



Induction of IgE antibody responses by protein allergens: inter-laboratory comparisons

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Received 16 April 2003; accepted 28 May 2003

Abstract

There is a growing interest in the development of methods for the evaluation of the allergenic potential of novel proteins. One approach is the measurement of specific IgE antibody production stimulated by systemic (intraperitoneal; ip) exposure of BALB/c strain mice. In the current investigations, inter-laboratory comparisons have been performed of IgE antibody production induced in mice by food proteins of differing sensitizing potential. Female BALB/c strain mice ($n = 5$) were exposed to 0.1% peanut agglutinin, an allergenic constituent of peanuts, to 2% ovalbumin (OVA), a major allergenic constituent of hens' egg, or to a protein considered to lack significant allergenicity, potato agglutinin (5%). Specific IgE antibody was measured by homologous passive cutaneous anaphylaxis assay and IgG and IgG1 antibody production was analysed by enzyme-linked immunosorbent assay (ELISA). Two independent experiments were conducted in each laboratory, but with all serological analyses 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 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 the two laboratories, titres ranged from 1:32 and 1:64 for peanut agglutinin, and from 1:8 and 1:32 for OVA. 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 by food proteins of differing allergenic potential is a relatively robust phenomenon and transferable between laboratories. Furthermore, these results provide additional evidence that the measurement of antibody (IgE) responses in BALB/c mice may allow discrimination between allergens and those materials that apparently lack allergenicity.

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Keywords: Protein allergenicity; IgE; IgG; Systemic exposure; Inter-laboratory comparisons; Predictive testing

1. Introduction

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. An

important requirement is to address concerns about potential allergenicity, and in particular whether the products of novel genes introduced into crop plants have the potential to induce allergic sensitization (Hollingworth et al., 2003; Lack et al., 2002; Taylor and Hefle, 2001).

In 1996, the first structured approach for the assessment of the allergenic potential of novel food proteins was developed jointly by the International Food Biotechnology Council (IFBC) and the International Life Sciences Institute (ILSI), Allergy and Immunology Institute (Metcalf et al., 1996). The proposed scheme took the form of a decision tree that included (but was not limited to) consideration of the serological identity of the novel protein with proteins known to be

Abbreviations: BSA; bovine serum albumin; ELISA; enzyme-linked immunosorbent assay; FAO; Food and Agriculture Organization; IFBC; International Food Biotechnology Council; Ig; immunoglobulin; ILSI; International Life Sciences Institute; OD; optical density; OVA; ovalbumin; PCA; passive cutaneous anaphylaxis; PBS; phosphate buffered saline; WHO; World Health Organization.

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allergenic in humans, structural similarity to (or amino acid sequence homology with) allergenic proteins, and resistance to proteolytic digestion in a simulated gastric fluid. Although this approach provides useful information, particularly in those cases where proteins are found to be homologous with known human allergens, it does not permit a direct evaluation of inherent sensitizing potential. As a consequence there has been a growing interest in the design, development and application of appropriate animal models (Kimber et al., 2003a; Kimber and Dearman, 2001a).

A special consultation panel to consider food allergenicity testing and possible revisions to the original IFBC/ILSI decision tree was convened in 2001 by the Food and Agriculture Organization (FAO) and World Health Organization (WHO) (2001). One of the conclusions of this panel was that sufficient evidence had accumulated to suggest that animal models may contribute valuable information regarding the allergenic potential of foods derived from biotechnology. Some investigators have explored the utility of rats (Knippels et al., 1998), dogs (Ermel et al., 1997) and swine (Helm et al., 2002) for the assessment of allergenic potential. An alternative approach is the development of mouse models of sensitization to food proteins (Dearman et al., 2001, 2002a; Li et al., 1999, 2000). These offer considerable 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. The strategy we have adopted is to measure inherent allergenic potential 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 (Dearman et al., 2001, 2002a). In this regimen, material 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. Specific IgG (and IgG subclass) antibody production is measured by enzyme-linked immunosorbent assay (ELISA) and specific IgE antibody responses assessed by homologous passive cutaneous anaphylaxis (PCA) assay. Although only a relatively limited number of proteins have been examined to date, marked and very significant differences in the ability of allergens and non-allergens to stimulate IgE responses have been demonstrated over a wide dose range, and under conditions where the same proteins are of equivalent overall immunogenicity in terms of IgG antibody production (Atherton et al., 2002; Dearman et al., 2002a; Kimber et al., 2003b).

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. In determining the utility of

this approach for safety assessment of protein allergenicity one consideration is whether the method is robust and will yield equivalent results when conducted in independent laboratories. In the current investigations we have therefore compared antibody responses induced in BALB/c strain mice by reference proteins in independent experiments conducted in two different laboratories. Antibody (IgG, IgG1 and IgE) responses induced by ovalbumin (OVA), a relatively common food allergen, and peanut agglutinin, a constituent of peanuts to which IgE antibody can be detected in 20–50% of peanut allergic individuals, were compared with those provoked by exposure to a material that is considered to lack significant allergenic activity, a purified potato protein, potato agglutinin (Burks et al., 1994; Metcalfe, 1985). Neither the peanut nor the potato agglutinin are mitogenic for mouse lymphoid cells, with culture of naïve lymph node cells in the presence of agglutinin failing to induce significant proliferation or cytokine expression (Betts et al., *in press*). Two separate experiments were conducted with each of the three proteins in both laboratories and all serological analyses were conducted in the one of the two laboratories. The decision to perform all of the antibody measurements in a single laboratory was made in order to distinguish between inter-laboratory differences in responses to the proteins per se, and potential differences resulting from variations in analytical method.

2. Materials and methods

2.1. Participating laboratories

Bayer CropScience Laboratory, Sophia Antipolis (Laboratory A) and Syngenta Central Toxicology Laboratory, Alderley Park, Macclesfield (Laboratory B) were the participating laboratories.

2.2. Animals

Young adult (8–12 weeks old) female BALB/c strain mice (Laboratory A, Iffa-Credo, L'Arbresle, France; Laboratory B, Harlan Seralab, Oxfordshire, UK) were used throughout these studies. Food and water were available *ad libitum*. The diet used in Laboratory A was pelleted and irradiated rodent diet A04C-10 (Usine d'Alimentation Rationnelle, Villemoisson-sur-Orge, France). The diet used in Laboratory B was pelleted Special Diet Services Rat and Mouse No. 1 Maintenance Diet comprising primarily cereal products (Special Diets Services, Witham, Essex). The ambient temperature was maintained between 20 and 24 °C and relative humidity was maintained between 40 and 70% with a 12 h light/dark cycle. All husbandry and procedures were conducted in accordance with the regulations

of the Guide for the Care and Use of Laboratory Animals (Public Health Service, National Institute of Health, No. 86-23, revised 1985) and the Guide du Journal Officiel des Communautés Européennes (L358, 18 December 1986, No. 86/609/EEC, 24 November 1986).

2.3. Test materials

Commercial preparations of ovalbumin (OVA; Grade V), peanut (*Arachis hypogea*) agglutinin and potato (*Solanum tuberosum*) agglutinin were obtained from Sigma Chemical Co., St Louis, MO.

2.4. Sensitization procedure

Groups of mice ($n=5$) received 0.25 ml of 2% OVA (5 mg per mouse), 0.1% peanut agglutinin (0.25 mg per mouse) or 5% potato agglutinin (12.5 mg per mouse) in phosphate buffered saline (PBS) by intraperitoneal injection. Seven days later treatment was repeated. Mice were exsanguinated 14 days after the initiation of exposure. Individual and pooled serum samples were prepared and stored at -20°C until analysis. All serum analyses were conducted at Laboratory B.

2.5. Anti-protein IgG and IgG1 antibody analysis

Protein-specific IgG and IgG1 antibodies were detected using enzyme-linked immunosorbent assay (ELISA). Plastic microtitre plates (Nunc, Copenhagen, Denmark) were coated with 100 $\mu\text{g}/\text{ml}$ of protein in PBS by overnight incubation at 4°C . The plates were blocked by incubation for a further 30 min at 37°C with 2% bovine serum albumin (BSA; Sigma) in PBS. Doubling dilutions of mouse serum samples (derived from individual animals; starting dilution 1:32) diluted in 1% BSA in PBS were added to consecutive wells and incubated for 3 h at 4°C . There followed a further incubation for 2 h at 4°C with peroxidase-labelled sheep anti-mouse IgG (Serotec, Kidlington, UK) diluted 1:4000 or peroxidase-labelled rat monoclonal anti-mouse IgG1 (Serotec) diluted 1:1000 with 1% BSA in PBS. Enzyme substrate (*o*-phenylenediamine and urea hydrogen peroxide) was added and the reaction stopped after 15 min by the addition of 0.5 M citric acid. Between each incubation, the plates were washed with PBS containing 0.05% Tween 20. Substrate conversion was 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 nm}) was greater than 0.5. For control serum derived from naïve (untreated) animals OD_{450 nm} readings never exceeded this value even at the maximum concentration tested. Data are expressed as mean and SE of IgG and IgG1 reciprocal titre (log₂) for each treatment group.

2.6. Measurement of anti-protein IgE antibody

The presence in mouse serum of IgE antibodies was detected by homologous PCA assay. Due to limitations on the amount of serum available, IgE titres were 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 were injected (30 μl) into the dermis of the ears of naïve recipient mice ($n=4$). Concurrent negative control serum samples (derived from naïve animals) diluted to an equivalent extent with physiological saline were incorporated into every PCA assay. Two days later, 0.25 mg of relevant protein, together with Evans Blue dye (1.25 mg), in 0.25 ml of physiological saline were injected intravenously. Thirty minutes following challenge, mice were killed and the diameter of cutaneous reactions measured. A positive (IgE) response was recorded if the challenge resulted in a >3 mm blue lesion in the skin in the majority of recipient animals, with antibody titre recorded as the highest dilution of serum resulting in a positive PCA reaction.

2.7. Statistical analyses

It was assumed that the data were normally distributed. Following logarithmic transformation, the data were analyzed by two-sided Student's *t*-test. For IgG and IgG1 antibody responses, reciprocal titres for the individual serum samples per protein and per laboratory were compared. For IgE antibody responses, differences in reciprocal titres for the pooled serum samples between proteins (independent of laboratory) were compared.

3. Results

The ability of food proteins of varying allergenic potential to induce IgG and IgE antibody production was examined in two independent laboratories. Antibody responses provoked following exposure to the allergens OVA (2%) and peanut agglutinin (0.1%) have been compared with those induced by treatment with a material considered to be lacking in significant allergenic potential, a purified potato protein, potato agglutinin (5%). These concentrations of proteins were chosen on the basis of previous experience to be immunogenic, eliciting in each case substantial IgG antibody responses (Atherton et al., 2002; Dearman et al., 2002a; Kimber et al., 2003b). Furthermore, dose response analyses have revealed that for these three proteins a normal dose response curve is observed both with respect to IgG and IgE production, with low doses resulting in

decreased (or absent) antibody production (Dearman et al., accepted for publication). Groups of BALB/c mice ($n=5$) received 0.25 ml of test protein by intraperitoneal injection on days 0 and 7. Control mice were untreated or received an equivalent volume of PBS alone. In each of the two laboratories, two independent experiments were conducted in parallel, with the same proteins, at the same concentrations. Fourteen days after the initiation of exposure, animals were exsanguinated and individual serum samples prepared and analyzed by ELISA (starting dilution 1:32) for IgG antibody production. For each protein examined, negative controls (naïve serum samples and those derived from PBS-treated mice) were tested concurrently and were invariably negative. IgG antibody titres (mean and S.E.) are displayed for each of the two separate experiments conducted in the two laboratories (Fig. 1). In both laboratories, all three proteins induced vigorous specific IgG antibody production in all mice in every experiment, confirming the immunogenic potential of the doses of protein selected. There was very little inter-experimental variation in IgG antibody production within each protein treatment group, regardless of which laboratory the serum samples derived from. IgG antibody responses provoked by peanut agglutinin were somewhat more vigorous than those stimulated following exposure to OVA or potato agglutinin. Statistical comparisons (Student's *t*-test) revealed that there were no significant differences between the two laboratories with respect to IgG antibody responses provoked by administration of 0.1% peanut agglutinin, 2% OVA or 5% potato agglutinin.

Serial doubling dilutions of serum samples pooled for each treatment group from each independent experiment were analyzed by PCA assay for IgE antibody (Fig. 2). As reported previously from studies conducted exclusively in Laboratory B, intraperitoneal administration of the allergens peanut agglutinin and OVA induced high titre IgE antibody responses, whereas treatment with potato agglutinin failed to stimulate vigorous IgE antibody production (Atherton et al., 2002; Dearman et al., 2002a; Kimber et al., 2003b). There was very little inter-laboratory variation in the titres of IgE antibody induced by each of the three proteins in the two independent laboratories. Thus, in the two experiments conducted in both Laboratory A and B, titres of 1:32 and 1:64 were recorded following exposure to 0.1% peanut agglutinin. There was somewhat more inter-laboratory variation observed in anti-OVA IgE responses, with titres of 1:8 and 1:16 recorded for the two experiments performed in Laboratory A, whereas a titre of 1:32 was achieved for both of the studies conducted in Laboratory B. Despite the vigorous IgG antibody responses stimulated by exposure to 5% potato agglutinin (cf. Fig. 1), this protein failed to provoke marked IgE antibody production in any experiments. In both of the studies conducted in Laboratory A, IgE antibody titres of 1 were recorded (positive with neat serum only), whereas in Laboratory B, there was no detectable IgE antibody in either experiment (negative PCA assay with neat serum). Indeed, statistical comparisons across proteins revealed that against a background of equivalent immunogenicity (no significant differences between proteins with respect

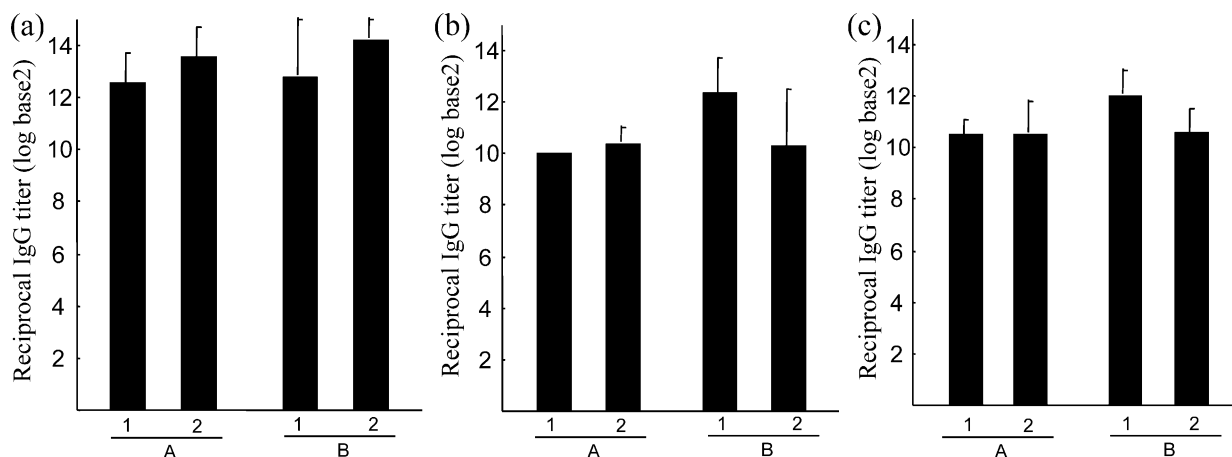


Fig. 1. IgG antibody responses following systemic exposure to 0.1% peanut agglutinin, 2% ovalbumin (OVA) or 5% potato agglutinin: inter-laboratory evaluations. Groups of mice ($n=5$) received 0.25 ml of (a) 0.1% peanut agglutinin, (b) 2% OVA or (c) 5% potato agglutinin by intraperitoneal injection on days 0 and 7. Two independent experiments 1 and 2 were performed in each of the two laboratories A and B. 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). Data are expressed as mean and SE of IgG reciprocal titre (log2) for each of the two independent experiments conducted in the two laboratories. For each protein, the statistical significance of differences in IgG titres between laboratories was compared by Student's *t*-test. There were no significant differences between laboratories for any of the three proteins examined.

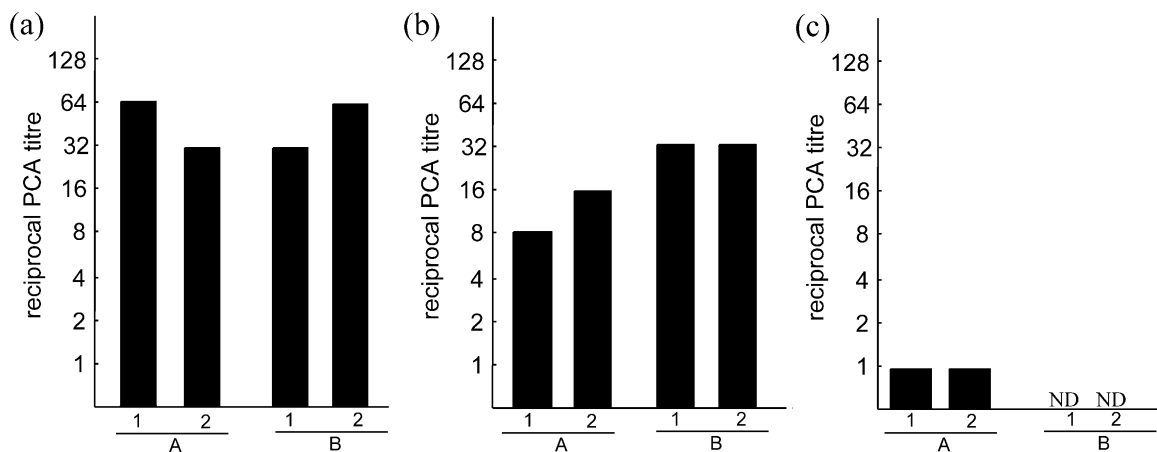


Fig. 2. IgE antibody responses following systemic exposure to 0.1% peanut agglutinin, 2% ovalbumin (OVA) or 5% potato agglutinin: inter-laboratory evaluations. Groups of mice ($n=5$) received 0.25 ml of (a) 0.1% peanut agglutinin, (b) 2% OVA or (c) 5% potato agglutinin by intra-peritoneal injection on days 0 and 7. Two independent experiments 1 and 2 were performed in each of the two laboratories A and B. Seven days later animals were exsanguinated, serum samples pooled per treatment group and tested for the presence of specific IgE antibody by homologous passive cutaneous anaphylaxis assay. Negative control serum samples (from naïve animals) were tested concurrently and were routinely negative (data not shown). Data are expressed as reciprocal IgE titre for each of the two independent experiments conducted in the two laboratories. ND=not detectable with neat serum.

to IgG antibody titres), intraperitoneal administration of 2% OVA to BALB/c strain mice resulted in significantly higher titre IgE antibody ($P<0.01$) than did identical exposure to 5% potato agglutinin. Significant differences in the ability of peanut agglutinin, compared with potato agglutinin, to stimulate IgE antibody were also recorded ($P<0.01$), although such were observed under conditions where significantly higher titres of IgG were found also ($P<0.01$).

In mice, it is well established that two kinds of homocytotropic (mast cell binding) antibody are produced which are both detectable in homologous PCA assay; IgG1 and IgE antibody (Inagaki et al., 1988; Lehrer and Vaughan, 1976). Although it has been demonstrated that these different classes of antibody possess different properties, the first being heat stable and active 2 h after injection whereas the latter is heat labile and active 48 h after injection (Lehrer and Vaughan, 1976), it was not possible (due to limitations on the availability of serum) to demonstrate routinely the heat lability of the PCA responses recorded. We have therefore also examined the ability of each of the proteins to induce specific IgG1 antibody production. Individual serum samples were analyzed by ELISA for IgG1 antibody expression. For each laboratory, the data from the two independent experiments have been combined and are displayed as mean (and SE) IgG1 titres for each protein for each laboratory in comparison with total specific IgG antibody titres (Fig. 3). Each protein when tested in both laboratories provoked substantial IgG1 antibody responses that in each case were of higher titre than the corresponding total IgG antibody responses, indicating that the detection antibody for the IgG1 ELISA has a higher sensitivity than does

the corresponding antibody in utilized in the ELISA for IgG. There were no significant differences observed between the two laboratories in either IgG or IgG1 antibody titres for each of the three proteins. Both IgG and IgG1 antibody responses induced by peanut agglutinin were significantly higher in titre ($P<0.01$) than those stimulated by exposure to either OVA or to potato agglutinin. It is of particular relevance, however, that there were no significant differences in either IgG or IgG1 antibody titres following administration of OVA and potato agglutinin, despite the fact that significantly higher titre IgE antibody was provoked by OVA compared with potato agglutinin ($P<0.01$).

4. Discussion

We have demonstrated previously (in experiments conducted exclusively in Laboratory B) that the measurement of IgE antibody responses in BALB/c strain mice shows considerable promise as a method for the identification of potential protein allergens, distinguishing them from those materials that apparently lack significant allergenic potential. An important step in the development and future wider acceptance of any toxicological test is to demonstrate the robustness of the approach and successful transfer of 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. Firstly, whether identical responses to the different proteins can be provoked in both laboratories, and secondly, whether the measurement of IgE by homologous PCA (an experimental technique that is

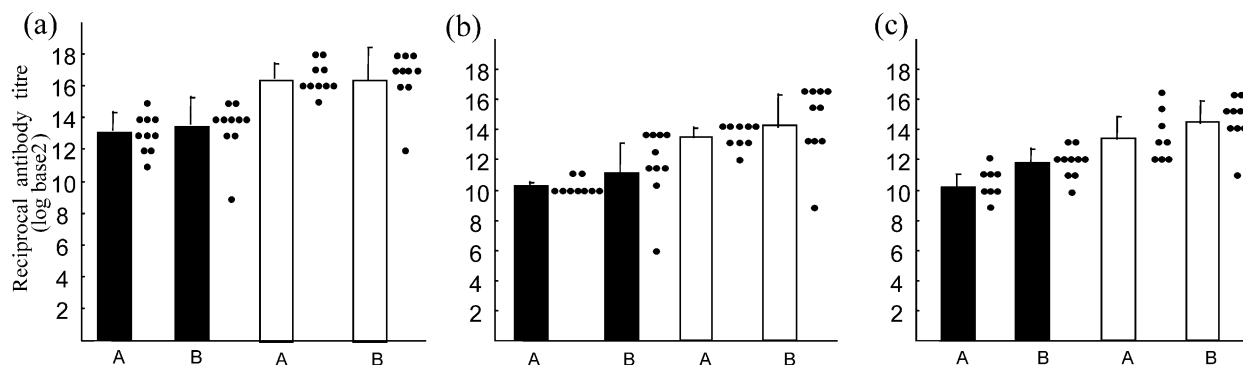


Fig. 3. IgG and IgG1 antibody responses following systemic exposure to 0.1% peanut agglutinin, 2% ovalbumin (OVA) or 5% potato agglutinin: inter-laboratory evaluations. Groups of mice ($n=5$) received 0.25 ml of (a) 0.1% peanut agglutinin, (b) 2% OVA or (c) 5% potato agglutinin by intraperitoneal injection on days 0 and 7. Two independent experiments 1 and 2 were performed in each of the two laboratories A and B. Seven days later animals were exsanguinated and individual serum samples tested for the presence of specific IgG and IgG1 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 (●) along with mean and SE of IgG (■) and IgG1 (□) reciprocal titres (log2) for each protein for the each of the two independent experiments conducted in the two laboratories. For each protein, the statistical significance of differences in IgG and IgG1 titres between laboratories was compared by Student's *t*-test. There were no significant differences between laboratories for any of the three proteins examined.

not trivial to master) yields equivalent results in both laboratories. In the first phase of this inter-laboratory collaboration we have chosen to separate these two aspects, in order to be able to differentiate between inter-laboratory differences in responses to the proteins, and potential differences in the measurement of such responses. We have therefore conducted two separate experiments using identical protocols in each of two independent laboratories A and B using three reference proteins, two known allergens (OVA and peanut agglutinin) and a protein considered to lack significant sensitizing potential, potato agglutinin. All serological analyses were conducted in the one of the two laboratories, Laboratory B, and employing the experimental design used here there was in fact insufficient serum available to be able to conduct such analyses in both laboratories.

Each of the three proteins induced vigorous IgG and IgG1 antibody responses, with no statistically significant differences in titres recorded between laboratories for each of the three proteins. Administration of peanut agglutinin and OVA each stimulated marked IgE antibody responses in every experiment. In the two laboratories, titres ranged from 1:32 and 1:64 for peanut agglutinin, and 1:8 and 1:32 for OVA. These IgE antibody responses are very similar to those recorded previously in repeat experiments conducted in Laboratory B. In each of three independent experiments, administration of 0.2% peanut agglutinin or of 2% OVA was characterized by high titre IgE antibody production, varying from 1:64 to 1:128, and from 1:32 to 1:64, respectively (Dearman et al., 2002a). 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. Furthermore, under these conditions where differential IgE antibody responses were stimulated, OVA and potato agglutinin induced responses of equivalent immunogenicity with respect to both IgG and IgG1 antibody titres. These very weak (or undetectable) IgE responses induced following exposure to relatively high concentrations of the negative control protein presumably reflect the sensitivity of the BALB/c strain mouse, as a high IgE responder strain, for the detection of IgE antibody production. These data provide additional evidence of the induction of divergent IgE antibody by food proteins of differing allergenic potential and indicate for the first time that the experimental approach is relatively robust and can be transferred successfully between laboratories. In subsequent investigations, the objective will be to examine whether the measurement of IgE by homologous PCA is a robust technique that can be transferred successfully between laboratories. As described above, the current strategy is to examine the immunogenicity (induction of IgG antibody responses) and allergenicity (induction of IgE antibody production) of each protein of interest following systemic exposure of BALB/c strain mice. There are reports of strain differences in immune responsiveness to particular proteins (Holt et al., 1981), so it is important to identify lack of allergenicity (IgE) against a background of immune recognition (IgG antibody production).

An issue that requires consideration is the selection of the end point used to determine allergenicity, the induction of specific IgE antibody as measured by homologous PCA assay. There is a requirement to measure specific IgE, rather than changes in the total serum concentration of this immunoglobulin because exposure to protein allergens does not appear to

increase total serum IgE levels (Hilton et al., 1994). Homologous or heterologous PCA assays are utilized by many investigators as there is agreement that the demonstration of biologically active IgE is important (Dearman et al., 2002a; Kimber et al., 2003a; Li et al., 1999, 2000). Other investigators use ELISA assays for the measurement of specific IgE; this technique analyses IgE content and does not address the biological activity of this class of immunoglobulin (Adel-Patient et al., 2000; Wang et al., 1999). In addition, as the concentration of serum IgE antibody is orders of magnitude lower than other antibody isotypes including IgG, when performing IgE ELISAs controls must be in place to prevent cross-reactivity of reagents with IgG or blocking effects of such antibody. These problems may be circumvented using a monoclonal anti-IgE capture antibody followed by the detection of antigen-specific IgE utilizing biotinylated or digoxigenin labelled antigen (Adel-Patient et al., 2000; Wang et al., 1999). This requirement for labelled antigen means that for each individual protein of interest, a separate ELISA protocol must be developed, including the preparation of a biotinylated or digoxigenin labelled antigen complex. These requirements do not lend themselves readily to the development of a routine predictive test. Another potential method for the measurement of IgE utilizes a rat basophilic leukemic cell line (RBL) that expresses Fc receptors for IgE and, once sensitized by protein-specific IgE, can be induced to degranulate by challenge with specific antigen, releasing radiolabelled serotonin. This method has been used to measure IgE antibodies induced by intranasal exposure to respiratory sensitizing detergent enzymes, but has not yet been validated for a wider range of proteins (Kawabata and Babcock, 1993). Indeed, we have some preliminary evidence that this method is unlikely to be suitable for the measurement of murine polyclonal IgE antibody responses as there appears to be a requirement for a minimum of 10 IgE binding epitopes per protein molecule for successful degranulation of the RBL cell (Dearman et al., 2002b).

As described above, we have elected to measure IgE anti-protein antibody as a function of mast cell binding in a homologous PCA assay. It is important to acknowledge that in mice, both IgG1 and IgE antibody isotypes are homocytotropic (binds to murine mast cells) (Inagaki et al., 1988; Lehrer and Vaughan, 1976). IgG1 and IgE antibodies can be distinguished on the basis of their differential heat stability and affinity for mast cells receptors (Lehrer and Vaughan, 1976). Not only do IgG1 antibodies dissociate more quickly than do IgE antibodies (active after 2 and 48 h, respectively) (Lehrer and Vaughan, 1976), but also the minimal amount of IgG1 antibody required to elicit an homologous PCA reaction is orders of magnitude higher than the minimal amount of IgE required (500–700 and 15 ng, respectively) (Ovary, 1982). The PCA protocol used in the

experiments described herein should be largely selective for murine IgE antibody, as a 48 h incubation step is utilized. Furthermore, we have confirmed that differential IgE responses are induced under conditions where equivalent IgG1 responses are stimulated by measuring IgG1 antibody expression by ELISA.

The purpose of the experiments described herein was to assess the intrinsic allergenic potential of proteins, identified as a function of the ability of the protein to induce IgE antibody production as part of an inter-laboratory collaboration. Although it could be argued that all proteins are potential allergens, if appropriate levels and conditions of exposure were achieved, in practice this appears not to be the case. Only a small proportion of the food proteins consumed regularly is associated with allergic disease, with most cases of food allergy in the USA and Western Europe associated with a relatively limited range of produce; most commonly peanuts, tree nuts, hens' egg, cows' milk, wheat, soybeans, fish and shellfish (Bush and Hefle, 1996; Hefle et al., 1996; Sampson, 1988; Young et al., 1994). These observations suggest that there is indeed a spectrum of allergenicity among food proteins. In common with other forms of atopic disease, the pathophysiology of food allergy is complex and inter-individual differences in susceptibility will be affected by a combination of exposure conditions (including age of first exposure, and the route, duration and magnitude of exposure), environmental factors, and genetic predisposition (Kimber and Dearman, 2001b). It is generally considered, therefore, that it will not prove possible to define a method using experimental animals that will accurately predict all aspects of the likely prevalence, persistence and severity of food allergy among human populations exposed to a novel allergen in the diet. Although this is undoubtedly true, the purpose of the approach described herein, and indeed the first step of any safety assessment process, is to identify accurately intrinsic hazard, or lack of it. In this case, the intrinsic hazard is identified as a function of the inherent potential of a protein to induce IgE antibody production; the next steps in the risk assessment process will be to characterize the nature of the hazard and to define potential risks to humans based on likely exposure patterns.

In conclusion, the data presented here suggest that the measurement of antibody (IgE) responses in BALB/c mice appears to identify allergens accurately and to distinguish them from those materials that apparently lack allergenicity. It must be acknowledged, however, that to date these studies have been conducted with a limited number of proteins and that further experience with a wider range of proteins of differing allergenic potential is required. Furthermore, these results demonstrate that this approach is relatively robust and can be transferred successfully between laboratories. Experience to date suggests that the measurement of antibody (IgE)

responses in BALB/c mice shows some promise for the discrimination between allergens and those materials that apparently lack allergenicity.

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