

Determination of protein allergenicity: studies in mice

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Abstract

There is a need to identify and characterize the allergenic potential of novel proteins introduced into genetically-modified crop plants. Although several approaches have already been described, none of these measures directly the ability of proteins to cause allergic sensitization. For this reason there has been a growing interest in the development of suitable animal models. This article describes experience to date with a method based upon assessment of serological (IgG and IgE antibody) responses induced in BALB/c strain mice by proteins. Comparisons have been made between intraperitoneal (i.p.) administration and exposure by gavage using both allergenic and non-allergenic proteins. The available data indicate that responses provoked by i.p. exposure permit the identification of proteins that have the inherent potential to induce IgE antibody production and allergic sensitization. Moreover, this approach also provides a rank order of proteins with respect to allergenic potency that apparently reflects what is known of their relative sensitizing activity in humans. By comparison, oral exposure of mice by gavage is somewhat less sensitive. On this basis it is proposed that the inherent sensitizing potential of novel proteins can be evaluated as a function of IgE antibody responses stimulated by parenteral (i.p.) exposure of BALB/c mice. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

With the development of genetically-modified crop plants there has been a growing interest in the approaches available to assess the potential allergenicity of novel gene products (Astwood et al., 1996; Kimber et al., 1997, 1999, 2000; Taylor, 1997; Wal and Pascal, 1998). To date the only systematic approach to be described resulted from

a collaboration between the International Life Sciences Institute (ILSI) Allergy and Immunology Institute and the International Food Biotechnology Council (IFBC) (Metcalf et al., 1996). What was proposed is a hierarchical testing strategy which incorporates consideration of the serological identity of the protein of interest with known human allergens, examination of amino acid homology with, and/or structural similarity to, allergenic proteins and measurement of the stability (digestibility) of the protein in a simulated gastric fluid (SGF) (Astwood et al., 1996; Metcalf et al., 1996; Gendel, 1998). It was recognized at the time that there were available no suitable animal mod-

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els that could be used in concert with the approaches summarized above. Such models would be of considerable benefit if they facilitated a more direct evaluation of the ability of proteins to induce allergic responses. Progress in this area has developed along a number of fronts and methods have been described based upon measurement of serological responses induced in rats, either in the presence or absence of adjuvant, following oral (gavage) or intraperitoneal (i.p.) administration of protein (Atkinson and Miller, 1994; Atkinson et al., 1996; Houben et al., 1997; Knippels et al., 1998, 1999, 2000; Miller et al., 1999).

In our laboratory, we have developed an alternative approach where the allergenic potential of test proteins is evaluated as a function of serological responses (IgG and IgE antibody production) elicited in BALB/c strain mice. Attention to date has focused largely upon characterization of humoral immune responses provoked by i.p. administration of protein in the absence of adjuvant. Employing this protocol, we have shown previously consistent differences in the vigour of specific IgE antibody responses induced in mice by ovalbumin (OVA), a potent respiratory and food allergen (Metcalf, 1985), and by bovine serum albumin (BSA), a protein that is considered to have a lesser allergenic potential (Hilton et al., 1994, 1997; Dearman et al., 2000).

There is currently some debate regarding the most appropriate route or routes of exposure for the purposes of hazard identification and characterization. Although in theory oral administration appears the most attractive and relevant, it is well known that exposure of rodents to proteins in this way can result in immunological tolerance. It is probably for this reason that it has been reported recently that gavage exposure is necessary for the stimulation of IgE antibody responses in rats. Exposure of rats to the same protein *ad libitum* in drinking water failed to stimulate IgE antibody production (Knippels et al., 1998). While there is no doubt that parenteral (i.p.) administration of protein avoids the development of oral tolerance and provides a clear indication of the inherent ability of proteins to induce IgE antibody responses, it must be acknowledged that the stimulation of IgE responses via this route of exposure

will not necessarily translate into sensitization when the same protein is encountered following oral ingestion. Both methods have some merit and it is possible that i.p. administration and gavage exposure would each be of value in an holistic testing strategy. For this reason we have more recently compared directly the serological responses of proteins when administered to mice intraperitoneally or by gavage. This investigation also provided an opportunity to consider the activity of additional proteins.

2. Experimental

The test proteins examined were peanut agglutinin, OVA and a crude extract of type II acid phosphatase from potato (potato acid phosphatase; PAP). Peanut agglutinin is a minor peanut allergen (Barnett et al., 1983; Burks et al., 1994), whereas for PAP there exists little evidence for allergenicity. Groups of BALB/c strain mice ($n = 6$) received a daily dose by gavage of 1 mg of protein in water, or in control animals an equivalent volume (0.25 ml) of water alone. Mice were exsanguinated 28 or 42 days following the initiation of exposure. Individual and pooled serum samples were prepared and stored at -20°C until analysis. Alternatively, groups of mice ($n = 5$) received 0.25 ml of various concentrations of protein (0.2% peanut agglutinin; 2% OVA or 10% PAP) in phosphate buffered saline by i.p. injection with the same treatment repeated seven days later. Fourteen, 28 or 42 days following the start of exposure mice were exsanguinated and serum prepared and stored as described above. Protein specific IgG antibodies were detected using enzyme-linked immunosorbent assays (ELISA), while IgE anti-protein antibody responses were measured by homologous passive cutaneous anaphylaxis (PCA) assay.

Gavage administration of peanut agglutinin resulted in vigorous IgG antibody responses at both time points (28 and 42 days) measured. Similar treatment of mice with either OVA or PAP resulted in weaker and more variable IgG responses (data not presented). Marked differences were observed between proteins with respect to the

proportion of IgE responders in each treatment group. In all mice examined, daily gavage exposure to peanut agglutinin for either 28 or 42 days was associated with IgE antibody responses. By contrast, OVA stimulated IgE production in only a minority of mice (two of six mice at 28 days and one of six mice at 42 days). No IgE antibody was detectable following 28 days of exposure to PAP and after 42 days only two of six mice displayed detectable IgE responses (Fig. 1). In addition to these differences in the proportion of IgE responder animals following treatment with the three proteins, analysis of IgE titres of pooled serum samples showed a similar relationship. Thus, relatively high titre (1/32) IgE responses were provoked by gavage administration of peanut agglutinin, whereas identical exposure to OVA or PAP stimulated no detectable or only low titre (positive with neat serum) IgE (Fig. 1).

A different pattern of responses was recorded following i.p. exposure of mice to the same proteins. All proteins induced IgG responses that were of maximal vigour 14 days following exposure and of generally higher titre than those observed after gavage administration (data not

presented). In common with gavage treatment, i.p. injection of 0.2% peanut agglutinin was characterized by IgE antibody production in all mice (with one exception at day 28) at all time points examined. Under the same conditions, exposure to 2% OVA was also associated with measurable IgE responses in the majority of animals. In contrast, most animals failed to mount detectable IgE responses in response to 10% PAP (Fig. 1). Variation in the percentages of responder animals in each group was reflected by differential IgE titres recorded in pooled serum samples. Intraperitoneal exposure to both peanut agglutinin and OVA induced high titre (1/16 or greater) IgE antibody at all timepoints investigated. In contrast, PAP failed to stimulate high titre IgE, with detectable antibody (positive with neat serum) induced only at 28 days following initiation of exposure.

3. General discussion

Collectively, the data summarized above reveal that there appear to be significant differences between the proteins examined with respect to IgE

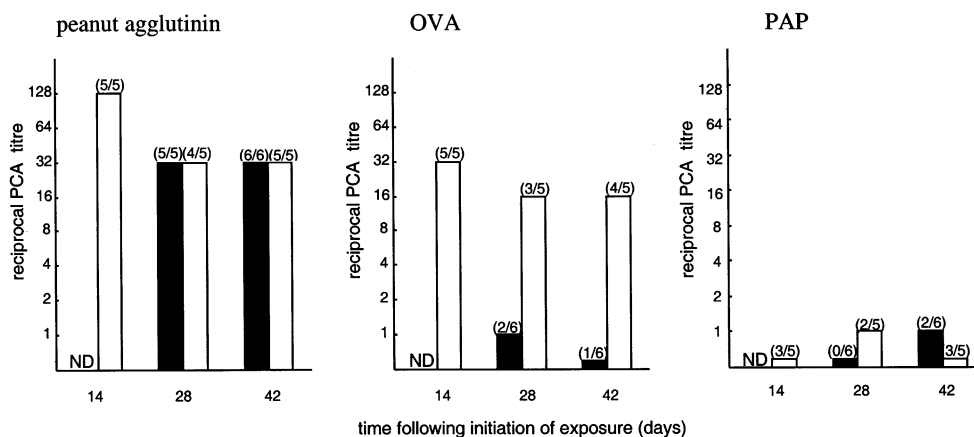


Fig. 1. Groups of mice ($n = 6$) received 1 mg of peanut agglutinin, ovalbumin (OVA) or potato acid phosphatase (PAP) in 0.25 ml of water daily by gavage (■). Additional groups of mice ($n = 5$) received 0.25 ml of 0.2% peanut agglutinin, 2% OVA or 10% PAP in phosphate buffered saline via intraperitoneal injection with treatment repeated 7 days later (□). Fourteen, 28 or 42 days after the initiation of treatment, mice were exsanguinated and individual and pooled serum samples were tested for the presence of specific IgE antibody by homologous PCA assay. IgE antibody titre is recorded as the highest dilution of pooled serum resulting in a positive PCA reaction. The number of IgE responders per group (positive PCA reaction recorded with neat serum from individual animals) is shown in parentheses. In every PCA assay, neat serum isolated from untreated (naïve) animals failed to provoke positive PCA reactions (data not shown). ND – not done.

Table 1
Properties of a range of food proteins^a

Protein	Concentration	Stability	IgG	IgE
Peanut agglutinin	0.2%	Yes	✓	1/128
OVA	2%	Yes	✓	1/32
BSA	2%	Yes (fragments)	✓	1/8
Potato agglutinin	10%	Yes	✓	–
Potato AP	10%	No	✓	–

^a The ability of a range of food proteins (peanut agglutinin; ovalbumin, OVA; bovine serum albumin, BSA; potato agglutinin; potato acid phosphatase, potato AP) to induce IgG and IgE antibody responses following systemic (intraperitoneal) exposure of BALB/c strain mice is shown. Also shown is the stability of the whole protein and protein fragments in SGF (as measured using a modification of the method described by Astwood et al. (1996)).

antibody production. By either route of exposure peanut agglutinin provoked very vigorous IgE responses; results consistent with the known allergenic potential of this protein (Barnett et al., 1983; Burks et al., 1994). Again consistent with its confirmed allergenic activity, OVA induced strong IgE responses when administered i.p. However, oral exposure to this protein was associated with only variable and low grade IgE production. By neither route did PAP elicit anything but weak IgE responses. The pattern of responses observed after parenteral exposure indicate a rank order of potency with respect to IgE responses (especially when the concentrations used for i.p. injection are considered) that appears to reflect what is known of the allergenic properties of these proteins, and the ability of the foods from which they derive to cause allergic sensitization in humans. The data from oral exposure studies are much less convincing because in this case the allergenic potential of OVA appears to have been substantially underestimated.

The characteristics of these and other proteins and the immune responses they induce in mice following i.p. administration are summarized in Table 1. The additional proteins considered here are BSA and potato agglutinin; the latter being viewed as lacking any significant potential for sensitization. It is clear from these data that while each of these is able to stimulate IgG antibody responses (and are therefore clearly immunogenic) only some proteins (peanut agglutinin, OVA and BSA) have been found to provoke IgE antibody production under the same conditions of expo-

sure. The implication is that foreign (and consequently immunogenic) proteins differ significantly with respect to their inherent potential to induce IgE responses. The assumption currently is that this variable ability to stimulate IgE antibody reflects the quality of response induced, and in particular, the differential development of functional subpopulations of T-lymphocytes that either favour or antagonize IgE production (Kimber and Dearman, 1997). The properties of proteins that dictate whether and to what extent they will elicit the quality of immune response necessary for the initiation and maintenance of IgE responses have yet to be defined (Huby et al., 2000). Two other important points can be drawn from Table 1. First, it is apparent that the possession of lectin activity does not per se confer on proteins allergenic activity. Thus, unlike peanut agglutinin, potato agglutinin fails to induce detectable IgE production. The second issue relates to the correlation between sensitizing potential and protein stability. It has been demonstrated that as a general rule known allergenic proteins are comparatively stable in a SGF (Astwood et al., 1996). It is assumed usually that resistance to digestion in the stomach ensures survival of the protein in an immunogenic form for sufficient time for recognition and processing by the immune system. However, as illustrated in Table 1, and as exemplified by potato agglutinin, not all stable proteins are allergenic. Clearly resistance to digestion will be of importance in the generation of mucosal immune responses when proteins are encountered by oral ingestion and this may ex-

plain why PAP lacks sensitizing potential and fails to induce IgE antibody when given by gavage. However, it is far more difficult to reconcile in terms of differential stability in SGF the very significant differences between proteins with respect to IgE responses when administered by i.p. injection. One speculation is that the comparative lability of proteins as measured in SGF actually reflects a more general and more important property of the protein than simple survival in the gastrointestinal tract. In this context, inherent resistance to proteolytic digestion may influence the way in which proteins are processed within cells for presentation to the immune system (Huby et al., 2000).

4. Conclusion

The available evidence suggests that the BALB/c strain mouse may provide an appropriate model for the identification and characterization of protein allergens. Certainly serological responses induced by i.p. administration appear to distinguish between allergenic proteins and other proteins which despite being immunogenic fail to provoke IgE antibody responses.

Moreover, at least to some extent, the vigour of induced IgE responses, particularly when considered in conjunction with dose levels, reflects what is known of the relative sensitizing potential of known human allergens. Although oral administration by gavage may be considered to reflect more accurately the relevant route of human exposure, the data summarized here indicate that this approach may not possess the sensitivity or reliability to provide an initial assessment of allergenic potential.

The utility of the i.p. exposure model for the purposes of hazard identification and characterization is currently being explored more thoroughly with a wider range of test proteins.

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