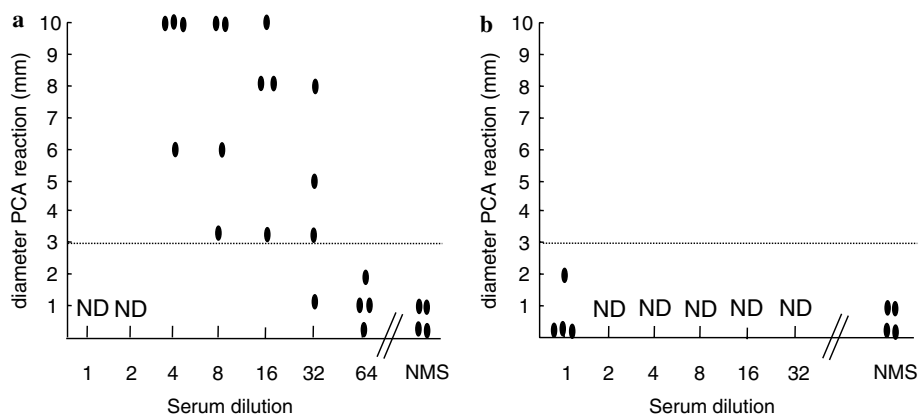


Fig. 3. IgE antibody responses following systemic exposure to 0.1% peanut agglutinin or 5% potato agglutinin : pooled samples. Groups of mice ($n = 5$) received 0.25 ml of (a) 0.1% peanut agglutinin or (b) 5% potato agglutinin by intraperitoneal injection on days 0 and 7. Seven days later animals were exsanguinated, serum pooled on a treatment group basis and tested for the presence of specific IgE by homologous passive cutaneous anaphylaxis assay. Negative control serum samples (from naïve animals; NMS) were tested concurrently. Each serum sample dilution was tested in 4 individual animals. Data are displayed as diameter of cutaneous reaction (mm) for each recipient mouse (●). Horizontal line denotes cut off for positivity at 3 mm. ND, not done.

responses of equivalent immunogenicity with respect to both IgG and IgG1 antibody titres. Administration of peanut agglutinin and OVA each stimulated marked IgE antibody responses in every experiment. In contrast, exposure to potato agglutinin failed to induce vigorous IgE production, with no detectable IgE (negative with neat serum), or titres of 1 (positive with neat serum only) recorded. These data demonstrate that the induction of IgE antibody in BALB/c strain mice by intraperitoneal administration of food proteins of differing allergenic potential is a relatively robust phenomenon and transferable between laboratories.

Perhaps the most contentious issue regarding the development of various animal models of food allergy is that of the preferred route of exposure. Although it might appear initially that oral administration represents the most appropriate route of exposure for a method designed to identify potential food allergens, this is not necessarily the case. For instance there is evidence that exposure via the diet or in drinking water is more likely in rodents to cause immunological hyporesponsiveness than it is sensitization. Thus, experience in rats has shown that *ad libitum* exposure to the known human allergen OVA failed to induce IgE antibody responses in Brown Norway strain rats [33]. We have demonstrated that even gavage exposure appears to be considerably less sensitive than is parenteral administration with respect to eliciting IgE antibody responses in BALB/c mice [34]. Although oral administration by gavage may be considered to reflect more accurately the relevant conditions of human exposure, the available evidence suggests that this experimental approach in mice may not possess the sensitivity or reliability to provide an initial assessment of intrinsic hazard (allergenic potential). For the purposes of toxicological evaluation, we have therefore chosen to focus on systemic (intraperitoneal) exposure.

Another issue that is worth addressing is the option for the use of adjuvant. The approach outlined herein has been to conduct these experiments in the absence of adjuvant, allowing assessment of the inherent potential of a given protein to induce allergic sensitization (IgE antibody responses). Other investigators have chosen to incorporate adjuvants, such as carrageenan or cholera toxin that are reportedly selective for IgE antibody responses, into immunization protocols [23,24,35,36]. While co-administration of adjuvant will undoubtedly increase the sensitivity of detection of IgE antibody responses to proteins, there is some concern that this may be at the cost of some loss in selectivity. What is not clear at present, however, is the extent to which the use of different adjuvants will perturb or modify the inherent properties of proteins to induce IgE antibody responses. That is, the use of adjuvant may compromise the ability to discriminate between proteins with respect to allergenic potential, possibly generating false positives and conferring the appearance of sensitizing potential on non-allergens. This issue is currently unresolved, as to date few non-allergenic proteins have been examined using this type of approach. There is some preliminary evidence to suggest that intraperitoneal exposure of mice to protein and carrageenan does indeed

result in some loss of selectivity of IgE responses to proteins [37]. The use of adjuvant may therefore represent a potential source of false positives in hazard identification.

4. Concluding remarks

There is considerable interest in the development and evaluation of approaches for the safety assessment of novel foods, and in particular in methods for characterization of allergenic potential. Various animal models have been suggested for the identification of intrinsic hazard, that is, the inherent potential of a protein to cause allergic sensitization. As yet, however, none of these methods has been validated or gained widespread acceptance. The approach described herein comprises systemic (intraperitoneal) exposure of BALB/c strain mouse to protein allergens with responses measured as a function of IgE antibody expression analyzed by homologous PCA. Investigations to date suggest that proteins provoke in mice variable immune responses; those with the potential to cause allergic sensitization stimulating IgE (and IgG) antibody production. In contrast, non-allergenic—but nevertheless immunogenic—proteins are associated with IgG antibody responses in the absence of significant IgE production. Furthermore, the approach is relatively robust and transferable between laboratories. Clearly, there is a need to interrogate the sensitivity and selectivity of this approach in greater detail using a wider range of allergenic and non-allergenic proteins. The evidence summarized above suggests that with continuing effort, it should be possible to develop mouse models that will be of value in identifying proteins with the inherent potential to cause allergic sensitization.

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