



Research Section

Immunogenic properties of rapidly digested food proteins following gavage exposure of mice: a comparison of ovalbumin with a potato acid phosphatase preparation

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Accepted 18 October 2001

Abstract

The ability of food proteins to resist digestion in simulated gastric fluid (SGF) correlates with allergenic potential. The purpose of the current investigations was to determine whether this association is due solely to the failure of unstable proteins to elicit an immune response when administered orally. We have examined immune responses induced in BALB/c mice by gavage administration of ovalbumin (OVA) and a crude potato protein extract (PPE) containing acid phosphatase activity. The stability of OVA and PPE in SGF was measured using sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis. The ability of these proteins to stimulate specific IgG and IgE antibody production in mice following parenteral (intraperitoneal; ip) or oral (gavage) exposure was compared using enzyme-linked immunosorbent and homologous passive cutaneous anaphylaxis assays, respectively. Both OVA and PPE induced specific IgG antibody responses when administered either by gavage or by ip injection. Parenteral, but not gavage, exposure to OVA was associated with robust IgE antibody responses. Administration of PPE failed to stimulate strong IgE production via either route of exposure. Differential stability in SGF was observed, with PPE being digested extremely rapidly (within 1 min), whereas OVA was more resistant. The strong association reported by others between stability in SGF and allergenic potential is unlikely to be solely due to orally-ingested labile proteins failing to provoke immune responses due to degradation in the stomach. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Protein allergenicity; Digestibility; IgE; IgG; Oral exposure; Systemic exposure

1. Introduction

There is a growing interest in the methods available for safety assessment of foods derived from genetically modified crop plants, with the potential for allergic sensitization being a major consideration (Kimber et al., 1997, 2000; Taylor, 1997; Wal and Pascal, 1998). The most detailed and systematic analysis of the approaches available for assessment of the allergenic potential of foods derived from genetically engineered crops was

described by Metcalfe et al. in 1996. The recommendations made included evaluation of the serological identity, and structural and sequence homology, of novel proteins with known human allergens (Metcalfe et al., 1996). Based on the observation that there exists a strong correlation between the ability of a protein to resist digestion in simulated gastric fluid (SGF) and its potential to induce allergic sensitization (Astwood et al., 1996), an *in vitro* assessment of the stability of proteins in SGF was also recommended. Many proteins that are confirmed human allergens are stable in SGF, sometimes for 60 min or more, while common plant proteins considered not to have allergenic potential are digested very rapidly, often within 15 s (Astwood et al., 1996). Examination of protein stability remains a central element of the safety assessment process, despite recent proposals that appropriate mouse and rat models may provide a more direct evaluation of allergenic sensitizing

Abbreviations: BSA, bovine serum albumin; GI, gastrointestinal; OVA, ovalbumin; PBS, phosphate buffered saline; PCA, passive cutaneous anaphylaxis; PPE, potato protein extract; SGF, simulated gastric fluid.

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potential (Atkinson and Miller, 1994; Atkinson et al., 1996; Hilton et al., 1997; Knippels et al., 1998; Dearman et al., 2000).

The correlation between protein stability in SGF and allergenic potential is assumed to be due to the fact that for sensitization to develop the protein must survive in the stomach for sufficient time to reach the gastrointestinal (GI) mucosa and initiate an immune response (Astwood et al., 1996; Metcalfe et al., 1996). While survival in the GI tract undoubtedly plays an important role, this may not be the sole explanation for the observed association between stability in SGF and sensitization potential. We have previously compared immune responses induced by parenteral exposure to ovalbumin (OVA) and bovine serum albumin (BSA). OVA is an acknowledged food allergen (Metcalfe, 1985), and has been shown also to cause respiratory allergy (Bernstein et al., 1987). By comparison, BSA has a lesser potential to cause sensitization, although there is some evidence for respiratory and GI allergy to this protein (Joliat and Weber, 1991; Fiocchi et al., 1995). Following either intraperitoneal (ip) or intranasal (in) exposure of mice to these proteins, under conditions where total IgG antibody responses were similar, OVA stimulated much higher levels of specific IgE antibody than did BSA. The subclass distribution of IgG antibody response was also consistent with the ability of OVA to provoke a more selective type 2 immune response (Hilton et al., 1997; Dearman et al., 2000). In independent investigations it was reported by Astwood et al. (1996) that OVA and BSA display differential stability in SGF. OVA remained intact for at least 60 min in SGF, whereas whole BSA was stable for only 0.5 min, with fragments of BSA detectable for up to 15 min (Astwood et al., 1996). Although the differential potential of OVA and BSA to induce IgE in mice appears to correlate with the relative stability of these proteins in SGF, it is hard to reconcile this with survivability in the GI tract given that the proteins have been administered by parenteral (ip or in) routes of exposure. We have therefore examined whether the lack of allergenic potential of SGF-labile proteins is due to their inability to induce specific immune responses following GI exposure.

BALB/c strain mice were exposed ip or by gavage to either OVA or a crude potato protein extract (PPE) containing acid phosphatase activity and IgG and IgE antibody responses were measured. The PPE has been reported previously to be very unstable in SGF (Astwood et al., 1996), and clinical and experimental studies suggest that potato proteins are not a significant cause of sensitization (Hefle et al., 1996; Jeannet-Peter et al., 1999). For these experiments gavage administration was chosen in favor of dietary exposure because it has been reported previously by others that, although gavage dosing with OVA is able to stimulate both IgG and IgE

responses in rats, ad lib. exposure to the same protein fails to provoke IgE antibody production (Knippels et al., 1998).

2. Materials and methods

2.1. Animals

Young adult (8–12 weeks old) female BALB/c strain mice (Harlan Seralab, Oxfordshire, UK) were used throughout these studies. Food and water were available ad lib. The diet used was 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 at 21 ± 2 °C and relative humidity was $55 \pm 10\%$ with a 12-h light/dark cycle. All experiments were carried out under the provisions of the UK Animals (Scientific Procedures) Act, 1986.

2.2. Test materials

Commercial preparations of ovalbumin (OVA; Grade V, 98% pure) and a crude PPE containing type II acid phosphatase enzymatic activity were obtained from Sigma Chemical Co. (St Louis, MO, USA). Analysis of PPE by Western blotting using a monoclonal antibody directed against patatin (kindly supplied by Dr. R. Goodman, Monsanto, St Louis, MO, USA), the major allergenic component of potato (Jeannet-Peter et al., 1999), revealed that this extract contained detectable amounts of this protein (data not shown). Pepsin was obtained from ICN Pharmaceuticals (Thame, Oxfordshire, UK).

2.3. Digestibility in simulated gastric fluid (SGF)

Solutions of test proteins (2 mg/ml in distilled water) were prepared immediately prior to analysis. Twenty μ l of test protein solution and 80 μ l of SGF solution (pepsin, 0.4% or 0.1% dissolved in 0.2% NaCl/0.7% HCl; pH 1.2) were incubated at 37 °C for 0, 1, 5, 15 or 60 min. Reactions were terminated by addition of 35 μ l 0.32 M sodium carbonate and tubes were incubated on ice for a minimum of 15 min. Sixty five μ l of gel loading buffer (0.25 M Tris–HCl pH 6.8, 10% sodium dodecyl sulfate (SDS), 25% glycerol, 6 mg/ml dithiothreitol, 0.3 mg/ml bromophenol blue) was added to each sample and tubes were heated to 90–100 °C for 4 min. All samples were allowed to cool to room temperature and were analyzed by discontinuous SDS–polyacrylamide gel electrophoresis, using a Tris–Tricine gel system optimized for detection of low molecular mass peptides (Schagger et al., 1987). Protein molecular weight markers (Bio-Rad Laboratories, Hertfordshire, UK) were

as follows: phosphorylase B (94.4 kDa); human serum albumin (66.2 kDa); ovalbumin (45 kDa); carbonic anhydrase (31 kDa); trypsin inhibitor (21.5 kDa); and lysozyme (14.4 kDa). Polypeptide markers (Bio-Rad) were as follows: triosephosphate isomerase (26.625 kDa); myoglobin (16.95 kDa); α -lactalbumin (14.437 kDa); aprotinin (6.512 kDa); oxidized insulin β chain (3.496 kDa); and bacitracin (1.423 kDa).

Gels were fixed for 30 min at room temperature in 50% trichloroacetic acid and stained for 1 h in PhastGel Blue R (Pharmacia Biotech, Buckinghamshire, UK) prepared according to manufacturers' specifications. Gels were destained with 20% ethanol/4% acetic acid. Digital images were obtained using a Dage MT1 CCD72 monochrome digital video camera (Imaging Research Inc., Brock University, Ontario, Canada).

2.4. Sensitization procedures

Groups of mice ($n=6$) received a daily dose by gavage of 1 mg of protein in 0.25 ml of water using a blunt-ended stainless-steel animal feeding needle. Animals were dosed for 28 or for 42 consecutive days. Alternatively, groups of mice ($n=5$) received 0.25 ml of various concentrations (w/v) of protein in phosphate buffered saline (PBS) by ip injection. Seven days later treatment was repeated. Mice were exsanguinated 28 or 42 days after the initiation of exposure. Individual and pooled serum samples were prepared and stored at -20°C until analysis.

2.4.1. Anti-protein IgG antibody analysis

Protein-specific IgG antibodies were detected using enzyme-linked immunosorbent assay (ELISA). Plastic microtiter 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% BSA (Sigma) in PBS. Doubling dilutions of mouse serum samples (derived from individual animals or pooled for each experimental group; starting dilution 1:50 or 1 in 10, respectively) 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 goat anti-mouse IgG (Nordic Immunology, Tilberg, The Netherlands) diluted 1:8000 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 titers are expressed as the highest dilution at which substrate conversion (OD450 nm) was greater than 0.5. For control serum derived from naive

(untreated) animals OD450 nm readings never exceeded this value even at the maximum concentration tested.

2.5. Measurement of anti-protein IgE antibody

The presence in mouse serum of IgE antibodies was detected by homologous passive cutaneous anaphylaxis (PCA) assay. In order to determine the number of IgE responder animals per treatment group, PCA assays were performed with individual serum samples (neat). Individual serum samples (neat) were injected (30 μl) into the dermis of the ears of naïve recipient mice ($n=2$). Owing to limitations on the amount of serum available, IgE titers were determined using serum samples pooled on a treatment group basis which comprised both 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 min 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 (pooled serum samples), with antibody titer recorded as the highest dilution of serum resulting in a positive PCA reaction. For individual serum samples, the mean diameter of the cutaneous reaction in the two recipient animals was recorded, and the serum sample was identified as positive for IgE antibody if the mean diameter of the challenge-induced cutaneous reaction was >3 mm.

3. Results

The lability of OVA and PPE to digestion in simulated gastric fluid (SGF) was investigated using a method based on that of Astwood and colleagues (1996). A representative experiment is displayed in Fig. 1. Using the concentration of pepsin (0.32%) described for the composition of SGF in The US Pharmacopoeia (2000), a proportion of the OVA whole protein was resistant to degradation for 5 min. A low molecular weight peptide degradation product (approximately 3 kDa) appeared within 1 min of exposure to pepsin but was no longer detectable after 5 min. Treatment with a lower concentration of pepsin (0.08%) resulted in greater stability of this low molecular weight fragment, which was first detectable after 5 min and persisted throughout the period of exposure (60 min). In

contrast, under the same conditions using either 0.32 or 0.08% pepsin, all components of the PPE were digested completely within 1 min to peptide fragments below the limits of detection (less than 1.423 kDa).

In parallel experiments the ability of these two proteins to induce IgG antibody responses following oral (gavage) or systemic (ip) exposure to BALB/c strain mice was compared. Animals received by gavage a daily

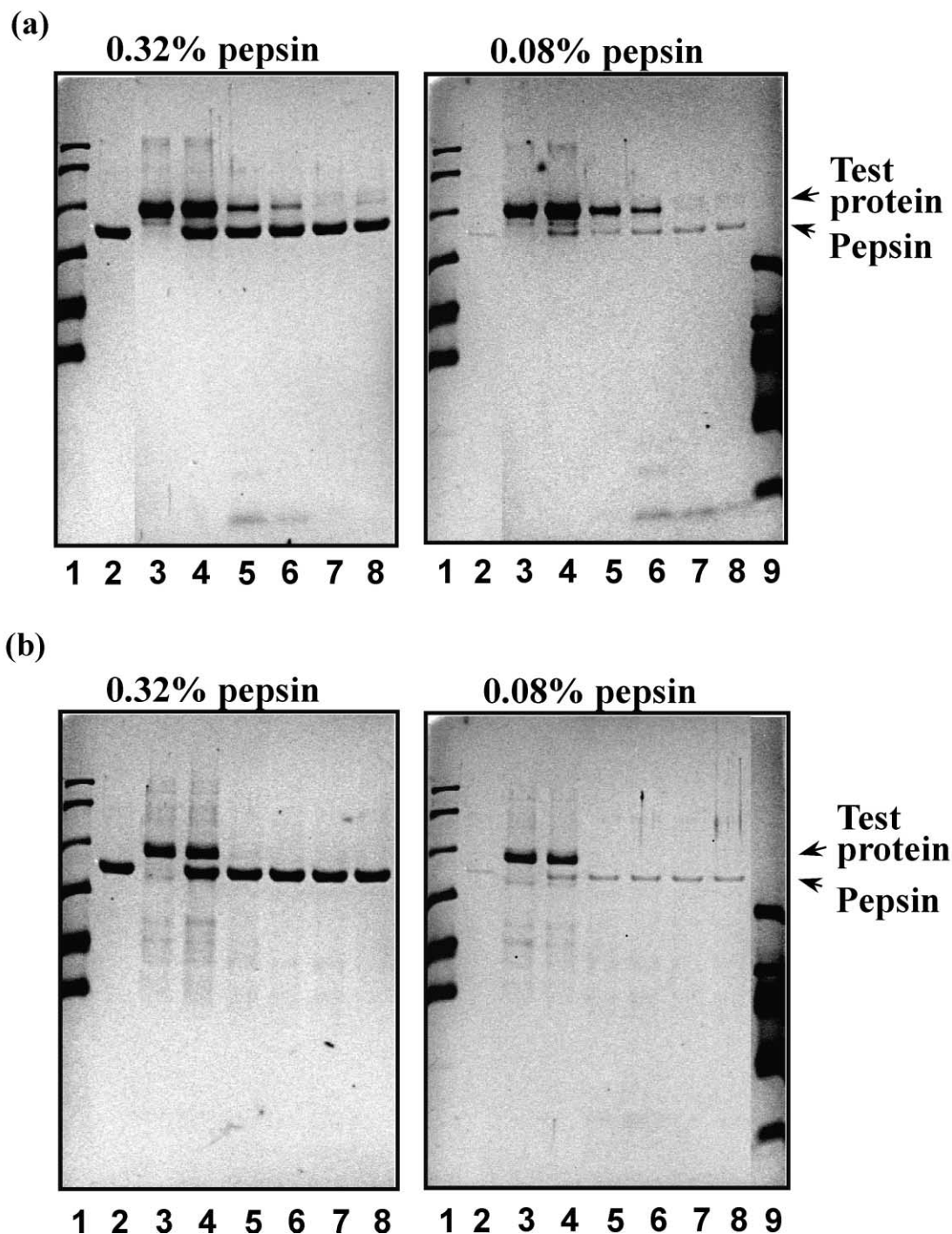


Fig. 1. Digestibility of ovalbumin (OVA) and crude potato extract (PPE) containing acid phosphatase enzymatic activity in simulated gastric fluid (SGF). OVA (a) or PPE (b) at 2 mg/ml were incubated for between 0 and 60 min in SGF containing 0.32 or 0.08% pepsin and samples analyzed by SDS-PAGE. Lane 1: high molecular weight markers (molecular mass 94.4, 66.2, 45, 31, 21.5 and 14.4 kDa); Lane 2: SGF alone; Lane 3: test protein alone; Lane 4: test protein in SGF, 0 min digestion; Lane 5: test protein in SGF, 1 min digestion; Lane 6: test protein in SGF, 5 min digestion; Lane 7: test protein in SGF, 15 min digestion; Lane 8: test protein in SGF, 60 min digestion; Lane 9: low molecular weight markers (molecular mass 26.6, 17, 14.4, 6.5, 3.5 kDa).

dose of 1 mg/ml of OVA or PPE in 0.25 ml of water for 28 or 42 consecutive days. Alternatively, mice were exposed ip on days 0 and 7 to 0.25 ml of 2% OVA or 10% PPE in PBS. Twenty eight or 42 days after the initiation of treatment, mice were exsanguinated and serum analyzed for the presence of specific antibody. Not surprisingly, systemic immunization by ip administration of both proteins provoked marked IgG antibody responses (Fig. 2, pooled serum samples; Fig. 3, individual serum samples). Gavage treatment with OVA also elicited a relatively high titer IgG response after 28 daily exposures, although a considerably less vigorous response was observed after 42 days (Fig. 2; pooled serum samples). This was a reflection of the considerable inter-animal variation in the antibody response (Fig. 3). Interestingly, despite the fact that PPE was completely degraded by exposure to SGF for less than 1 min, administration of this protein via gavage exposure nevertheless resulted in the stimulation of detectable IgG antibody. Indeed, after 42 daily gavage treatments, relatively high titer IgG was observed in 50% of the animals (Figs. 2 and 3), reaching similar levels to those induced by ip administration.

In subsequent analyses, the influence of route of exposure upon the capacity of OVA and PPE to provoke IgG and IgE antibody responses was investigated. For these measurements, the presence of specific IgG and IgE antibody was determined in serum samples derived from individual mice in order to determine the number of responder animals per group. In addition, IgG and IgE antibody titers were determined in serum

samples pooled on an experimental group basis to determine the overall vigor of responses (Fig. 4). Very robust antibody responses were provoked by ip administration of OVA, with all animals displaying positive IgG antibody responses at both 28 and 42 days after initiation of exposure and the majority of animals positive for IgE antibody (60 and 80%, respectively). In addition, relatively high titer IgG and IgE antibody responses were recorded for pooled serum samples. Identical treatment with PPE stimulated comparable IgG antibody production, with all animals expressing specific IgG antibody at both time points. Anti-PPE IgE antibody production was much weaker and somewhat more variable, with only 60 and 40% of animals having detectable specific IgE. Analysis of pooled serum samples revealed the presence of low titer IgE (positive with neat serum only) at 28 days, or no detectable IgE at 42 days. Gavage administration of OVA resulted in lower titer IgG antibody production than did ip injection of the same protein, and some animals failed to produce detectable antibody, particularly after 42 daily treatments. IgE responses to orally administered OVA were also markedly lower than those achieved following systemic administration, with only a minority of animals positive at either time point. Gavage treatment with PPE also failed to stimulate a robust IgE response. However, despite the lability of this protein to digestion in SGF, substantial IgG antibody responses were provoked after 28 and 42 days of exposure, with 67 and 50% of the animals, respectively, displaying positive IgG responses.

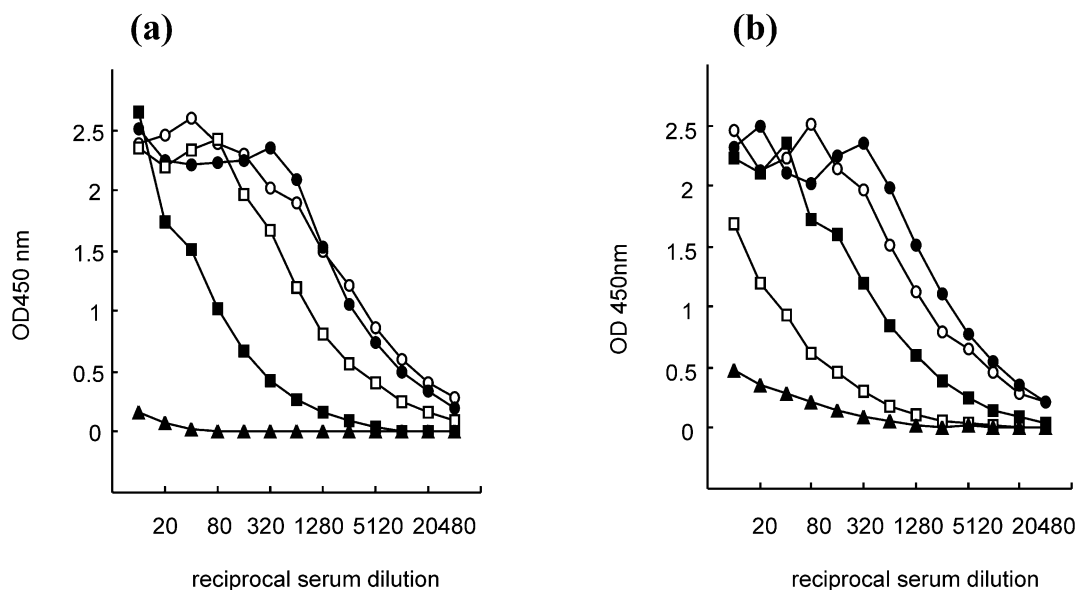


Fig. 2. Influence of route of exposure to ovalbumin (OVA) or crude potato extract (PPE) containing acid phosphatase enzymatic activity on IgG antibody production; pooled serum samples. Groups of mice ($n=6$) received 0.25 ml of water containing 1 mg of OVA (a) or PPE (b) by gavage daily ($\square$, $\blacksquare$). Alternatively, animals ($n=5$) received 0.25 ml of 2% OVA (a) or 10% PPE (b) in PBS by intraperitoneal injection with treatment repeated 7 days later ($\circ$, $\bullet$). Twenty-eight (open symbols) and 42 (closed symbols) days after the initiation of exposure, mice were exsanguinated and pooled serum samples tested for protein-specific IgG antibody by ELISA. Negative control serum derived from naive untreated mice represented by ($\blacktriangle$).

4. Discussion

The analyses described here show that both OVA and PPE induce specific IgG antibody responses in mice when administered either by gavage or by ip injection, but that only ip exposure to OVA was associated with strong IgE antibody production. The differential potential of OVA and PPE to stimulate IgE antibody responses when administered parenterally is consistent with their relative ability to induce allergic sensitization. Ovalbumin is a major egg allergen, eggs being one of the eight foodstuffs that cause the majority of confirmed food-related allergies in western Europe (Metcalf, 1985; Hefle et al., 1996). In contrast, although the crude potato protein extract contains patatin, the major allergen in potato and which represents up to 40% of total protein in potatoes, the potato is a rare cause of IgE-mediated allergic reactions (Metcalf, 1985; Hefle et al., 1996; Jeannet-Peter et al., 1999). Following gavage exposure, neither OVA nor PPE induced consistent or strong IgE responses.

The important observation is that PPE is immunogenic in mice and that its ability to provoke a specific IgG antibody response is not lost upon oral administration. However, PPE was digested fully during treatment for 1 min with SGF containing either 0.08 or 0.32% pepsin. Previous independent investigations have reported that PPE can actually be completely digested within 15 s in SGF (Astwood et al., 1996). In the present studies, whole OVA was resistant to digestion for up to 5 min in SGF containing 0.32% pepsin and fragments persisted for up to 60 min in SGF containing 0.08% pepsin. Astwood et al. (1996) reported that OVA was stable for at least 60 min in SGF with 0.32% pepsin. Despite these differences, in both laboratories OVA was substantially more resistant than PPE to pepsin digestion. Although the OVA was of greater than 98% purity, the PPE was a crude extract containing contaminating proteins including patatin. Nevertheless, all the proteins contained in the PPE preparation were digested completely by SGF within 1 min.

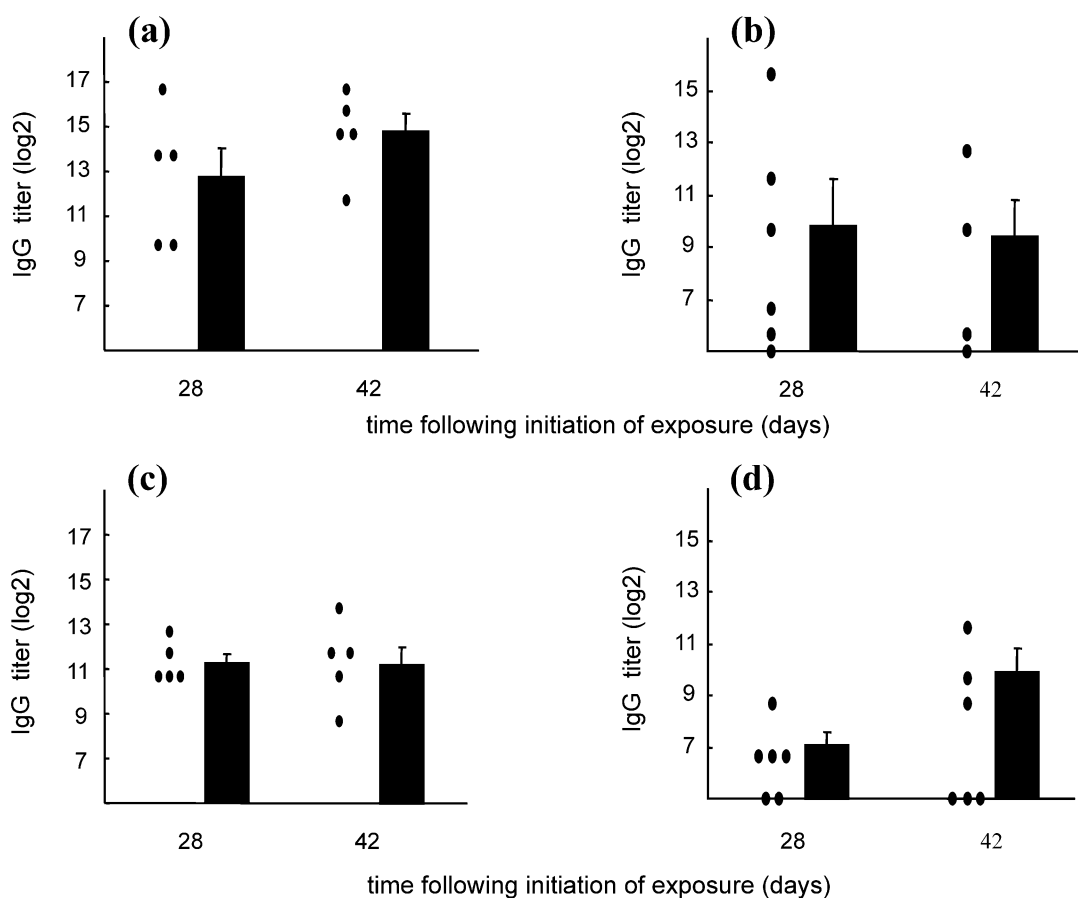


Fig. 3. Influence of route of exposure to ovalbumin (OVA) or crude potato extract (PPE) containing acid phosphatase enzymatic activity on IgG antibody production; individual serum samples. Groups of mice ($n=5$) received 0.25 ml of 2% OVA (a) or 10% PPE (c) in PBS by intraperitoneal injection with treatment repeated 7 days later. Alternatively, animals ($n=6$) received 0.25 ml of water containing 1 mg of OVA (b) or PPE (d) by gavage daily. Twenty eight and 42 days after the initiation of exposure, mice were exsanguinated and individual serum samples tested for protein-specific IgG antibody by ELISA. IgG titer (log2) for each serum sample is represented by an individual marker (●), mean and SE per treatment group for IgG responder animals are displayed also.

These results show that a protein which is clearly very labile in SGF, and which should therefore be digested rapidly in the gastric environment, is able to elicit a substantial IgG antibody response when administered orally (by gavage). It may be that gavage dosing provides some opportunity for exposure to protein by other routes, such as via the trachea, although this is unlikely to be sufficient to provide for such a substantial IgG antibody response. The failure of antigenic proteins to stimulate immune responses when given continuously in drinking water is likely to be due to the development of immunological tolerance (Strobel and Mowat, 1998). Exposure by gavage, rather than in drinking water, may prevent tolerance induction (possibly by provision of a concurrent inflammatory stimulus) (Strobel and Mowat, 1998) and result in an immunogenic response, rather than clonal anergy or active suppression. Indeed, antigen recognition and internalization in the gastrointestinal tract can be very efficient. Two main routes are recognized: the uptake of antigen by M cells overlying Peyer's patches and internalization by intestinal epithelial cells that are in intimate contact with T lymphocytes and which have been shown recently to have the ability to act as antigen presenting cells (Hershberg and Mayer, 2000). In both cases, antigen uptake is likely to be a rapid process with kinetics of internalization

which are consistent with the survival of apparently labile proteins, or their peptides, in the GI tract. This may explain why a labile protein such as PPE can elicit an IgG response following gavage administration.

It is relevant to speculate as to the ways in which protein stability may influence sensitizing activity. Individual proteins differ with respect to their ability to induce IgE antibody production and to cause allergic sensitization, processes which are favored by the development of selective Th2-type responses. It is not unreasonable, therefore, to hypothesize that operationally allergenic proteins are those that have the potential to provoke the selective development of Th2-type cells. Non-allergenic (but nevertheless immunogenic) proteins will instead stimulate preferential type 1 immune responses (Dearman et al., 2000). It can be postulated further that it is the processing and subsequent presentation of protein antigens to the immune system that have the greatest influence on the quality of immune response induced (Kapsenberg et al., 1998). Certainly, the nature of the peptide antigen displayed by antigen presenting cells in association with major histocompatibility complex gene products, and the interaction of this complex with the T lymphocyte receptor for antigen, are important determinants of selective T cell development (Constant et al., 1995; Kumar et al., 1995;

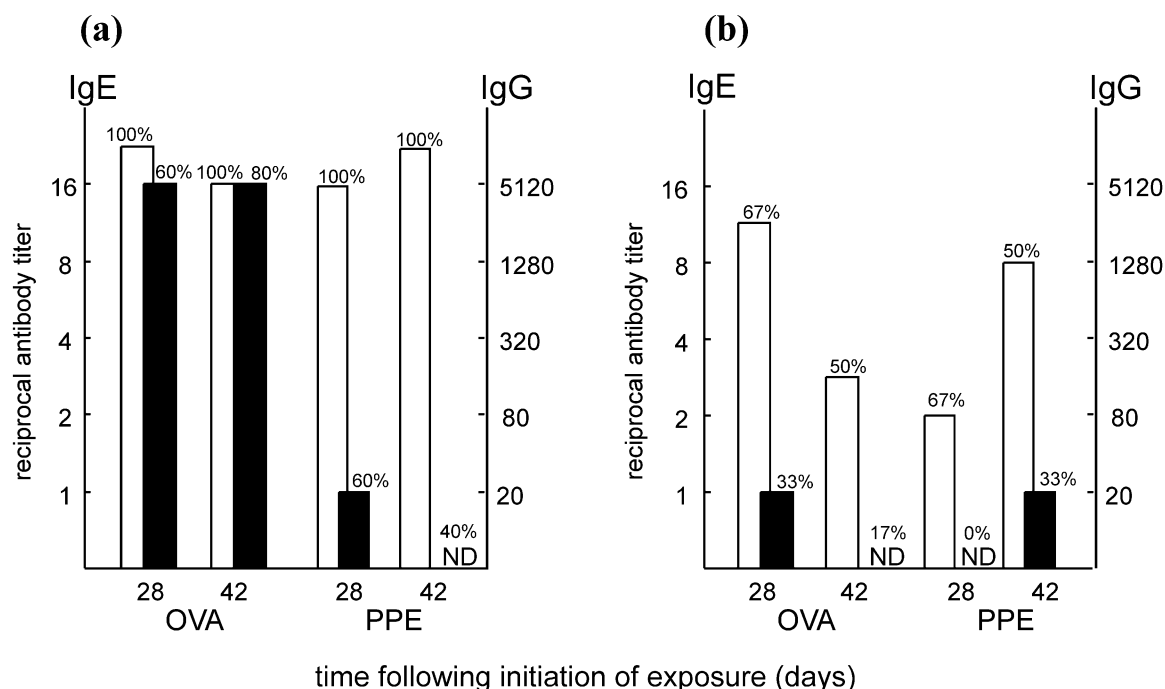


Fig. 4. Influence of route of exposure to ovalbumin (OVA) or crude potato extract (PPE) containing acid phosphatase enzymatic activity on IgG and IgE antibody production: inter-animal variation. Groups of mice ($n=5$) received 0.25 ml of 2% OVA or 10% PPE in PBS by intraperitoneal injection with treatment repeated 7 days later (a). Alternatively, animals ($n=6$) received 0.25 ml of water containing 1 mg of OVA or PPE by gavage daily (b). Twenty eight and 42 days after the initiation of exposure, mice were exsanguinated and individual and pooled serum samples tested for protein-specific IgG antibody by ELISA and for protein-specific IgE antibody by PCA. Pooled serum samples were utilized to derive IgG (□) and IgE (■) antibody titers on an experimental group basis. In every experiment, pooled serum from naive (untreated) animals was tested concurrently and were negative (data not shown). The percentages of responders in each group are shown. ND = not detectable.

Pfeiffer et al., 1995). This suggests in turn that the nature and/or kinetics of protein processing by antigen presenting cells will also impact on the selectivity of T lymphocyte responses. It has been shown that protein epitope presentation is directed by preferential proteolytic cleavage at sites of instability (Landry, 1997). This suggests that the differential susceptibility of proteins to intracellular enzymatic digestion could therefore influence directly the nature of epitopes displayed and presented to T lymphocytes and the quality of immune response generated. The nature and/or kinetics of antigen processing, determined in part by the inherent stability of the protein, would therefore be one factor favoring the development of type 2 T cell development. Inherent stability alone would not be sufficient to confer allergenic potential and indeed not all stable proteins are able to induce allergic sensitization.

In conclusion, the evidence presented here suggests that the association between stability in SGF and allergenic potential may not be solely a function of the survivability of proteins in the GI tract. We propose that stability in SGF may instead reflect resistance to cleavage of the protein by intracellular proteases during processing for presentation to T lymphocytes, and that the nature of such processing and the peptide(s) displayed will influence whether an immune response of the quality necessary for allergic sensitization will develop. That the stability of proteins in SGF may have significance beyond survivability in the GI tract is consistent with the observations that resistance to food processing and heat denaturation are common properties of protein allergens (Nordlee et al., 1981; Herian et al., 1993; Taylor and Lehrer, 1996). Irrespective of whether the association between stability in SGF and the potential for allergic sensitization is a reflection of extracellular digestion in the GI tract and/or intracellular processing by antigen presenting cells, assessment of the survival of proteins in SGF currently represents a central and essential component of allergenicity testing. The views expressed here do not question the importance of the correlation observed previously between allergenicity and stability in SGF for the identification of sensitizing proteins; the suggestion is simply that such correlations do not only reflect survival in the GI tract.

References

- Astwood, J.D., Leach, J.N., Fuchs, R.L., 1996. Stability of food allergens to digestion in vitro. *Nature Biotechnology* 14, 1269–1273.
- Atkinson, H.A.C., Johnson, I.T., Gee, J.M., Grigoriadou, F., Miller, K., 1996. Brown Norway rat model of food allergy: effect of plant components on the development of oral sensitization. *Food and Chemical Toxicology* 34, 27–32.
- Atkinson, H.A.C., Miller, K., 1994. Assessment of the Brown Norway rat as a suitable model for investigation of food allergy. *Toxicology* 91, 281–288.
- Bernstein, D.I., Smith, A.B., Moller, D.R., Gallagher, J.S., Tar-Ching, A., London, M., Kopp, S., Carson, G., 1987. Clinical and immunologic studies among egg processing workers with occupational asthma. *Journal of Allergy and Clinical Immunology* 80, 791–797.
- Constant, S., Pfeiffer, C., Woodward, A., Pasqualini, T., Bottomly, K., 1995. Extent of T cell receptor ligation can determine the functional differentiation of naïve CD4⁺ T cells. *Journal of Experimental Medicine* 182, 1591–1596.
- Dearman, R.J., Caddick, H., Basketter, D.A., Kimber, I., 2000. Divergent antibody isotype responses induced in mice by systemic exposure to proteins: a comparison of ovalbumin with bovine serum albumin. *Food and Chemical Toxicology* 38, 351–360.
- Fiocchi, A., Restani, P., Riva, E., Qualizza, R., Bruni, P., Restelli, A.R., Galli, C.L., 1995. Meat allergy: 1. Specific IgE to BSA and OSA in atopic beef sensitive children. *Journal of the American College of Nutrition* 14, 239–244.
- Hefle, S.L., Nordlee, J.A., Taylor, S.L., 1996. Allergenic foods. *Critical Reviews in Food Science and Nutrition* 36 (S), S69–S89.
- Herian, A.M., Taylor, S.L., Bush, R.K., 1993. Allergenic reactivity of various soybean products as determined by RAST inhibition. *Journal of Food Science* 58, 385–388.
- Hershberg, R.M., Mayer, L.F., 2000. Antigen processing and presentation by intestinal epithelial cells—polarity and complexity. *Immunology Today* 21, 123–128.
- Hilton, J., Dearman, R.J., Sattar, N., Basketter, D.A., Kimber, I., 1997. Characteristics of antibody responses induced in mice by protein allergens. *Food and Chemical Toxicology* 35, 1209–1218.
- Jeannot-Peter, N., Piletta-Zanin, P.A., Hauser, C., 1999. Facial dermatitis, contact urticaria, rhinoconjunctivitis, and asthma induced by potato. *American Journal of Contact Dermatitis* 10, 40–42.
- Joliat, T.L., Weber, R., 1991. Occupational asthma and rhinoconjunctivitis from inhalation of crystalline bovine albumin powder. *Annals of Allergy* 66, 301–304.
- Kapsenberg, M.L., Hilken, C.M.U., Wierenga, E.A., Kalinski, P., 1998. The role of antigen-presenting cells in the regulation of allergen-specific T cell responses. *Current Opinion in Immunology* 10, 607–613.
- Kimber, I., Atherton, K.T., Kenna, J.G., Dearman, R.J., 2000. Predictive methods for food allergenicity: perspectives and current status. *Toxicology* 147, 147–150.
- Kimber, I., Lumley, C.E., Metcalfe, D.D., 1997. Allergenicity of proteins. *Human and Experimental Toxicology* 16, 516–518.
- Knippels, L.M.J., Penninks, A.H., Spanhaak, S., Houben, G.F., 1998. Oral sensitization to food proteins: a Brown Norway rat model. *Clinical and Experimental Allergy* 28, 368–375.
- Kumar, V., Bhardwaj, V., Soares, L., Alexander, J., Sette, A., Sercarz, E., 1995. Major histocompatibility complex binding affinity of an antigenic determinant is critical for differential secretion of interleukin 4/5 or interferon γ by T cells. *Proceedings of the National Academy of Sciences of the U.S.A.* 92, 9510–9514.
- Landry, S.J., 1997. Local protein instability predictive of helper T-cell epitopes. *Immunology Today* 18, 527–532.
- Nordlee, J.A., Taylor, S.L., Jones, R.T., Yunginger, J.W., 1981. Allergenicity of various peanut products as determined by RAST inhibition. *Journal of Allergy and Clinical Immunology* 68, 376–382.
- Metcalfe, D.D., 1985. Food allergens. *Critical Reviews in Allergy* 3, 331–349.
- Metcalfe, D.D., Astwood, J.D., Townsend, R., Sampson, H.A., Taylor, S.L., Fuchs, R.L., 1996. Assessment of the allergenic potential of foods derived from genetically-engineered crop plants. *Critical Reviews in Food Science and Nutrition* 36, S165–S186.
- Pfeiffer, C., Stein, J., Southwood, S., Ketelaar, H., Sette, A., Bottomly, K., 1995. Altered peptide ligands can control CD4 T lymphocyte differentiation in vivo. *Journal of Experimental Medicine* 181, 1569–1574.

- Schagger, H., von Jagow, G., 1987. Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Analytical Biochemistry* 166, 368–379.
- Strobel, S., Mowat, A.McI., 1998. Immune responses to dietary antigens: oral tolerance. *Immunology Today* 19, 173–181.
- Taylor, S.L., 1997. Food from genetically modified organisms and potential for food allergy. *Environmental Toxicology and Pharmacology* 4, 121–126.
- Taylor, S.L., Lehrer, S.B., 1996. Principles and characteristics of food allergens. *Critical Reviews in Food Science and Nutrition* 36, S91–S118.
- The United States Pharmacopeia, 2000. Vol. 24. The National Formulary 18. Simulated Gastric Fluid. Board of Trustees, United States Pharmacopeial Convention, Rockville.
- Wal, J.M., Pascal, G., 1998. Benefits and limits of different approaches for assessing the allergenic potential of novel foods. *Allergy* 53, 98–100.