

Inter-laboratory comparisons of assessment of the allergenic potential of proteins in mice

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ABSTRACT: Assessment of the potential allergenicity of novel proteins, including those expressed in genetically modified plants, is an important issue. In previous studies, we have shown that the IgE measurement induced by systemic exposure of BALB/c mice to a range of proteins correlates broadly with what is known of their allergenic potential in humans. The approach used a homologous passive cutaneous anaphylaxis (PCA) assay that reflects IgE-dependent biological activity and is of sufficient sensitivity to detect IgE production in the absence of adjuvant. In previous studies, the immunization phase was conducted independently in two separate facilities, and the subsequent analytical work (PCA) conducted in a single facility. The purpose here was to further evaluate the transferability of this approach. To this end, BALB/c mice were exposed to a range of doses of peanut agglutinin or ovalbumin, allergenic proteins of peanut and hen's egg, respectively, in two independent laboratories. Serial doubling dilutions of serum pooled for each treatment group were analyzed for specific IgE. At higher doses of allergen very similar, or identical, IgE titers were achieved in both laboratories, although at lower doses, responses were somewhat more variable. These data demonstrate that, although technically demanding, the measurement of protein allergen-induced IgE antibody production in mice using PCA is relatively robust and is transferable and reproducible between laboratories. This approach may provide a useful tool for the safety assessment of novel proteins and suggests that continued evaluation of the approach with a wider range of protein allergens and non-sensitising proteins is justified. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: protein allergenicity; IgE; IgG; systemic exposure; inter-laboratory comparisons; PCA

Introduction

Anaphylaxis mediated by IgE antibody is a life-threatening allergic reaction, and food is one of the most common responsible allergens in the outpatient setting (Wang and Sampson, 2007). Other symptoms of food allergy include nausea and vomiting, skin symptoms such as acute urticaria and angioedema, and may involve the respiratory tract causing allergic rhinitis and asthma (Sampson, 1999). Thus food allergy is an important human health issue. The possibility that novel proteins might be allergenic, particularly those derived from genetically modified (GM) plants, was recognized in the early 1990s. In 1996, the International Food Biotechnology Council (IFBiC), the International Life Sciences Institute (ILSI) and the Allergy and Immunology Institute (Metcalfe *et al.*, 1996) jointly developed the first structured approach for assessing the allergenic potential of novel proteins. This approach, which took the form of a decision tree, was mainly focused on the source of the gene, biochemical characterization of the protein (including resistance to digestion in simulated gastric fluid), homology searches to known allergens and serum screening tests. Although this scheme provides reasonable assurance that the protein is not similar to known allergens, inherent sensitizing potential is not evaluated directly. This has led to the suggestion that the development of appropriate animal models may provide additional valuable information regarding novel sensitizing potential.

Such a conclusion was reached in 2001, when an Expert Consultation of the Food and Agriculture Organization (FAO) and World Health Organization (WHO) (2001) proposed possible revisions to the original IFBiC/ILSI decision tree. More recently,

the experts on the *Codex Alimentarius* Commission (2003) expressed their interest in the development of *in vivo* models for assessing the allergenicity potential of novel proteins as part of the assessment strategy. In parallel, the use of such models has been encouraged worldwide by scientists and regulators. For instance, the scientific panel from the European Food Safety Authority (2006) stated that:

"... animal models are certainly also useful tools for the assessment of the sensitizing potential of newly expressed proteins, i.e. their capacity to induce an allergic immune response with the synthesis of specific IgE in individuals that have never been exposed to those proteins nor to proteins that cross react with them. The development of animal models should be encouraged and, once validated, their use may increase the body of evidence to support a conclusion..."

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The use of several different species has been proposed, including rats (Knippels *et al.*, 1998), dogs (Ermel *et al.*, 1997), swine (Helm *et al.*, 2002) and mice (Adel-Patient *et al.*, 2000; Li *et al.*, 2000; Yano *et al.*, 2002; Kozai *et al.*, 2006; Dearman and Kimber, 2007; Bowman and Selgrade, 2008). The mouse models offer considerable advantages compared with other animal models. In particular, a variety of inbred and congenic high-IgE responder strains, as well as various immunological and molecular reagents, are available commercially. Moreover, epitope similarities between mouse and human suggest that the mouse may represent a valid model for the understanding of human food allergy (McClain and Bannon, 2006). The method that is explored herein relies on the measurement of IgG specific antibodies as a marker of immunogenicity and of IgE-specific antibodies as a marker of allergenicity. This experimental approach is designed to provide maximal specificity and sensitivity for the purposes of hazard identification. To this end, BALB/c mice were used, this strain being predisposed to mount vigorous IgE antibody responses (Dearman *et al.*, 2003a, b; Dearman and Kimber, 2007). In order to avoid the possibility of tolerance induction associated with oral administration, exposure was systemic (intraperitoneal injection). In order to reflect accurately the inherent immunologic and allergenic properties of test proteins, exposure was performed in the absence of adjuvant. If such a method is to provide for a test for assessment of allergenic potential, then it is necessary to demonstrate that the assay will yield equivalent results when conducted in independent laboratories. In previous studies we have shown that the immunization phase (production of serum antibodies) can be transferred successfully between laboratories (Dearman *et al.*, 2003b). In two independent laboratories, equivalent IgG and IgE antibody responses were generated following exposure of BALB/c strain mice to reference allergens ovalbumin (OVA) and peanut agglutinin or to the non-allergen potato agglutinin (Dearman *et al.*, 2003b). In these experiments, the analytical methods [specific IgG antibody by enzyme-linked immunosorbent assay (ELISA) and specific IgE antibody by homologous passive cutaneous anaphylaxis (PCA) assay] were conducted in one laboratory in order to distinguish between inter-laboratory differences in responses to the proteins *per se*, and potential differences resulting from variations in analytical method. In the current investigation, we have focused on the transferability of the analytical method for specific IgE antibody, the homologous PCA assay, which is known to be technically challenging. IgE antibody is measured as a function of the ability of the serum to transfer immediate-type hypersensitivity reactions to the skin of naive recipient mice, requiring intradermal injection of small volumes of serum into the dorsa of the ears (Dearman and Kimber, 2007). Serum samples derived following immunization of BALB/c strain mice with various concentrations of the reference allergens OVA or peanut agglutinin were pooled on a treatment group basis, aliquotted and analyzed for specific serum IgE antibody in two independent laboratories.

Materials and Methods

Participating Laboratories

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

Animals

Young adult (8–12 weeks old) female BALB/c strain mice (Laboratory A, Charles River, 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 (Scientific Animal Food and Engineering, formerly named Usine d'Alimentation Rationnelle, Augy, 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, UK). 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 du Journal Officiel des Communautés Européennes* (L358, 18 December 1986, no. 86/609/EEC 24 November 1986) for Laboratory A and the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health, 1985) for Laboratory B.

Test Materials

Commercial preparations of ovalbumin (OVA; Grade V) and peanut agglutinin (*Arachis hypogea*) were obtained from Sigma Chemical Co., St Louis, MO, USA. They were highly purified (>99%). In particular, the endotoxins were removed and their absence was confirmed by a Limulus Amebocyte Lysate assay (QCL-1000 kit, BioWhittaker/Cambrex Corporation).

Sensitization Procedure

The concentrations of proteins used in these experiments (0.2, 1 or 2% OVA or 0.01, 0.05, 0.1% peanut agglutinin) were chosen on the basis of the results obtained in previous experiments (Dearman *et al.*, 2003a, b). Groups of five mice received 0.25 ml of proteins in phosphate-buffered saline (PBS) by intraperitoneal injection. Treatment was repeated 7 days later. Mice were exsanguinated 14 days after the initiation of exposure. Individual and pooled serum samples were prepared and stored at –20 °C until analysis.

Anti-Protein IgG Antibody Analysis

Protein-specific IgG antibodies were detected by ELISA. Plastic microtiter plates (Nunc, Copenhagen, Denmark) were coated with 100 µg/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) diluted in 1% BSA in PBS were added to consecutive wells and incubated for 1 h at 37 °C in Laboratory A or for 3 h at 4 °C in Laboratory B. This was followed by a further incubation for 2 h at 4 °C with peroxidase-labelled sheep anti-mouse IgG (Serotec, Kidlington, UK) diluted 1:4000 with 1% BSA in PBS. Enzyme substrate (o-phenylenediamine and hydrogen peroxide) was added and the reaction stopped after 15 min by the addition of 0.5 M citric acid. Between incubations, the plates were washed with PBS containing 0.05% Tween 20. Substrate conversion was measured as optical density at 450 nm (optical density, OD, 450 nm) using an automated reader (Flow Laboratories, Irvine, UK). Antibody titers were calculated as the highest

dilution at which substrate conversion (OD 450 nm) was greater than 0.5. For control serum derived from naive (untreated) animals OD 450 nm readings never exceeded this value even at the maximum concentration tested. Data are expressed as reciprocal titer (log₂) for each individual serum sample.

Measurement of Anti-Protein IgE Antibody

The presence of IgE antibodies in mouse serum was detected by homologous PCA assay. Owing to limitations on the amount of serum available, IgE titers were determined using serum samples pooled on a treatment group basis. In some treatment groups, this may comprise both IgE negative and positive individual serum samples. Pooled serum samples neat or diluted to various extents with PBS were injected (30 μ l) into the dermis of the ears of naive recipient mice ($n = 4$). Concurrent negative control serum samples (derived from naive animals), diluted with PBS, were incorporated into every PCA assay. Two days later, the appropriate quantity of the relevant protein (OVA or peanut agglutinin), together with Evans Blue dye (1.25 mg), in 0.25 ml of physiological saline was injected intravenously. Thirty minutes

following challenge, mice were sacrificed and the diameter of cutaneous reactions in the ears was measured. A positive IgE response was recorded at a given serum dilution if the challenge resulted in a 3 mm or greater blue lesion in the skin of 50% or more of recipient animals and the antibody titer was recorded as the highest dilution of serum resulting in a positive PCA reaction.

Results

Concentrations of OVA (2, 1 and 0.2%) and peanut agglutinin (0.1, 0.05 and 0.01%) were chosen that had been shown previously to stimulate specific IgE antibody production, ranging from relatively low titer (1 in 2) to high titer (1 in 64) responses (Dearman *et al.*, 2003a, b). In two independent experiments, groups of 5 BALB/c mice received various concentrations of protein by intraperitoneal injection on days 0 and 7. Fourteen days after the initiation of exposure, individual serum samples were prepared and aliquotted with some aliquots used to prepare serum pools on a treatment group basis. Individual serum samples were analyzed for specific IgG antibody by ELISA in Laboratory B (Fig. 1). For each protein examined, negative controls

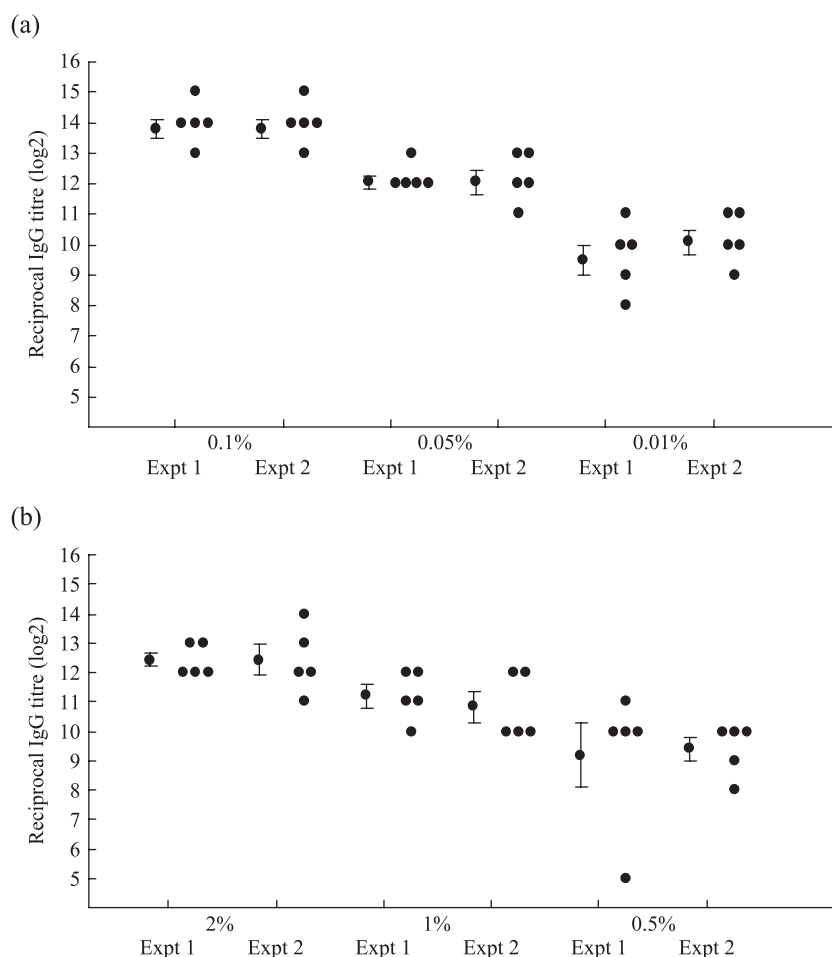


Figure 1. Specific IgG antibody responses induced by OVA and peanut agglutinin: dose-response analyses. Groups of mice ($n = 5$) received 0.25 ml of (a) 0.1, 0.05 or 0.01% peanut agglutinin or (b) 2, 1 or 0.2% OVA by intraperitoneal injection on days 0 and 7. Two independent experiments 1 and 2 were performed. Seven days later, animals were exsanguinated and individual serum samples were tested for the presence of specific IgG antibody by enzyme-linked immunosorbent assay in Laboratory B. Negative control serum samples (from naive animals) were tested concurrently and were routinely negative (data not shown). Data are expressed as IgG reciprocal titer (log₂) for individual serum samples (●) with mean and SE of IgG for the two independent experiments displayed.

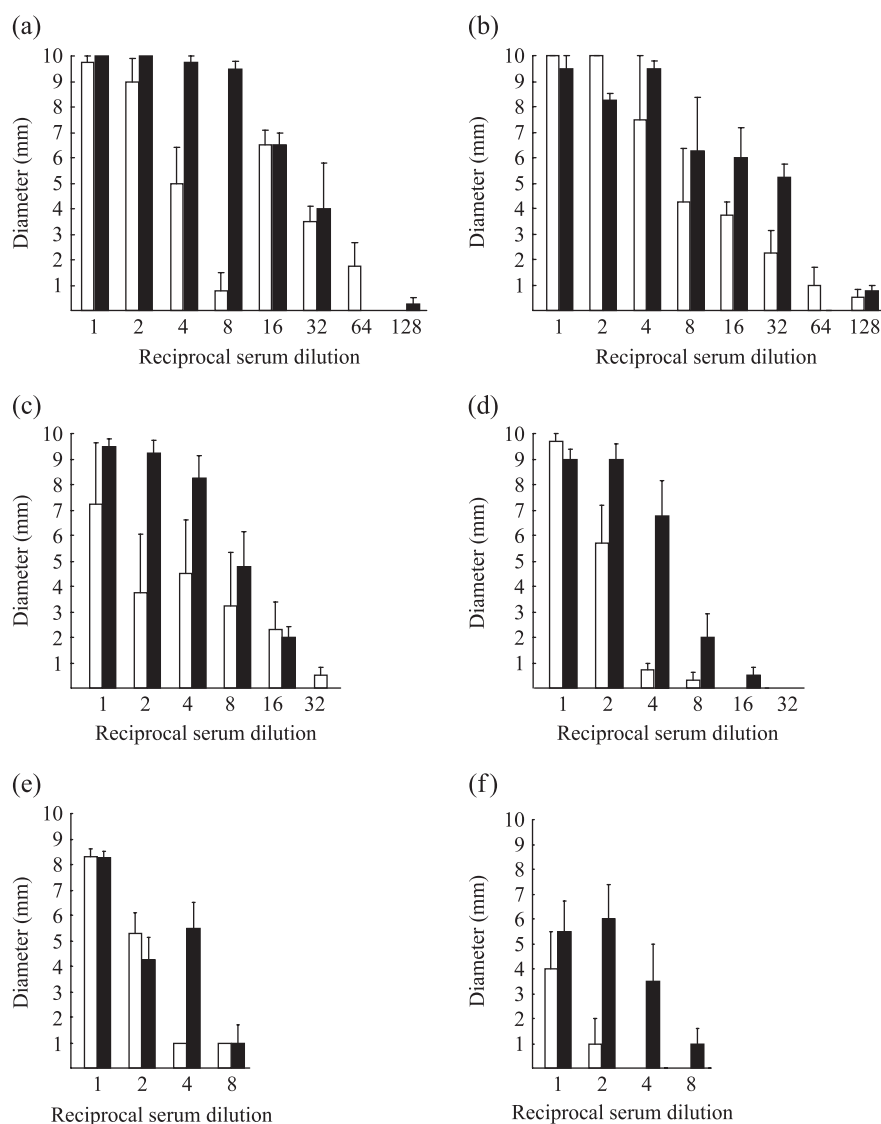


Figure 2. Measurement of OVA-specific IgE antibody responses: inter-laboratory variation. Serum samples from mice exposed to 2% OVA (a and b), 1% OVA (c and d) and 0.2% OVA (e and f) were pooled on a treatment group basis. Naive recipient mice ($n = 4$) received intradermal injections into the dorsum of both ears of various concentrations of sera diluted in PBS (ranging from neat to 1 in 128 dilution). Control intradermal injections of naive mouse serum were tested concurrently and were routinely negative (data not shown). The mean and SE diameter (mm) of PCA reactions induced in individual animals are displayed for each serum dilution determined in Laboratory A (□) and Laboratory B (■) for experiments 1 (a, c and e) and 2 (b, d and f).

(naive serum samples) were invariably negative (data not shown). In accordance with results obtained in previous experiments (Dearman *et al.*, 2003b), the two proteins induced specific IgG antibody production in a dose-dependent fashion in both experiments, confirming the immunogenic potential of the selected proteins at the doses tested. There was little inter-experimental variation in IgG antibody production within each protein treatment group. IgG antibody responses provoked by peanut agglutinin were somewhat more vigorous than those stimulated following exposure to OVA. Comparisons revealed that there were no differences between the two experiments with respect to IgG antibody responses provoked by administration of 0.2, 1 or 2% OVA or 0.01, 0.05, 0.1% peanut agglutinin. Furthermore, the titers observed following treatment with 2% OVA or 0.1% peanut agglutinin were similar to

those observed in the previous experiments (Dearman *et al.*, 2003a, b).

Subsequently, pooled serum samples derived from the same two independent experiments were analyzed for specific IgE content by homologous PCA assay in both Laboratory A and Laboratory B (Figs 2 and 3). A range of serial doubling dilutions from neat to 1 in 128 were used, with the full range of dilutions used for serum samples isolated from mice exposed to high concentrations of OVA and peanut and a smaller dilution range utilized for low dose OVA and peanut samples. Each dilution of serum was injected into the ear of four recipient mice and following challenge with protein, the diameter of the skin reaction was recorded. Results are displayed as the mean and SE of the diameter of the cutaneous response for each serum dilution as recorded in both of the two independent laboratories for sera

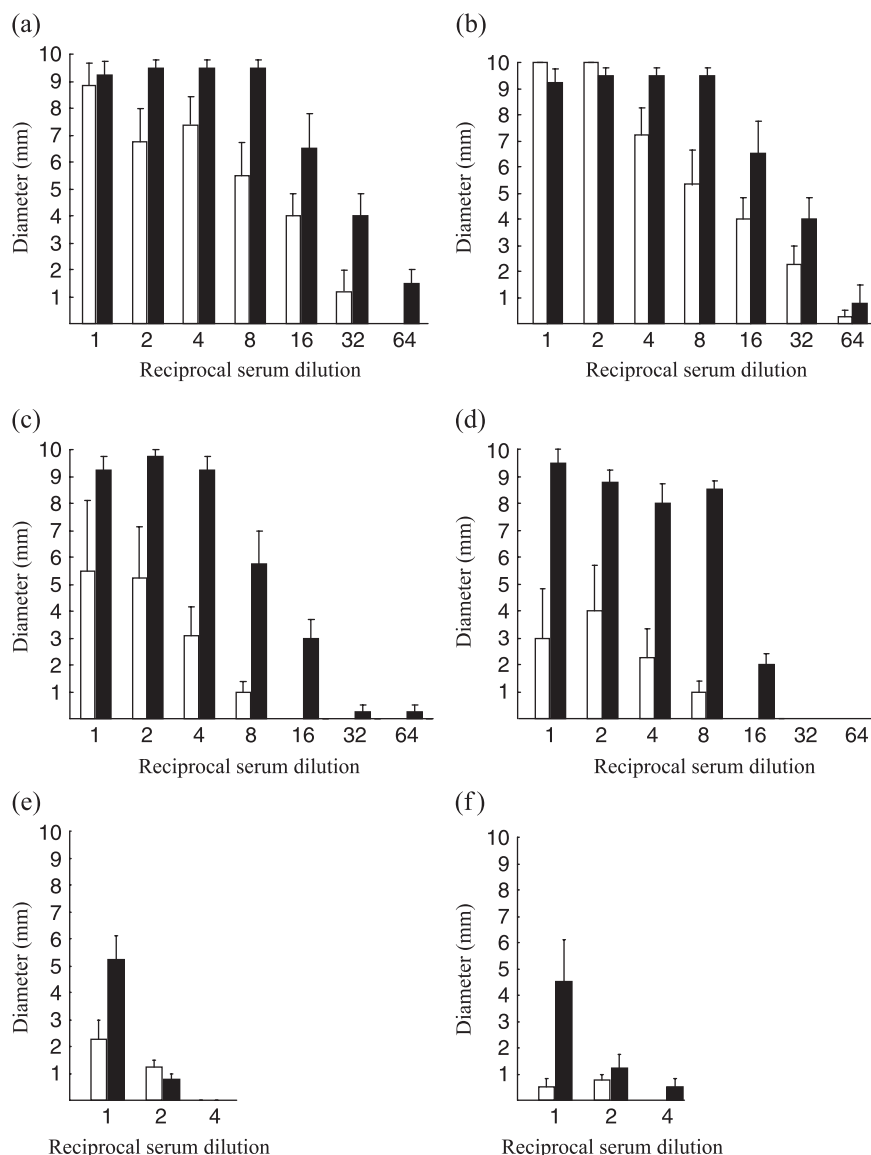


Figure 3. Measurement of peanut agglutinin-specific IgE antibody responses: inter-laboratory variation. Serum samples from mice exposed to 0.1% peanut agglutinin (a and b), 0.05% peanut agglutinin (c and d) and 0.01% peanut agglutinin (e and f) were pooled on a treatment group basis. Naive recipient mice ($n = 4$) received intradermal injections into the dorsum of both ears of various concentrations of sera diluted in PBS (ranging from neat to 1 in 128 dilution). Control intradermal injections of naive mouse serum were tested concurrently and were routinely negative (data not shown). The mean and SE diameter (mm) of PCA reactions induced in individual animals are displayed for each serum dilution determined in Laboratory A (□) and Laboratory B (■) for experiments 1 (a, c and e) and 2 (b, d and f).

derived from OVA (Fig. 2) and peanut agglutinin-treated animals (Fig. 3). Very vigorous IgE antibody responses were detected in serum derived from mice treated with 2% OVA, with maximal responses (approximately 10 mm diameters) recorded in both laboratories for both experiments with neat serum and serum diluted 1 in 2 [Fig. 2(a, b)]. In three out of four analyses, a marked dose-response relationship was observed with IgE responses reaching threshold levels (3 mm diameters) at a dilution of 1 in 32. In the remaining analysis (Laboratory A, experiment 1), positive IgE responses were detected at serum dilutions of ranging from neat to 1 in 32, with the exception of the 1 in 8 serum dilution, which was below the threshold of positivity. Less vigorous responses were recorded for serum derived from animals

exposed to 1% OVA, with relatively strong reactions recorded for dilutions from neat to 1 in 8 and approaching threshold levels at the 1 in 16 dilution. Somewhat lower diameters were recorded with the same sera analyzed in Laboratory A, and such were also more variable. Titers of 1 in 16 and 1 in 2 were achieved in Laboratory A, with titers of 1 in 8 and 1 in 16 observed for the same sera in Laboratory B. Considerably weaker dermal reactions were provoked with serum derived from animals exposed to the low dose OVA (0.2%) in both laboratories. Values remained above the threshold for dilutions down to 1 in 4 (titers of 1 in 4 for both experiments) for Laboratory B, whereas in Laboratory A the same samples titrated out more rapidly [titers of 1 (positive with neat serum only) and 1 in 2 achieved].

A similar pattern of results was observed for sera derived from mice treated with peanut agglutinin (Fig. 3). In both laboratories and for both experiments, high-dose peanut agglutinin (0.1%) resulted in maximal IgE responses (approximately 10 mm diameters) at serum dilutions from neat to 1 in 4 (and to 1 in 8 in Laboratory B). Weaker but positive reactions were recorded in both laboratories at serum dilutions of 1 in 32 and 1 in 16, resulting in titers of 1 in 32 recorded for both experiments in both laboratories. At the mid dose of 0.05% peanut agglutinin, serum samples titrated out more rapidly and only Laboratory B achieved maximal skin reactions (diameters of 10 mm) at dilutions from neat down to 1 in 8 or 1 in 4. Titres of 1 in 4 and 1 in 4, and 1 in 8 and 1 in 16 were recorded in Laboratories A and B respectively. The lowest dose of peanut agglutinin (0.01%) stimulated relatively weak IgE responses, with neither laboratory recording maximal dermal reactions at any serum concentration tested. In Laboratory A, administration of neat serum in only one out of the two experiments resulted in a positive dermal response being recorded [Fig. 3(e, f)]. Thus titers of 1 (positive with neat serum) and 0 (not detectable with neat serum) were recorded for Laboratory A, whereas titers of 1 were observed in both experiments in Laboratory B. In general, somewhat higher titers of IgE were recorded in Laboratory B than in Laboratory A, and the extent of intra- and inter-experimental variation was higher in Laboratory A.

Discussion

The purpose of the current investigations was to examine the reliability of an approach for the prospective identification of protein with allergenic potential. In a previous publication, the focus was on the transferability of the immunization phase of the experimental method (Dearman *et al.*, 2003b). Mice were exposed by intraperitoneal injection to the allergens OVA and peanut agglutinin and to the non-allergen potato agglutinin in two independent laboratories and the sera analyzed for specific IgG and IgE antibody in one laboratory. These data demonstrated that very similar responses to the different proteins were elicited in both laboratories, indicating that the allergen-selective induction of IgE antibody is a relatively robust phenomenon (Dearman *et al.*, 2003b). In the current series of experiments, the intra- and inter-laboratory reproducibility of the analytical phase of the approach was evaluated. In this context, the important question was whether the measurement of IgE by homologous PCA, a technically challenging method, would yield equivalent results in both laboratories.

In initial experiments, it was confirmed that the selected doses of the two allergens induced vigorous dose-dependent IgG antibody responses, with no differences in titers recorded between experiments. Indeed, the results were similar to those observed in previous experiments (Dearman *et al.*, 2003a, b) and confirmed that the mice have been exposed to sufficient test proteins to stimulate the immune system. The same sera samples were utilized for measurement of specific IgE responses by homologous PCA assay in both laboratories. Although this method is known to be technically challenging, it is also recognized to be accurate for detecting specific biologically active IgE responses (Hilton *et al.*, 1994; Li *et al.*, 2000; Dearman *et al.*, 2002; Kimber *et al.*, 2003). In the current investigations, administration of high-dose peanut agglutinin (0.1%) or OVA (2%) stimulated marked and very similar IgE antibody responses in both experiments. At the mid and low doses of allergen (1 and 0.2% OVA and 0.05 and 0.01% peanut

agglutinin), somewhat more variation was observed in IgE titers, particularly between laboratories, although both laboratories still identified these doses of protein as allergenic (inducing detectable IgE) with the exception of one experiment in Laboratory A at the lowest dose of peanut agglutinin tested. In general, Laboratory B apparently had a higher sensitivity for IgE detection than did Laboratory A, with higher titers and less inter-animal variation recorded, particularly at low-dose allergen. However, at the higher doses, this variation resulted in only one doubling dilution difference in titers. The differences between laboratories in sensitivity may be mainly related to the fact that the mice used as recipients in the PCA assay are from different suppliers and may vary in sensitivity due to mast cell numbers or histamine sensitivity. This could also be attributed to other experimental conditions as the diet compositions, which are different in the two laboratories. However, the larger extent of inter-animal variation observed in Laboratory A compared with Laboratory B and the fact that the differences were more apparent in serum with low levels of IgE suggest that some of the variation may be due to the fact that Laboratory B has had considerably more experience in performing the assay.

Taken together, these data suggest that the measurement of IgE antibody by PCA assay is a relatively robust test and is transferable between laboratories provided there is adequate training. This confirms that the differences with regard to the animal supplier, exposure to different diets and other environmental conditions as well as technical personnel have only a relatively minor impact on the results. Given that the assay is technically demanding and requires some investment in the training of personnel (Dearman and Kimber, 2007) and furthermore requires the use of recipient animals, it is appropriate to give some consideration as to whether there are other methods that could be employed for the measurement of specific IgE. There are various methods available; however, whichever one is employed, the primary technical hurdle that must be surmounted is that of competition with specific IgG antibody that generally exceeds IgE antibody concentrations by at least 3-log orders of magnitude (Plebani *et al.*, 1986; Wachholz *et al.*, 2004). Provision must be made against the blocking effects of IgG antibody, leading to potential underestimation of IgE antibody, and the cross reactivity of anti-IgE antibody reagents with IgG antibody, leading to false positive results.

Standard ELISA-based assays are generally inappropriate for this purpose due to the relatively limited amount of allergen available as capture molecules for IgE detection, such that the excess of allergen-specific IgG in sera results in competition for allergen binding and therefore compromises the sensitivity of the assay. IgE capture ELISA techniques can be used to concentrate IgE antibody, but to determine specific IgE antibody such method must be used in conjunction with allergen labeled with biotin or enzyme (Plebani *et al.*, 1986; Wachholz *et al.*, 2004). This approach requires that, for each allergen, a separate labeled allergen preparation must be synthesized. Another issue with ELISA methodologies is that they often fail to correlate with tests that measure the biological activity of specific IgE (Niederberger *et al.*, 2001; Saevik *et al.*, 2003). This emphasizes the importance of assessing biological activity as well as quantity of allergen-specific IgE, such as is measured in the PCA (Lehrer and Vaughan, 1976; Dearman *et al.*, 2003a, b).

The principle of the PCA assay is that serum is administered intradermally and any IgE antibody present in the transferred

serum will bind to FcεR on the surface of tissue mast cells in the skin of the host animals. Recipient animals are then challenged with an intravenous injection of the test protein together with Evans Blue dye. If IgE antibody specific for the test protein is present then it will be crosslinked, causing the local degranulation of mast cells and the release of inflammatory mediators, including agents that cause vasodilation. In such circumstances, the blue dye injected with the protein antigen is able to extravasate, resulting in a blueing of the skin, which is the end point measured in this assay. In mice, both IgG1 and IgE antibody isotypes are homocytotropic, i.e. bind murine mast cells; Lehrer and Vaughan, 1976; Baeza and Zubeldia, 2007). However, mast cell binding IgG1 and IgE antibodies have differential affinity for mast cells receptors with only IgE antibodies remaining bound after 48 h, the incubation time of the assay, ensuring the specificity of the method (Lehrer and Vaughan, 1976). More recent studies have therefore focused on using allergen-induced activation of these IgE-bearing cells as surrogate *in vitro* assays to detect biological activity of allergen-specific IgE. One such method is the rat basophilic leukemia cell (RBL) assay. The RBL cell line possesses FcεR, which can selectively bind rat or murine IgE antibody and can be stimulated by crosslinking with protein allergen to degranulate, releasing granules, which have been preloaded with radiolabeled serotonin. The relative sensitivity of the RBL assay, and of the homologous PCA assay for the measurement of specific IgE antibody has been investigated (Dearman *et al.*, 2005). Polyclonal anti-OVA IgE antisera raised in the presence or absence of adjuvant were also assessed for activity in the RBL assay. The RBL assay was unable to detect polyclonal antibodies that have low affinity or that have a low epitope density, and thus lack the necessary sensitivity for the detection of anti-protein antibodies derived in the absence of adjuvant. These data demonstrated that there are serious limitations for the use of the RBL assay as an *in vitro* replacement for the PCA assay as it is insufficiently sensitive for the routine analysis of specific polyclonal IgE antibody responses such as those that are induced by exposure to complex protein allergens (Dearman *et al.*, 2005).

Although only a relatively limited number of proteins have been examined to date, experience suggests that the measurement of IgE antibody responses in BALB/c mice serves to identify food allergens accurately, and to distinguish them from those materials that apparently lack significant allergenic potential. Furthermore, the results of the current series of experiments reveal that the methodology is robust and transferable between laboratories. The development of an effective animal model that mimics all aspects of human food allergy is challenging, because of the complexity of food allergy, the causes of which are multifactorial and poorly understood. A number of factors are known to influence the susceptibility to the development of food allergies. Such include the age and strain of animals, route of exposure, dose and frequency of antigen administration (McClain and Bannon, 2006). Continuing improvements in this animal model will provide higher confidence in its potential applicability. In particular, the sensitivity and selectivity of this approach should be further investigated in greater detail using a wider range of allergenic and non-allergenic proteins. Although it is generally considered that a single *in vivo* model will not predict all aspects of the food allergy among human populations exposed to a novel allergen in the diet, this animal model could be useful as a general screening tool for characterizing and identifying intrinsic hazard, or lack of it.

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