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Divergent Antibody Isotype Responses Induced in Mice by Systemic Exposure to Proteins: a Comparison of Ovalbumin with Bovine Serum Albumin

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Abstract—Whereas many foreign proteins are immunogenic, only a proportion is associated commonly with allergy, having the potential to induce the quality of immune response necessary for IgE antibody production and the development of immediate type hypersensitivity reactions in the gastrointestinal and/or respiratory tracts. In the context of toxicological evaluations there is a need to identify those properties that confer on proteins the ability to provoke allergic reactions. The characteristics of antibody responses induced in BALB/c strain mice following administration of ovalbumin (OVA), a significant human allergen, have been compared with those provoked by bovine serum albumin (BSA), a protein considered to have more limited allergenic potential. Intranasal or intraperitoneal (ip) administration of BSA or OVA elicited vigorous IgG and IgG1 antibody responses. Differential IgE antibody production was observed, however, with OVA stimulating relatively high IgE antibody titres at all doses tested whereas no or low titre IgE antibody was detected following exposure to BSA. Furthermore, a differential capacity for IgG2a antibody responses was observed, with only BSA provoking high titres of this IgG subclass. The relative quality of induced responses was equivalent following administration of these proteins via mucosal (in) tissue or via a non-mucosal (ip) route of exposure. IgG2a antibody production is promoted by the type 1 cytokine interferon γ (IFN- γ), whereas IFN- γ and the type 2 cell product interleukin 4 exert reciprocal antagonistic effects on IgE antibody responses. Although cytokine expression patterns were not analysed in this series of experiments, the differential IgE and IgG subclass antibody responses induced by BSA and OVA are consistent with the preferential activation of T helper (Th) 1- and Th2-type cells, respectively. These data indicate that proteins can provoke in mice characteristic antibody (IgE and IgG) isotype profiles suggestive of discrete T lymphocyte responses and that such differences may be associated with variable allergenic activity. © 2000 Elsevier Science Ltd. All rights reserved

Keywords: protein allergy; IgE; IgG subclasses; Th cell subpopulations.

Abbreviations: BSA = bovine serum albumin; ELISA = enzyme-linked immunosorbent assay; IFN- γ = interferon γ ; IL = interleukin; NGS = normal goat serum; OVA = ovalbumin; PBS = phosphate buffered saline; PCA = passive cutaneous anaphylaxis; Th cell = T helper cell.

INTRODUCTION

Allergy resulting from exposure to proteins is a significant environmental and occupational health problem and an important toxicological issue. Asthma and rhinitis associated with inhalation exposure to environmental protein aeroallergens, such as pollen

and house dust mite, are common. In occupational settings, although baker's asthma is the most well studied form of occupational food allergy (Bernstein and Malo, 1993), respiratory sensitization caused by egg proteins and enzymes has been well documented (Bernstein *et al.*, 1987; Blair Smith *et al.*, 1987; Bossert *et al.*, 1988; Franz *et al.*, 1971; Juniper *et al.*, 1977; Juniper and Roberts, 1984; Sarol *et al.*, 1990; Shapiro and Eisenberg, 1971). It is clear also that ingested proteins contained within a variety of food products (including milk and milk

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products, eggs and some fish, crustacea, legumes, tree nuts, cereal grains and fruits) are able to induce allergic sensitization among susceptible individuals. The symptoms of food allergy can range from oral allergy syndrome, where the symptoms are mild and confined to the oral cavity (Ortolani *et al.*, 1988), to gastrointestinal distress, and sometimes systemic anaphylaxis (Brandtzaeg, 1997; Ferguson, 1997; Kimber *et al.*, 1997; Metcalfe, 1985; Taylor *et al.*, 1987). Consideration of food allergy has recently assumed greater toxicological significance with the need to assess the allergenic potential of foods derived from genetically modified crop plants and fruits (Kimber *et al.*, 1997; Metcalfe *et al.*, 1996b; Taylor, 1997).

In the context of toxicological evaluation, there have been attempts to develop methods in guinea pigs and mice for the identification and characterization of protein aeroallergens, and in particular detergent enzymes (Kawabata *et al.*, 1996; Ritz *et al.*, 1993; Robinson *et al.*, 1996, 1997, 1998; Sarlo *et al.*, 1997). There have been some attempts also to address the question of the potential for food allergenicity. A systematic approach has been described in which considerations of allergenic activity are based on immunological cross-reactivity, or amino acid sequence homology, with known food allergens, supplemented by examination of protein stability (Metcalfe *et al.*, 1996a). With respect to the latter measurements, Astwood and colleagues have reported an association between allergenic potential and the resistance of proteins to digestion in a simulated gastric fluid (Astwood *et al.*, 1996). Attempts to evaluate more directly the potential of proteins to cause food allergy have relied primarily on the assessment of immune responses induced in Brown Norway strain rats, often in the presence of adjuvant (Atkinson *et al.*, 1996; Atkinson and Miller, 1994; Houben *et al.*, 1997).

Despite these proposed experimental methods, there remains a need for a robust predictive test method that will allow the accurate characterization of allergenic activity and which is based upon an appreciation of the immunological variables that determine differences between proteins with respect to potential allergenicity. We have described previously one approach to considering the characteristics that confer on proteins allergenic activity that is based on comparison of immune responses induced in BALB/c strain mice by ovalbumin (OVA) and bovine serum albumin (BSA) (Hilton *et al.*, 1997). The former is a component of egg protein that has been shown to cause respiratory allergy in a proportion of exposed workers in egg processing plants (Bernstein *et al.*, 1987; Blair Smith *et al.*, 1987) and is an acknowledged common food allergen (Metcalfe *et al.*, 1985). By comparison, BSA displays significantly less allergenic activity, although some limited potential of this protein to cause respiratory sensitization (Joliat and

Weber, 1991) and gastrointestinal allergy (Fiocchi *et al.*, 1995) has been reported. In previous investigations it was found that under conditions of ip exposure or intranasal administration where both proteins induced in mice specific total IgG and IgG1 antibody responses, there were marked differences between OVA and BSA with respect to their ability to provoke IgE antibody production. Whereas OVA routinely stimulated IgE antibody responses, BSA either failed to induce IgE antibody production altogether or, in one instance, induced only a low titre response (Hilton *et al.*, 1994, 1997). The inference drawn from those investigations was that OVA and BSA elicit in mice qualitatively variable immune responses, with the ability to provoke IgE antibody production being associated with allergenic potential. The purpose of the studies described here was to explore further the characteristics of immune responses induced in mice by OVA and BSA with particular reference to the production of specific IgG2a antibody; an antibody isotype that is known in mice to be regulated in reciprocal fashion to IgE.

MATERIALS AND METHODS

Animals

Young adult (8–12 wk 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).

Test materials

Bovine serum albumin (BSA; Grade V, 98–99%), ovalbumin (OVA; Grade V, 98%) and carrageenan iota (type V, from *Eucheuma spinosa*) were obtained from Sigma Chemical Co. (St Louis, MO, USA).

Sensitization procedures

Groups of mice ($n = 5$ or 10) received $250 \mu\text{l}$ of 1, 2 or 5% (w/v) protein in phosphate buffered saline (PBS) by ip injection. 7 days later treatment was repeated. Alternatively, groups of mice ($n = 10$) received $5 \mu\text{l}$ per nostril of 5% (w/v) protein in PBS following iv injection of Saffan anaesthetic (Pitman-Moore, Harefield, Middlesex, UK) for restraint. The treatment was repeated 2 and 9 days later. 14 days following the initiation of exposure, all mice were exsanguinated by cardiac puncture. In some experiments, animals ($n = 5$) received a single ip injection of $250 \mu\text{l}$ of 2% (w/v) protein in PBS containing 0.25 mg carrageenan and were exsanguinated by cardiac puncture 28 days after initiation of exposure. Individual and pooled serum samples were prepared and stored at -20°C until analysis.

Measurement of protein-specific IgG, IgG1 and IgG2a antibody

Protein-specific IgG, IgG1 and IgG2a antibodies were detected using enzyme-linked immunosorbent assay (ELISA). Plastic microtitre plates (Nunc, Copenhagen, Denmark) were coated with 100 µg of BSA/ml or OVA in PBS by overnight incubation at 4°C. The plates were blocked by incubation for a further 30 min at 37°C with 5% normal goat serum (NGS) in PBS. Doubling dilutions of mouse serum samples (derived from individual animals or pooled for each experimental group; starting dilution 1:25) diluted in 0.5% NGS in PBS were added to consecutive wells and incubated for 3 hr at 4°C. There followed a further incubation for 3 hr at 4°C with peroxidase-labelled goat anti-mouse IgG (Nordic Immunology, Tilberg, The Netherlands) diluted 1:8000 with 0.5% NGS in PBS or a further incubation for 2 hr at room temperature with peroxidase-labelled rat monoclonal anti-mouse IgG1 or IgG2a (Serotec Ltd, Oxford, UK) diluted 1:2000 with 0.5% NGS 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). Where sets of serum samples have been subjected to within and between group comparisons, ELISA analyses were performed on the same day for all of the samples.

Measurement of protein-specific IgE antibody

The presence in the serum of IgE antibodies was detected by homologous passive cutaneous anaphylaxis (PCA) assay. Pooled serum samples were diluted serially in physiological saline and injected (30 µl) into the dermis of the ears of naive recipient mice ($n = 4$). 2 days later, 0.25 mg of protein, together with Evans Blue dye (1.25 mg), in 250 µl of physiological saline were injected iv. 30 min following challenge, mice were killed and the diameter of cutaneous reactions measured. Antibody titre was recorded as the highest dilution of pooled serum resulting in a measurable (>3 mm) blue lesion in the skin in the majority of recipient animals.

Statistical analyses

For each ELISA dilution curve for each individual animal, the area under the curve was calculated. The areas were considered using analysis of covariance following a log transformation to stabilize the variance. Least-squares means for each group were calculated. Unbiased estimates of differences between groups were provided by comparisons of each treatment groups least squares mean. For pooled serum samples, the area under each curve

was calculated and considered using analysis of covariance as described above. Such differences were tested statistically using a two-sided Student's *t*-test based on the error mean square in the analysis (SAS, 1989). Differences were considered to be statistically significant at the 1% level ($P < 0.01$).

RESULTS

Comparison of the isotype distribution of antibody responses induced by intraperitoneal exposure to BSA and OVA

In preliminary experiments, the isotype distribution of antibody responses provoked by ip administration of BSA or OVA was compared. Animals ($n = 5$) were exposed to 2% solutions of protein and 14 days after the initiation of exposure, mice were exsanguinated, serum samples pooled and doubling dilutions prepared for each experimental group. The presence of IgG, IgG1 and IgG2a antibodies was measured by ELISA (Fig. 1; three independent experiments) and IgE antibody production was analysed by PCA. As reported previously (Hilton *et al.*, 1994, 1997), BSA and OVA, under these conditions of exposure, displayed a differential capacity to induce the production of IgE antibody. Treatment with BSA failed to provoke measurable IgE antibody (undiluted serum from mice exposed to BSA did not elicit a positive PCA reaction in any of the three experiments) whereas in each case OVA stimulated detectable IgE antibody (experiment 1, titre of 1/4; experiment 2, titre of 1/2; experiment 3, titre of 1/8). Under the same conditions of exposure, however, both proteins provoked vigorous IgG and IgG1 antibody responses, with BSA stimulating somewhat higher levels of antibody production (both with respect to maximal optical density readings and the serum dilutions at which substrate conversion was detected) than those induced by administration of equivalent amounts of OVA protein. Interestingly, a striking difference was observed with respect to IgG2a antibody responses. In each of the three experiments shown, exposure only to BSA resulted in substantial production of this subclass of IgG antibody. Treatment with OVA stimulated little IgG2a antibody expression despite provoking high titre total IgG and IgG1 antibody responses. Even under conditions where a marked IgG2a antibody response was observed (administration of 2% BSA), however, production of this subclass of IgG was much less vigorous than either total IgG or IgG1 antibody production, both with respect to maximal optical density readings and the dilution of serum at which measurable substrate conversion occurred (Fig. 1). It should be noted that in independent experiments (not shown) the integrity of the ELISA method used for the detection of IgG2a anti-OVA antibodies was verified. This was achieved using serum

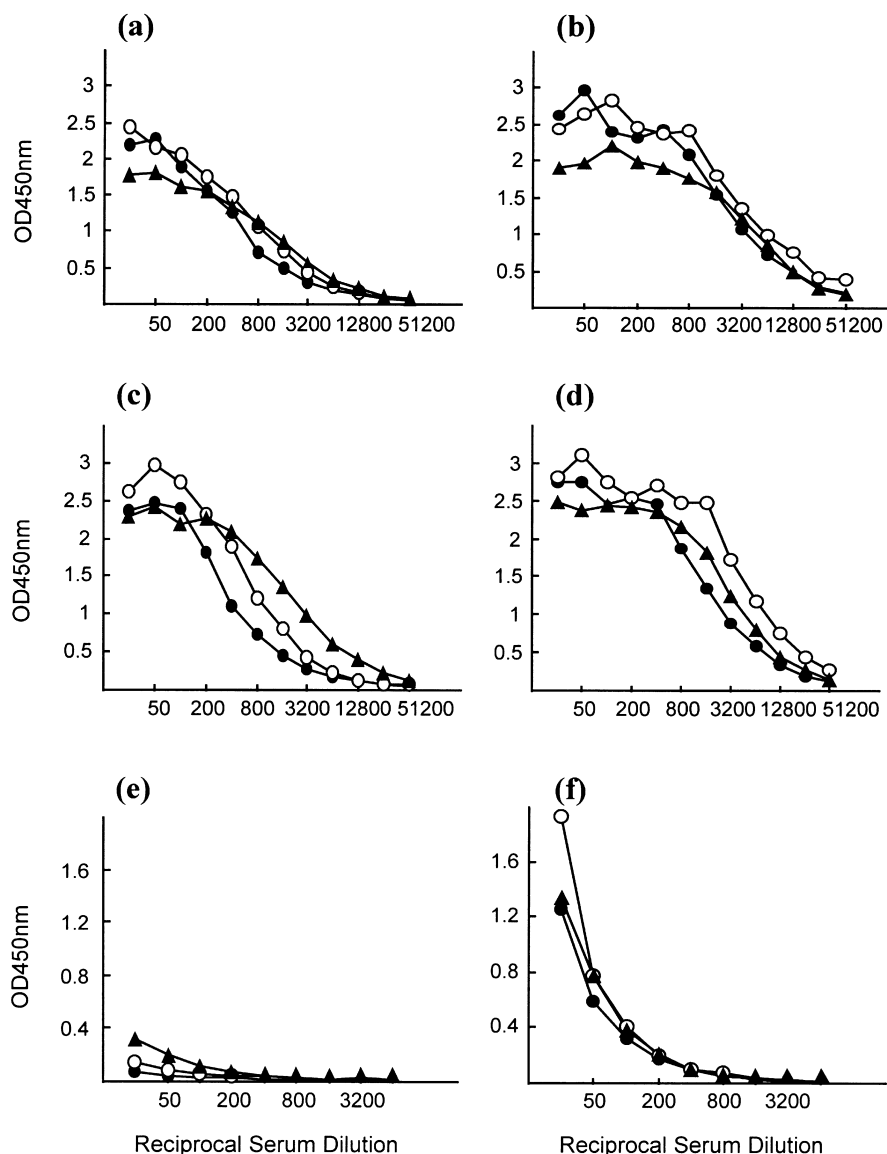


Fig. 1. IgG, IgG1 and IgG2a antibody production following intraperitoneal exposure to OVA or BSA. Groups of mice ($n = 5$) received $250 \mu\text{l}$ of 2% of OVA (a, c and e) or BSA (b, d and f) in PBS via intraperitoneal injection with treatment repeated 7 days later. 14 days after the initiation of exposure, mice were exsanguinated and pooled sera were tested for protein specific IgG (a and b), IgG1 (c and d) and IgG2a (e and f) antibody by ELISA. Results from three independent experiments are shown (●, Experiment 1; ○, Experiment 2; ▲, Experiment 3).

drawn from mice immunized ip with OVA in the presence of the adjuvant carrageenan; treatment which has been found previously in this laboratory to result in enhanced IgE and IgG (including IgG2a) antibody responses (Dearman *et al.*, 1999).

The isotype distribution of the antibody responses detected in pooled serum samples derived from BSA or OVA treated animals in the three independent experiments was remarkably consistent. In order to assess inter-animal variation in antibody production, in subsequent experiments analyses were performed on individual serum samples. Animals ($n = 10$) were exposed ip to 2% of BSA or OVA as described above and individual serum

samples analysed for IgG1 and IgG2a antibody production (Fig. 2). Some inter-animal variations in the levels of both anti-OVA and anti-BSA IgG1 antibodies were observed, with in each case a minority of animals failing to mount a vigorous IgG1 antibody response. As seen previously with pooled serum samples (cf Fig. 1), IgG1 responses to BSA tended to be somewhat more pronounced than those provoked by OVA. However, statistical analyses of the two populations revealed that the spread of IgG1 antibody responses provoked by BSA and OVA was not statistically significantly different. IgG2a antibody responses were considerably more variable. As seen previously (cf Fig. 1)

administration of OVA and BSA resulted in differential production of this subclass of IgG, with BSA stimulating relatively high titre IgG2a responses in the majority of animals, whereas only low levels of anti-OVA IgG2a antibody were observed. Statistical comparisons of anti-BSA and anti-OVA IgG2a antibody responses revealed that under conditions of equivalent IgG1 antibody responses, BSA and OVA displayed a statistically significant ($P > 0.001$) differential capacity to stimulate the production of IgG2a antibody.

Dose-response analyses of protein-specific antibody responses

Dose-response analyses were performed in order to determine whether the differences in antibody isotype production observed in response to BSA and OVA were quantitative rather than qualitative and therefore dependent simply on the strength of the immunogenic stimulus. Animals were exposed ip to various concentrations of BSA or OVA (1, 2 or 5%) and pooled serum samples analysed for the presence of IgG1 and IgG2a antibody by ELISA (Fig. 3; two independent experiments) and IgE anti-

body by PCA (Table 1; two independent experiments). Although there was some inter-experimental variation in antibody responses, in each experiment there was a pronounced dose-dependent increase in IgG1 anti-BSA and anti-OVA antibody production, with exposure to 5% of either protein provoking a substantial increase in IgG1 antibody expression compared with administration of 1% of material. IgG2a antibody responses provoked by BSA also showed a marked dose dependence, with 2 and 5% BSA provoking considerably more vigorous IgG2a responses than treatment with 1% BSA. Exposure to increasing concentrations of OVA under conditions where marked differences in immunogenicity with respect to IgG1 antibody responses were observed failed to induce a similar increase in IgG2a antibody production. As reported previously (Hilton *et al.*, 1997), the differential quality of immune responses induced by BSA and OVA with respect to IgE antibody responses was observed at higher doses also (Table 1) with BSA provoking no detectable or low titre IgE antibody only whereas OVA consistently stimulated relatively

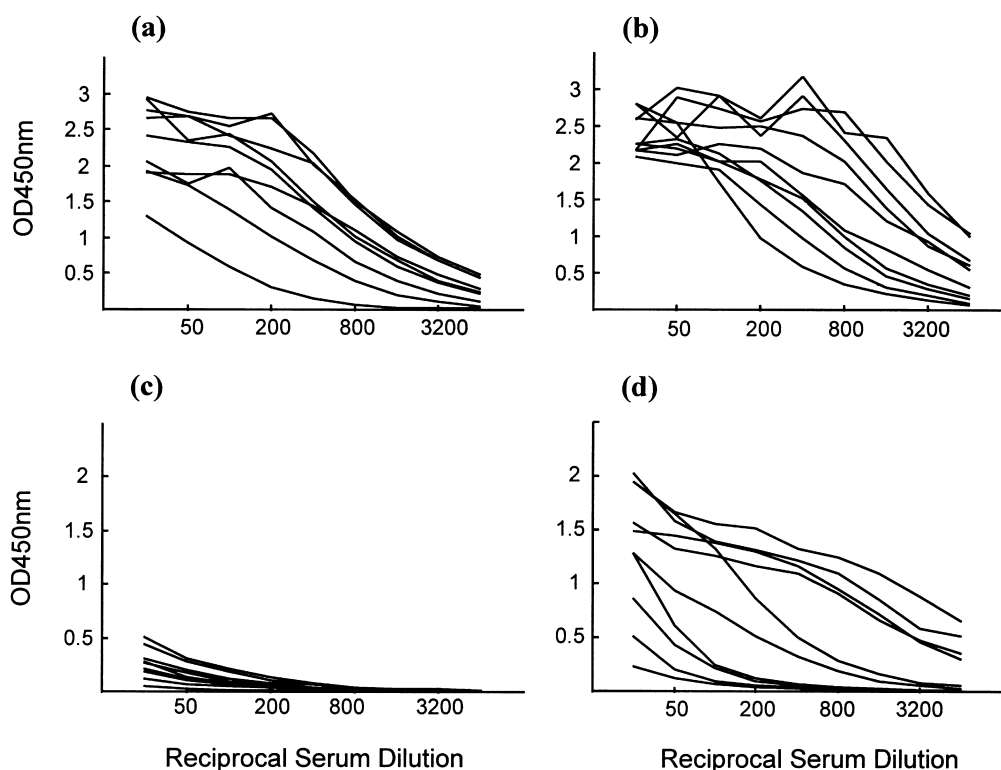


Fig. 2. Inter-animal variation in IgG1 and IgG2a antibody responses following intraperitoneal exposure to OVA or BSA. Groups of mice ($n = 10$) received $250 \mu\text{l}$ of 2% of OVA (a and c) or BSA (b and d) in PBS via intraperitoneal injection with treatment repeated 7 days later. 14 days after the initiation of exposure, mice were exsanguinated and individual serum samples were tested for protein specific IgG1 (a and b) and IgG2a (c and d) antibody by ELISA. Each individual line represents an individual serum sample. No statistically significant difference between anti-BSA and anti-OVA IgG1 titres (a vs b) was observed ($P = 0.313$). Anti-BSA and anti-OVA IgG2a titres (a vs d) were significantly different ($P < 0.001$).

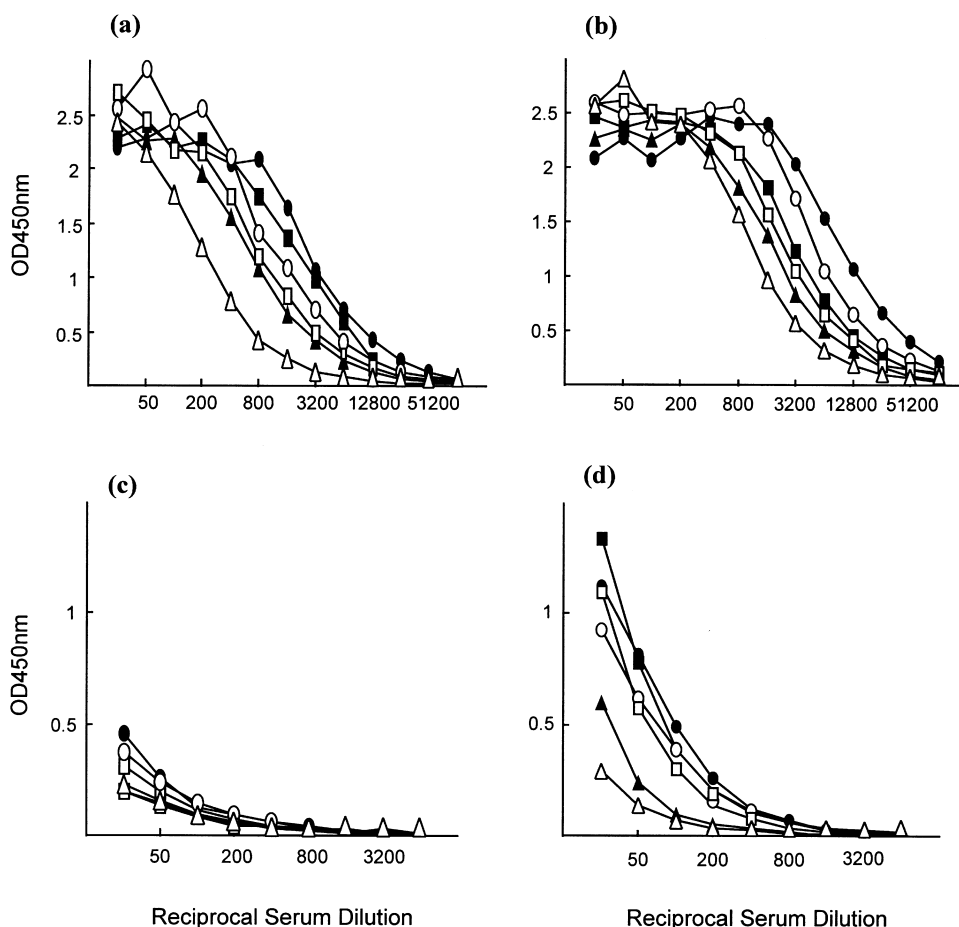


Fig. 3. Dose response of IgG1 and IgG2a antibody production following intraperitoneal exposure to OVA or BSA. Groups of mice ($n = 5$) received $250 \mu\text{l}$ of 5% ($\bullet$, $\circ$), 2% ($\blacksquare$, $\square$) or 1% ($\blacktriangle$, $\triangle$) of OVA (a and c) or BSA (b and d) in PBS via intraperitoneal injection with treatment repeated 7 days later. 14 days after the initiation of exposure, mice were exsanguinated and pooled sera were tested for protein specific IgG1 (a and b) and IgG2a (c and d) antibody by ELISA. Results from two independent experiments are shown (closed symbols, Experiment 1; open symbols, Experiment 2).

high titre IgE antibody production at all doses tested.

Influence of route of exposure on antibody isotype distribution

It has been suggested previously that the route of exposure may influence the quality of immune response induced, with exposure through mucosal surfaces such as the gastrointestinal or respiratory tracts preferentially stimulating T helper (Th) 2 type responses (Daynes *et al.*, 1990; Stumbles *et al.*, 1998). We have therefore compared the characteristics of antibody responses elicited in mice following ip exposure to OVA and BSA with those observed after intranasal administration (Fig. 4). As observed previously, ip exposure to 1% of protein resulted in a differential capacity to provoke detectable IgG2a antibody responses, with only administration of BSA resulting in IgG2a antibody expression. Consistent with previous experiments (Hilton *et al.*, 1997), intranasal application of pro-

tein resulted in less vigorous antibody responses than those provoked by ip exposure. However, under conditions where OVA and BSA stimulated equivalent levels of IgG and IgG1 antibody responses, detectable IgG2a antibody production was only observed following exposure to the latter protein. Also consistent with previous experiments, intranasal administration of BSA failed to provoke measurable IgE antibody production whereas a positive PCA reaction was recorded with undiluted serum from OVA-treated animals.

DISCUSSION

Two important conclusions can be drawn from the data presented here. The first is that, under the conditions of exposure employed, and irrespective of whether the proteins were administered by ip injection or intranasal instillation, OVA displayed a significantly greater potential to elicit IgE antibody production. This difference was not attributable to

Table 1. IgE production following intraperitoneal exposure to BSA and OVA; a dose-response analysis

Protein concn (%, w/v)	Reciprocal antibody titre			
	BSA		OVA	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
5	1	2	16	8
2	—	1	8	8
1	—	—	2	1

Groups of mice ($n = 5$) received $250 \mu\text{l}$ of 1%, 2% or 5% protein in PBS via intraperitoneal injection with treatment repeated 7 days later. 14 days after the initiation of treatment, mice were exsanguinated and pooled sera were tested for the presence of specific IgE antibody by PCA. IgE antibody titre is recorded as the highest dilution of serum resulting in a positive PCA reaction. A negative PCA reaction with neat serum is represented by a dash. Two independent experiments are shown.

quantitative variations in overall immunogenicity because OVA and BSA each stimulated strong total IgG and IgG1 antibody responses with, if anything, BSA provoking more vigorous IgG production. These data confirm and extend previous reports of the variable potential of OVA and BSA to elicit IgE responses in BALB/c strain mice (Hilton *et al.*, 1994, 1997). Furthermore, the data indicate that this differential activity is not confined to a single route of exposure and applies to both mucosal and non-mucosal sites. This is a relevant consideration since there is evidence that, in some instances, the quality of immune response induced by an antigen is dependent on the route of exposure, and importantly whether encounter with antigen is via a mucosal or non-mucosal tissue (Daynes *et al.*, 1990; Stumbles *et al.*, 1998).

The second conclusion is that against this background of comparable IgG and IgG1 responses and variable IgE responses, OVA and BSA exhibit clear differences in their ability to provoke the production of IgG2a subclass antibodies. IgG2a responses elicited by exposure of mice to BSA were considerably more vigorous than those associated with exposure to OVA. In accord with the variable IgE responses observed, differential stimulation of IgG2a antibodies by OVA and BSA was independent of the route of exposure. Taken together, these data support a hypothesis that, under conditions of comparable overall immunogenicity, OVA and BSA elicit in mice qualitatively distinct immune responses characterized by a reciprocal potential to induce IgE and IgG2a antibody production. It is relevant to consider these responses in the context of the immunobiological processes that serve to regulate the isotype distribution of induced antibodies.

It has been well established that in mice (and in other species) the integrity of IgE antibody production is regulated by cytokines. The initiation and maintenance of IgE responses are dependent upon the availability of interleukin (IL)-4 (Finkelman *et al.*, 1986, 1988b), whereas another cytokine, interferon γ (IFN- γ) antagonizes IgE antibody production (Finkelman *et al.*, 1988a). These

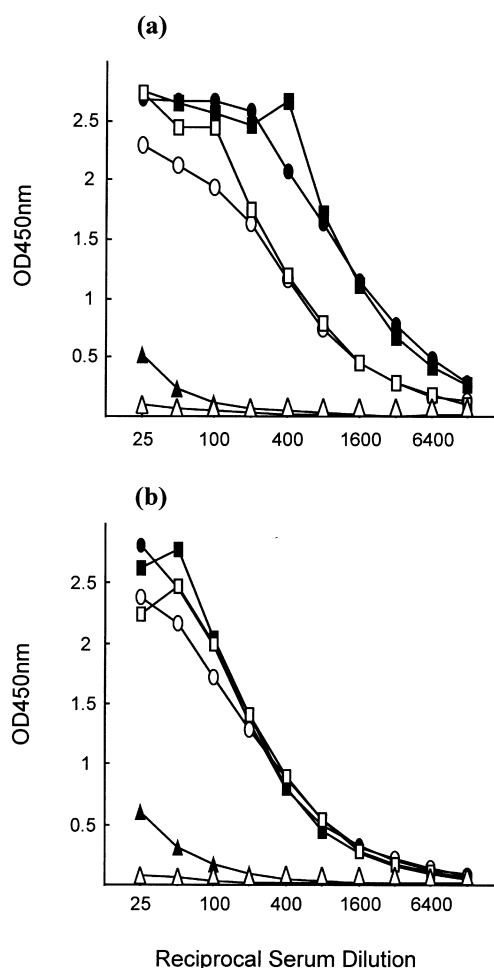


Fig. 4. Influence of route of exposure on IgG subclass distribution. Groups of mice ($n = 10$) received $250 \mu\text{l}$ of 1% of BSA (closed symbols) or OVA (open symbols) in PBS by intraperitoneal injection with treatment repeated 7 days later (a). Alternatively, animals ($n = 10$) received $5 \mu\text{l}$ per nostril of 5% of BSA (closed symbols) or OVA (open symbols) in PBS with treatment repeated 2 and 9 days later (b). 14 days after the initiation of exposure, mice were exsanguinated and pooled sera were tested for protein-specific IgG ($\bullet$, $\circ$), IgG1 ($\blacksquare$, $\square$) and IgG2a ($\blacktriangle$, $\triangle$) antibody by ELISA. Results of a single representative experiment are shown.

immunoregulatory cytokines are the products, respectively, of Th1 and Th2 cells, these being subpopulations of CD4⁺ Th cells (Mosmann *et al.*, 1986; Mosmann and Coffman, 1989). This leads to the speculation that the qualitatively variable responses induced by OVA and BSA are associated with and, at least in part, dependent on the selective development of Th subpopulations, with OVA favouring Th2 responses and the production of IL-4 and BSA eliciting preferential Th1-type responses and the production of IFN- γ . The variable IgG2a antibody responses provoked by these proteins lend further support to this hypothesis.

In addition to their reciprocal regulation of IgE responses, the cytokine products of Th cell subpopulations also serve to influence the expression of other antibody isotypes (Finkelman *et al.*, 1990; Snapper and Mond, 1993). Of particular relevance is the fact that Th1 cells and IFN- γ promote class switching to, and the production of, IgG2a antibody (Finkelman *et al.*, 1988a; Snapper and Mond, 1993; Snapper and Paul, 1987; Stevens *et al.*, 1988). The fact that BSA was shown to provoke more vigorous IgG2a production than did OVA provides supplementary evidence that the former protein appears to be associated with a selective Th1-type immune response and the production of IFN- γ . Similarly, the data suggest that, in contrast, exposure to OVA causes a preferential Th2-type response where the predominance of IL-4 over IFN- γ production would be permissive for IgE responses, but not IgG2a at a level comparable with BSA.

A precedent for the stimulation of variable immune responses consistent with differential Th cell development is to be found with chemical allergens. It has been shown that chemical allergens, such as 2,4-dinitrochlorobenzene and oxazolone, which cause contact sensitization but which apparently do not induce sensitization of the respiratory tract, fail to stimulate in mice IgE responses, but are associated instead with strong IgG2a antibody production. Conversely, other chemical allergens, such as trimellitic anhydride and phthalic anhydride, which are known causes of respiratory sensitization provoke in mice IgE responses, but induce only comparatively weak IgG2a production (Dearman and Kimber, 1991, 1992).

Drawing these elements together, the proposal is that the variable responses stimulated by OVA and BSA are secondary to the development following immunization of divergent T cell responses wherein the balance of available cytokines determines the patterns of antibody isotype production observed. The induction of preferential Th2-type responses by OVA would be consistent with more vigorous allergenic activity exhibited by this protein. The implication is that an important characteristic of protein allergens is the ability to initiate immunologic signals that will direct selective development of Th2 cells. The corollary is that proteins which fail to do

this and which instead are associated with Th1-type responses will not stimulate IgE antibody production and, as a consequence, will lack allergenic activity.

That the nature of immune responses provoked in mice by OVA is to a large extent determined by the characteristics of T lymphocyte subpopulations and their secreted products receives support from recent investigations performed by Kim *et al.* (1997). These authors demonstrated in BALB/c strain mice that whereas immunization with OVA alone resulted in little IFN- γ production and only weak IgG2a antibody responses, exposure of animals to a fusion protein of ovalbumin with the cytokine IL-12 resulted in strong IgG2a responses and vigorous IFN- γ secretion (Kim *et al.*, 1997). The significance of these data is that IL-12 is known to play a pivotal role in promoting selective Th1 responses at the expense of Th2-type activity (Trinchieri, 1993).

The assumption is that the variable responses induced by OVA and BSA are determined partly by differences in their processing and subsequent presentation by professional antigen presenting cells and that this itself is secondary to differences in protein structure. What physicochemical properties will favour the generation of Th2-type responses and IgE production is far from clear, although there is evidence to suggest that modification of the structure and/or properties of OVA will impact on the characteristics of induced immune responses (Peng *et al.*, 1998a,b).

Finally, it is important to emphasize that while the observed differences between OVA and BSA with respect to induced immune responses are robust, it is not known at which point the characteristics of such responses diverge or for how long they persist. To address this, more detailed kinetic analyses will be required. Nor is it known to what extent OVA and BSA will serve as paradigms for other proteins of differing allergenic potential. Notwithstanding these precautionary notes, the evidence presented here establishes that proteins of varying allergenic potential provoke in mice qualitatively discrete immune responses. The challenge now is to determine whether the immunological properties of OVA if shared with other protein allergens can be utilized in the toxicological evaluation of sensitizing activity.

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