



Characteristics of Antibody Responses Induced in Mice by Protein Allergens

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Abstract—Whereas many foreign proteins are immunogenic, only a proportion is also allergenic, having the capacity to induce the quality of immune response necessary to support the production of IgE antibody. We have demonstrated previously that intraperitoneal administration to mice of proteins such as ovalbumin (OVA) or the industrial enzyme *A. oryzae* lipase, which possess significant allergenic potential, stimulates the production of both IgG and IgE antibody. Identical exposure to bovine serum albumin (BSA), a protein with limited potential to cause immediate respiratory or gastrointestinal hypersensitivity reactions, induced IgG responses only. In the current investigations, the quality of immune responses induced following exposure to these proteins via mucosal tissue (intranasal) has been compared with those provoked following administration via a non-mucosal (intraperitoneal) route of exposure. Intranasal or intraperitoneal administration of BSA, OVA or *A. oryzae* lipase elicited in each case vigorous IgG and IgG1 antibody responses. For all three proteins, at every concentration tested, and via both routes of exposure, IgG1 antibody titres paralleled closely IgG titres. However, the three materials displayed a differential potential to provoke IgE responses and this correlated with their known allergenic potential in humans. Thus, OVA and *A. oryzae* lipase stimulated strong IgE antibody responses, whereas BSA provoked low titre IgE only at the highest concentration tested (5% administered intraperitoneally). The quality of induced responses was not affected by the route of exposure. It would appear, therefore, that the stimulation of IgG and IgG1 antibody responses is a reflection of protein immunogenicity whereas protein allergenicity is associated with the induction of strong IgE responses. © 1997 Elsevier Science Ltd. All rights reserved.

Abbreviations: BSA = bovine serum albumin; ELISA = enzyme linked immunosorbent assay; NGS = normal goat serum; OVA = ovalbumin; PBS = phosphate buffered saline; PCA = passive cutaneous anaphylaxis; RAST = radioallergosorbent test.

INTRODUCTION

The development of allergic hypersensitivity to occupational, environmental and food proteins encountered through the respiratory and/or gastrointestinal tracts is an important health issue. Such include allergies to common foods including egg, crustacea and legumes such as peanut (Metcalf, 1985; Metcalfe *et al.*, 1996), asthma to aeroallergens, including house dust mite and pollens such as birch or ragweed, and occupational respiratory sensitization among egg-processing workers (Bernstein *et al.*, 1987; Blair Smith *et al.*, 1987) or those exposed to proteolytic enzymes used in the detergent industry (Franz *et al.*, 1971; Juniper and Roberts, 1984; Shapiro and Eisenberg, 1971) or to other commercial enzymes (Bossert *et al.*, 1988). The allergic hypersensitivity reactions induced by such proteins are mediated by IgE antibody-depen-

dent mechanisms. Thus, for example, in individuals with symptoms of respiratory allergy to egg proteins, commonly ovalbumin (OVA), or to detergent enzymes, skin prick testing generally elicits immediate type cutaneous wheal and flare reactions and significant respiratory responses are observed following specific bronchial provocation challenge (Bernstein *et al.*, 1987; Blair Smith *et al.*, 1987; Franz *et al.*, 1971; Juniper *et al.*, 1977). In addition, protein allergen-specific serum IgE antibodies are detected frequently using radioallergosorbent tests (RAST) or enzyme-linked immunosorbent assays (ELISA) (Bernstein *et al.*, 1987; Blair Smith *et al.*, 1987; Sarlo *et al.*, 1990).

There have been various attempts to characterize immune responses to protein allergens in experimental animals. In the majority of these, to generate a strong and persistent protein-specific IgE response, animals have been exposed systemically to protein in the presence of materials such as *Bordetella pertussis* or alum—substances that are

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known to act as adjuvants for IgE antibody responses (Katz, 1980). These experiments have encompassed exposure to whole allergenic proteins such as OVA (Hayglass and Stefura, 1991; Oshiba *et al.*, 1996; Yang and Hayglass, 1993), extracts of allergens like ragweed pollen (Hayglass and Stefura, 1991; Sur *et al.*, 1996; Yang and Hayglass, 1993) and purified dominant epitopes of allergens such as, for example, the major birch pollen allergen, rBet vi (Bauer *et al.*, 1997). Alternative approaches have employed endpoints other than the analysis of serum IgE antibody. In the guinea pig, which is very sensitive to protein-induced anaphylactic reactions (Griffiths-Johnson and Karol, 1991), the major anaphylactic antibody is IgG1. The production of protein-specific IgG1 antibody in guinea pigs following inhalation or intratracheal exposure has been utilized with some success for the ranking of the respiratory sensitizing potential of detergent enzymes (Ritz *et al.*, 1993; Sarlo *et al.*, 1991). Other investigators have analysed antibody responses induced in mice following intratracheal or intranasal exposure to detergent enzymes (Kawabata *et al.*, 1996; Robinson *et al.*, 1996). While IgE responses were weak and somewhat variable, particularly after intranasal administration of protein allergens, consistent dose-response relationships were observed for the production of specific IgG1 antibody, an isotype which in mice is regulated similarly, but not identically, to IgE. Thus, conditions under which IgE production is provoked, such as infection with the nematode parasite *Nippostrongylus brasiliensis*, also result in vigorous IgG1 responses (Finkelman *et al.*, 1986). In addition, IgE and IgG1 antibody responses are promoted by T helper 2 (Th2) cell activation and production of the Th2-cell cytokine interleukin 4 (IL-4) (Stevens *et al.*, 1988). However, the influence of IL-4 on IgG1 antibody production is bimodal, with high concentrations of this cytokine being suppressive for IgG1, but not IgE, antibody responses (Snapper *et al.*, 1988).

We have demonstrated previously that systemic (ip) exposure to protein allergens (that do not possess proteolytic properties) provokes the appearance of specific IgE antibody (Hilton *et al.*, 1994). Ip administration to mice of OVA, a major allergenic component of egg protein which causes respiratory allergy in exposed workers (Bernstein *et al.*, 1987; Blair Smith *et al.*, 1987) and is a common food allergen (Metcalf, 1985; Renz *et al.*, 1993), stimulated the production of specific IgE antibody and a vigorous IgG response. Exposure to an industrial enzyme, a lipase from *Aspergillus oryzae*, caused the same pattern of antibody expression. Administration under identical conditions of bovine serum albumin (BSA), an immunogenic protein that appears to have only a limited potential to cause respiratory sensitization (Joliat and Weber, 1991) and allergy via the gastrointestinal tract (Fiocchi *et al.*, 1995), failed to elicit the production of detectable IgE antibody. In contrast, protein-specific IgG

antibody titres comparable with those observed following treatment with OVA or *A. oryzae* lipase were observed.

We have now examined the influence of route of exposure to proteins on the induction of antibody responses. It has been reported that the elicitation of immune responses is influenced markedly by the lymphoid microenvironment, with Th2 type responses predominating in lymphoid tissue draining the site of mucosal surfaces such as the respiratory or gastrointestinal tracts (Daynes *et al.*, 1990; Taguchi *et al.*, 1990). In the present series of experiments we have therefore compared the quality of immune responses provoked in mice following ip exposure to OVA, *A. oryzae* lipase or BSA with those observed following delivery via the intranasal route. We have extended our analyses also to include measurement of IgG1 subclass antibody level production.

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 with approximately 2.5% animal protein.

Test materials

Bovine serum albumin (BSA) and ovalbumin (OVA) were obtained from Sigma Chemical Co. (Dorset, UK). The lipase from *Aspergillus oryzae* was provided by Unilever Environmental Safety Laboratory (Bedford, UK).

Sensitization procedure

Groups of mice ($n = 10$) received 250 μ l of various concentrations of test material in phosphate buffered saline (PBS) via ip injection. 7 days later treatment was repeated. Alternatively, groups of mice ($n = 10$) received 5 μ l per nostril of various concentrations of test material 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 and serum prepared and stored at -20°C until analysis.

Measurement of protein-specific IgG and IgG1 antibody

Protein-specific IgG and IgG1 antibodies were detected using enzyme-linked immunosorbent assays (ELISA). Plastic microtitre plates were coated with 100 μ g/ml BSA or OVA or 50 μ g/ml *A. oryzae* lipase 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 in duplicate 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 peroxidase-labelled rat monoclonal anti-mouse IgG1 (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 addition of 0.5 M citric acid. Between each incubation, plates were washed with PBS containing 0.05% Tween 20. Substrate conversion was measured as optical density at 450 nm using an automated reader (Flow Laboratories, Irvine, UK). Antibody titres were expressed as the reciprocal of the serum dilution at which half-maximal OD 450 nm readings (of the serum sample) were observed. Background OD 450 nm readings from serum samples derived from control (untreated) animals were less than 0.075 (ELISAs for BSA and OVA) or less than 0.25 (ELISA for lipase) at the maximum serum concentration.

Measurement of protein-specific IgE antibody

The presence in the serum of IgE antibodies was detected by passive cutaneous anaphylaxis (PCA). Pooled serum samples were diluted serially in physiological saline and injected (30 µl) into the dermis of the ears of naive recipient mice. 2 days later, 0.25 mg protein, together with Evans Blue dye (1.25 mg) in 250 µl 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.

RESULTS

Comparison of antibody responses induced by intranasal or ip exposure to proteins

In preliminary experiments, we examined serum antibody responses following i.p. or intranasal exposure to OVA, *A. oryzae* lipase or BSA. 14 days following the initiation of treatment, mice were exsanguinated, serum samples pooled and serial doubling dilutions prepared for each experimental group. The presence of IgG and IgG1 antibodies was measured by ELISA (Fig. 1, representative experiment). As reported previously (Hilton *et al.*, 1994), ip administration of 1% solutions of all three materials stimulated vigorous IgG antibody responses, with significant substrate conversion observed at dilutions of below 1 in 12,800 (Fig. 1a). In addition, under these conditions of exposure, the proteins each provoked the production of specific

IgG1 antibody, with, in each case, similar dilution profiles to those observed for specific IgG antibody (Fig. 1b). Intranasal exposure of animals to 1% solutions of these materials also induced significant expression of specific IgG and IgG1 antibody (Fig. 1c, d). Levels of antibody were, however, considerably lower than those stimulated by ip administration of the same protein, both with respect to maximal optical density readings, and the serum dilutions at which significant substrate conversion was detected. Intranasal treatment with 5% solutions of OVA, BSA and *A. oryzae* lipase resulted in substantial IgG and IgG1 antibody production (Fig. 1e, f). Antibody levels comparable with those induced by ip exposure to protein were not achieved, however. As reported previously (Hilton *et al.*, 1994), no significant cross-reactivity was detected between BSA and OVA, with similar low levels of background staining observed with BSA antisera and OVA substrate, and *vice versa*, to those recorded with naive mouse serum or serum derived from control animals that had received PBS alone (data not shown).

The presence of protein-specific IgE antibody in these serum samples was measured by PCA assay. Titres of IgE antibody are recorded in Table 1 (Expt 1) with the IgG and IgG1 titres (at which half-maximal OD450 readings were observed) displayed in Fig. 1. As illustrated in Fig. 1, ip administration of BSA provoked substantial IgG and IgG1 antibody production; however, under these conditions of exposure IgE antibody was not detectable. Intranasal administration of either 1% or 5% BSA induced lower titres of IgG and IgG1 antibody and again failed to stimulate the production of measurable IgE. Thus, undiluted serum from mice exposed to BSA by either route did not elicit a PCA reaction. In contrast, either ip or intranasal treatment with OVA resulted in the appearance of detectable IgE antibody in addition to IgG and IgG1 antibody production. Exposure via the ip route resulted in higher titre IgG and IgE antibody responses. A similar pattern of antibody expression was induced following treatment with the industrial enzyme, *A. oryzae* lipase. With respect to IgE antibody production, this material was the most active, inducing titres of 1 in 32 and 1 in 16 after ip and intranasal exposure, respectively.

The differential ability of these proteins to stimulate specific IgE antibody responses regardless of route of exposure was confirmed in further experiments. Animals received ip injections or intranasal application of 1% or 5% solutions of proteins, respectively. IgG and IgG1 antibody titres of pooled serum samples were determined by ELISA and IgE antibody analysed by PCA assay (Table 1; Expts 2 and 3). Consistent with previous experiments, exposure to BSA via either route resulted in vigorous IgG and IgG1 antibody production, but failed to stimulate detectable IgE antibody expression. In both experiments, however, intranasal or ip administration of OVA or *A. oryzae* lipase

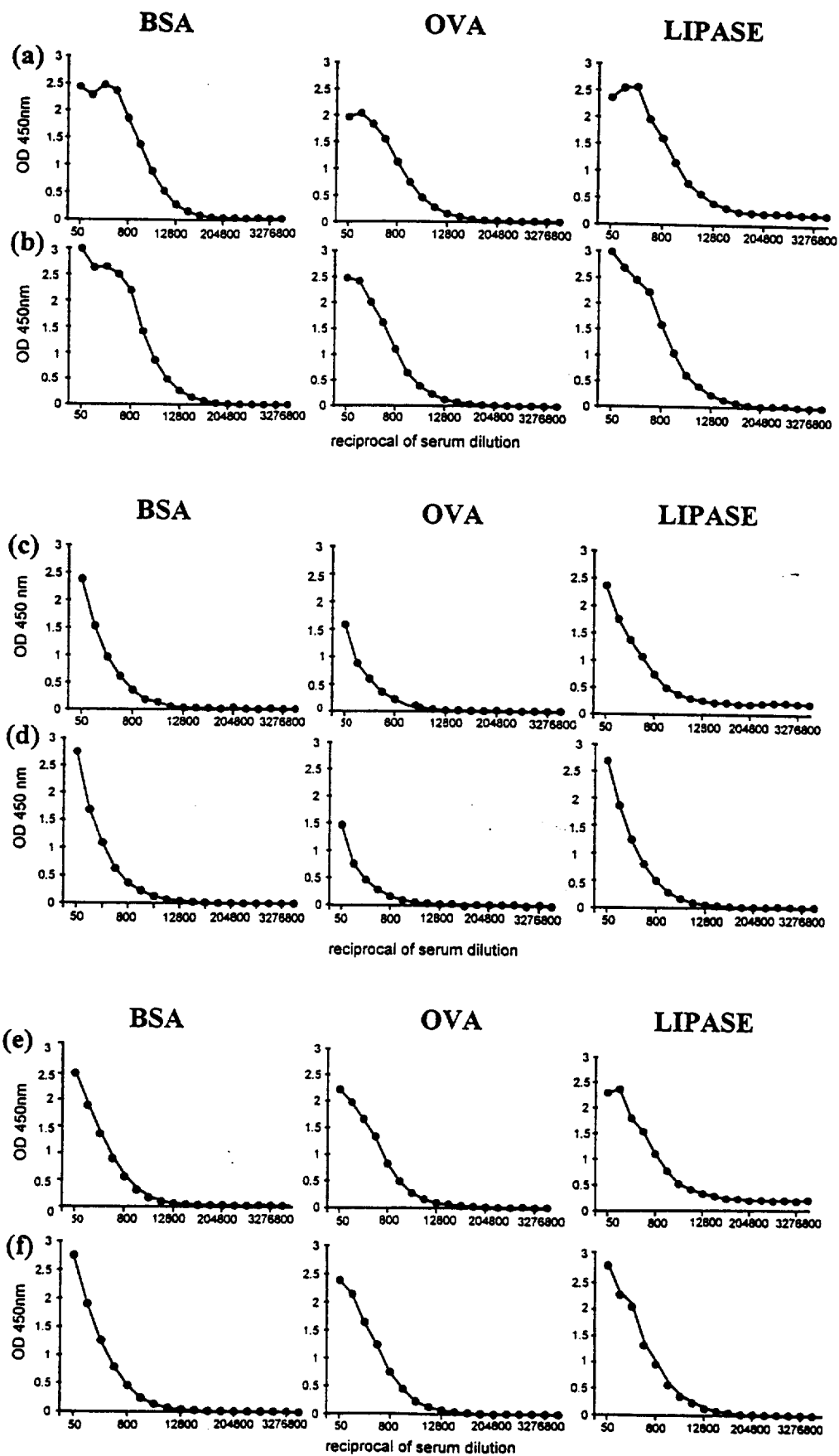


Fig. 1 [Caption opposite].

Table 1. IgG, IgG1 and IgE production in three independent experiments following exposure to BSA, OVA or *A. oryzae* lipase

Antibody class		Reciprocal antibody titre								
		BSA			OVA			Lipase		
		ip 1%	in 1%	in 5%	ip 1%	in 1%	in 5%	ip 1%	in 1%	in 5%
IgG	Expt 1	800	50	100	400	50	200	400	100	400
	Expt 2	800	ND	100	400	ND	200	400	ND	400
	Expt 3	800	ND	400	200	ND	200	400	ND	400
IgG1	Expt 1	400	50	50	200	50	200	400	50	100
	Expt 2	400	ND	50	200	ND	200	400	ND	100
	Expt 3	800	ND	400	100	ND	400	400	ND	200
IgE	Expt 1	—	—	—	4	1	1	32	8	16
	Expt 2	—	ND	—	4	ND	1	32	ND	16
	Expt 3	—	ND	—	4	ND	1	16	ND	32

ip = intraperitoneal in = intranasal ND = not determined

Groups of mice ($n = 10$) received $250 \mu\text{l}$ of 1% of protein in PBS via ip injection with treatment repeated 7 days later, or $5 \mu\text{l}$ per nostril of 1% or 5% of protein in PBS with treatment repeated 2 and 9 days later. 14 days after the initiation of exposure, mice were exsanguinated and pooled sera were tested for protein-specific IgG and IgG1 antibody by ELISA or analysed for the presence of specific IgE antibody by PCA assay. IgG and IgG1 antibody titres are expressed as the highest dilution at which half-maximal OD 450 nm readings were observed. 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.

Table 2. IgG and IgG1 production recorded in individual serum samples following intranasal exposure to BSA, OVA and *A. oryzae* lipase

Animal no.		Reciprocal antibody titre for individual animals					
		BSA		OVA		Lipase	
		IgG	IgG1	IgG	IgG1	IgG	IgG1
1		1600	1600	400	400	800	400
2		1600	1600	400	200	800	400
3		400	200	200	200	400	400
4		200	200	200	100	400	400
5		200	200	100	50	400	400
6		100	200	100	50	400	200
7		100	100	100	50	400	100
8		100	50	50	50	200	100
9		100	50	50	50	200	100
10		50	50	50	25	200	100
Mean titre		445	425	165	118	420	260
Median titre		100–200	200	100	50	400	200–400

Groups of mice ($n = 10$) received $5 \mu\text{l}$ per nostril 5% of protein in PBS with treatment repeated 2 and 9 days later. 14 days after the initiation of exposure, mice were exsanguinated and individual sera were tested for protein-specific IgG and IgG1 antibody by ELISA. IgG and IgG1 antibody titres are expressed as the highest dilution at which half-maximal OD 450 nm readings were observed.

provoked measurable IgE and IgG antibody production. There was little inter-experimental variation in IgG and IgE titres for all three materials, with the exception of intranasal exposure to BSA, where IgG and IgG1 titres varied from 1 in 100 to 1 in 400 and 1 in 50 to 1 in 400, respectively. Despite this variation, it is clear that with respect to IgG and IgG1 antibody responses, BSA is at least as immunogenic as OVA and *A. oryzae* lipase under these conditions and routes of exposure.

Inter-animal variation in IgG and IgG1 antibody production following intranasal exposure

Given the variation in mean IgG1 titre following intranasal exposure to BSA, analyses were per-

formed on individual serum samples. Mice ($n = 10$ per group) received 5% solutions of BSA, OVA or *A. oryzae* lipase delivered intranasally. 14 days after the initiation of exposure, animals were exsanguinated, individual serum samples prepared, serially diluted and analysed for IgG and IgG1 antibody by ELISA (Table 2). Titres are expressed as the reciprocal of the dilution at which half-maximal OD 450 nm readings were obtained. There was some variation in individual titres, particularly for BSA-stimulated animals where IgG and IgG1 titres varied from 1 in 1600 to 1 in 50. Responses to OVA and *A. oryzae* lipase were somewhat more consistent, with IgG titres ranging from 1 in 400 to 1 in 50 and 1 in 800 to 1 in 200, respectively, while IgG1

Fig. 1. IgG and IgG1 antibody production following exposure to BSA, OVA or *A. oryzae* lipase. Groups of mice ($n = 10$) received $250 \mu\text{l}$ of 1% of protein in PBS via ip injection with treatment repeated 7 days later (a) and (b); or $5 \mu\text{l}$ per nostril of 1% (c) and (d); or 5% (e) and (f) of protein in PBS with treatment repeated 2 and 9 days later. 14 days after the initiation of exposure, mice were exsanguinated and pooled sera were tested for protein-specific IgG antibody (a)(c) and (e) and IgG1 antibody (b)(d) and (f) by ELISA.

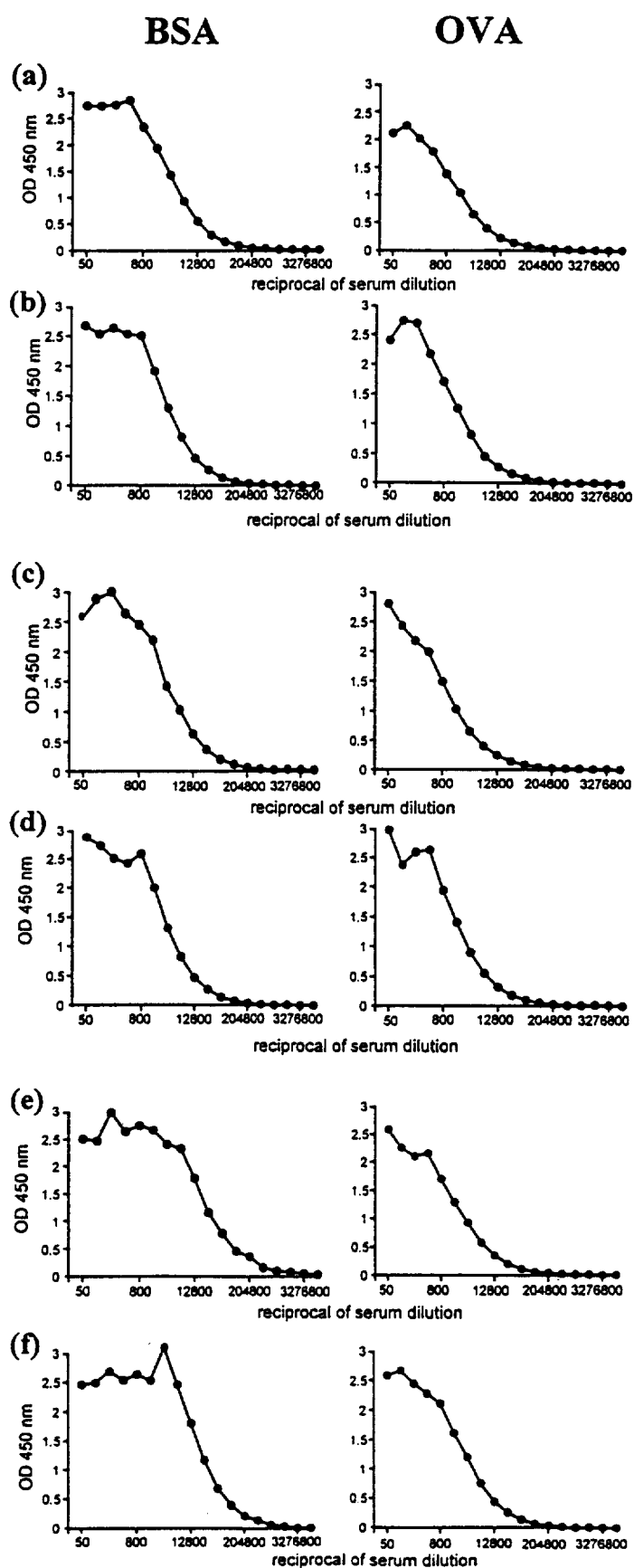


Fig. 2 [Caption opposite].

Table 3. IgG, IgG1 and IgE production following i.p. exposure to BSA and OVA; a dose-response analysis

Antibody class	Reciprocal antibody titre					
	BSA			OVA		
	1%	2%	5%	1%	2%	5%
IgG	1600	1600	6400	400	400	800
IgG1	800	800	6400	400	400	800
IgE	—	—	1	—	2	8

Groups of mice ($n = 10$) received 250 μ l of 1%, 2% or 5% protein in PBS via ip 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 and IgG1 antibody by ELISA or analysed for the presence of specific IgE antibody by PCA assay. IgG and IgG1 antibody titres are expressed as the reciprocal of the highest dilution at which half-maximal OD 450 nm readings were observed. 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.

titres ranged from 1 in 400 to 1 in 25 and 1 in 400 to 1 in 100, respectively. Interestingly, in all cases IgG1 antibody responses for individual animals paralleled closely IgG antibody titres. Thus, for instance, BSA-exposed animal number 1 displayed high titre IgG antibody (1 in 1600) and a correspondingly high IgG1 titre (1 in 1600), while OVA-treated animal number 8, for example, expressed low levels of IgG (1 in 50) and IgG1 (1 in 50). Analysis of mean and median antibody titres for each treatment group revealed that BSA was at least as immunogenic as OVA and *A. oryzae* lipase with respect to both IgG and IgG1 antibody production.

Dose-response analyses of protein-specific antibody production

Experiments were performed to determine whether the differential quality of immune responses induced by exposure to BSA and OVA with respect to IgE antibody production was observed at higher doses also. Animals received ip injections of 1, 2 or 5% solutions of proteins. 14 days after initiation of exposure, mice were exsanguinated and pooled serum samples prepared for each treatment group. Doubling serial dilutions of serum were prepared and analysed for the presence of IgG and IgG1 antibody by ELISA and IgE antibody by PCA assay. The dilution curves for IgG and IgG1 antibody production are illustrated in Fig. 2 and IgG and IgE antibody titres recorded in Table 3. Ip exposure to increasing concentrations of BSA or OVA resulted in increasingly vigorous IgG and IgG1 antibody responses, with respect to the lowest serum concentrations at which maximal OD 450 readings were observed and at which significant substrate conversion was detected (Fig. 2). This elevation in antibody production was reflected also by an increased titre (half-maximal OD 450 nm readings), from 1 in 1600 to 1 in 6400 for anti-BSA

IgG and from 1 in 400 to 1 in 800 for anti-OVA IgG which were paralleled by increased IgG1 antibody titres (Table 3). Anti-OVA IgE titres also increased markedly with exposure concentration. In the experiment illustrated in Table 3, ip exposure to 1% OVA failed to elicit detectable IgE, although titres as high as 1 in 4 were recorded in previous experiments (cf. Table 1). Treatment with 2% and 5% OVA stimulated IgE antibody expression with titres of 1 in 2 and 1 in 8, respectively. As seen previously, (cf. Table 1) exposure to 1% BSA failed to induce measurable IgE antibody. Administration of 2% solutions of BSA also failed to stimulate IgE antibody production. Treatment with 5% of BSA, however, did result in a weak IgE antibody response, with undiluted serum eliciting a positive PCA reaction.

Finally, experiments were performed to determine whether the strong association between IgG and IgG1 antibody production was observed also at lower protein exposure concentrations, equivalent to those used by other investigators (Robinson *et al.*, 1996). Mice received intranasal applications of 1, 0.1, 0.01 and 0.001% of BSA, OVA or *A. oryzae* lipase. Exposure to concentrations of OVA or *A. oryzae* lipase of below 1% failed to induce the expression of detectable IgG or IgG1 antibody under these conditions (data not shown). Antibody production following treatment with BSA is illustrated in Fig. 3. Exposure to both 1% and 0.1% BSA stimulated the expression of detectable IgG1 and IgG antibody, although antibody levels achieved following treatment with 0.1% BSA were considerably lower. In each case, however, IgG and IgG1 titres were comparable.

DISCUSSION

The data available from the present investigations demonstrate that ip or intranasal administration of

Fig. 2. Dose response of IgG and IgG1 antibody production following ip exposure to BSA and OVA. Groups of mice ($n = 10$) received 250 μ l of 1% (a) and (b); 2% (c and d); or 5% (e) and (f) of protein in PBS via ip 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 antibody (a)(c) and (e); and IgG1 antibody (b)(d) and (f) by ELISA.

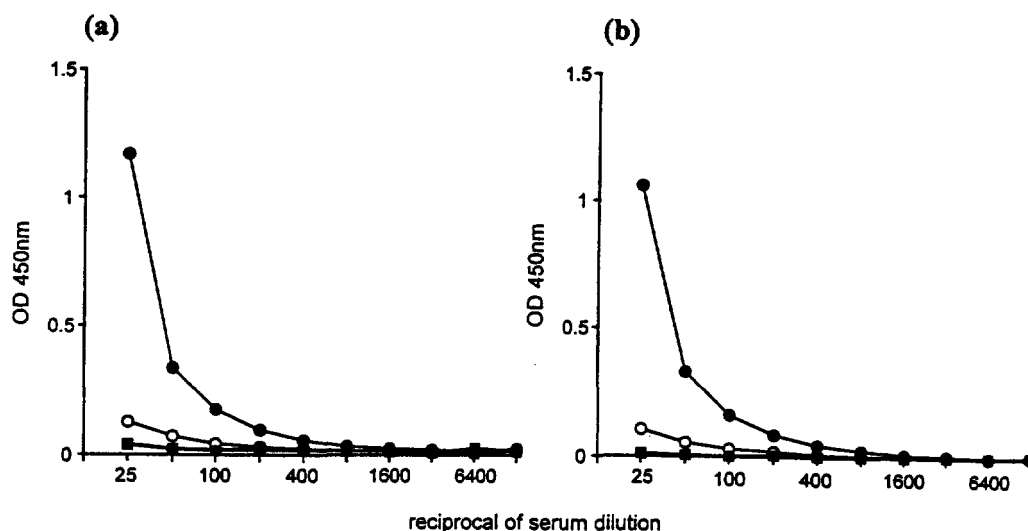


Fig. 3. Dose response of IgG and IgG1 antibody production following intranasal exposure to BSA. Groups of mice ($n = 10$) received $5 \mu\text{l}$ per nostril 1% (●), 0.1% (○), 0.01% (■) and 0.001% (□) BSA in PBS with treatment repeated 2 and 9 days later. 14 days after the initiation of exposure, mice were exsanguinated and pooled sera were tested for protein-specific IgG (a) and IgG1 (b) antibody by ELISA.

immunogenic or allergenic proteins induces vigorous IgG and IgG1 antibody responses. Titres of protein-specific IgG1 antibody paralleled closely those observed for IgG, via both routes of exposure for all three proteins and at every concentration tested. Under these conditions of exposure, the three materials displayed considerable differences in potency with respect to the induction of specific IgE responses, which apparently reflect their differential allergenic potential in humans. Thus *A. oryzae* lipase, an industrial enzyme which is a potent occupational respiratory sensitizer (Bossert *et al.*, 1988), provoked high titre IgE antibody production (1 in 32 and 1 in 16) following administration ip or intranasally at 1% or 5% concentrations, respectively. Exposure to OVA, a common food (Metcalf, 1985; Renz *et al.*, 1993) and respiratory allergen (Bernstein *et al.*, 1987; Blair Smith *et al.*, 1987), also resulted in significant IgE responses, with lower titres than those elicited by treatment with lipase. In contrast, administration of BSA, an immunogenic protein which causes respiratory or gastrointestinal hypersensitivity reactions relatively rarely (Fiocchi *et al.*, 1995; Joliat and Weber, 1991), stimulated a weak IgE antibody response only at the highest concentration of protein (5%) given via ip injection. Such absent or low titre anti-BSA IgE responses were observed, moreover, under conditions whereby BSA was at least as immunogenic as OVA or *A. oryzae* lipase with respect to IgG and IgG1 antibody production. Protein allergenicity was associated therefore with strong IgE antibody responses, whereas vigorous IgG and IgG1 reactions were a reflection apparently of protein immunogenicity.

Delivery of the proteins via a mucosal (intranasal) or a non-mucosal (ip) route did not affect the

quality of immune response induced. Thus, intranasal or ip administration of all three materials stimulated significant IgG and IgG1 antibody production, while only OVA and *A. oryzae* lipase provoked strong IgE antibody responses via both routes of exposure. The main difference between the antibody responses elicited by the two exposure routes is apparently one of magnitude, particularly for BSA and OVA. Thus ip exposure to BSA or OVA at 5% concentrations induced more vigorous IgG and IgG1 antibody responses compared with intranasal exposure to the same concentration; IgE antibody titres were increased also, from undetectable to detectable in neat serum, and from a titre of 1 to a titre of 1 in 8 for BSA and OVA, respectively. The differential immunogenicity observed between the two routes of exposure is likely to be a function primarily of the variation in the total amount of material that can be applied. For example, animals treated ip with a 5% solution of protein receive 25 mg protein each in total compared with 1.5 mg protein following intranasal exposure. These amounts of protein are higher than those used by other investigators to provoke IgG1 antibody responses following intranasal exposure (Robinson *et al.*, 1996; Robinson and Kawabata, 1997). Proteolytic enzymes at doses of as low as $1 \mu\text{g}$ per mouse induced detectable IgG1 antibody responses. In those experiments, however, proteins were co-administered with a detergent matrix which apparently serves as an adjuvant, increasing antibody levels by up to fourfold. Furthermore, these investigators chose a mouse strain, BDF1 (a C57B16/DBA2 hybrid), which was a high responder with respect to intranasally-induced IgG1 antibody production compared with BALB/c strain mice

which were shown to be low responders under the same conditions.

Of particular interest is the observation that protein-specific IgE antibody responses could be elicited in the absence of adjuvant. While exposure to proteins in the presence of adjuvants like alum or *Bordetella pertussis* stimulates persistent and high titre IgE responses, such methods of enhancing IgE production can alter the nature of induced immune responses. Thus, for example, topical exposure of mice to the chemical allergen dinitrochlorobenzene (DNCB), a potent contact sensitizer which apparently lacks the potential to cause IgE-mediated reactions, fails to provoke the production of type 2 cytokines (Dearman *et al.*, 1997) or the elicitation of IgE antibody (Dearman and Kimber, 1991). However, IgE responses can be induced to the same antigenic determinant, dinitrophenyl (DNP), by administration of DNP-protein conjugate in the presence of alum adjuvant (Hagan *et al.*, 1989). In the absence of such agents, we have demonstrated that the vigour of induced IgE responses correlated with protein allergenicity. No such relationship was observed for the induction of protein-specific IgG1 antibody responses via either route of exposure. While the elicitation of IgG1 antibody responses in mice following intranasal exposure may have the potential to predict the relative allergenicity of proteolytic detergent enzymes (Robinson *et al.*, 1996; Robinson and Kawabata, 1997), from the data described herein it is clearly not a universal phenomenon for proteins that do not have enzymatic properties.

Given the observed differential induction of IgE responses, it is important to consider the specificity and selectivity of the PCA assay employed for the measurement of IgE antibody. It has been demonstrated previously that other isotypes of murine immunoglobulin, IgG1 subclass antibody, have the capacity to bind to Fc receptors on murine mast cells, and crosslinking of such antibody with allergen can trigger mast cell degranulation (Ovary *et al.*, 1975). In addition, passive sensitization of naive animals with IgG1 antibody has been reported to result in increased airway responsiveness following inhalation challenge (Oshiba *et al.*, 1996). However, the affinity of mast cell receptors for murine IgG1 antibody is such that this immunoglobulin disassociates quickly *in vivo*, hence PCA assays which employ, as in the experiments described herein, a 48 hr passive sensitization step, are specific for IgE antibody (Ovary *et al.*, 1975). This is apparent in the current studies, in which high titre IgG1 antibody responses are observed in the absence of detectable protein-specific IgE (cf. 1 and 2% ip administration of BSA).

In conclusion, the present investigations have shown that administration to mice of proteins that are of comparable immunogenicity with respect to IgG and IgG1 antibody responses display considerable variation in their ability to provoke IgE antibody production which apparently reflects their

allergenic potential in humans. The differential induction of protein-specific IgE antibody responses was observed regardless of whether administration was via a mucosal (intranasal) or non-mucosal (ip) route of exposure. While further experience with a wider range of proteins is required, analyses of IgG1 and IgE antibody responses induced following ip exposure to proteins have considerable potential for the study of the relative allergenicity and immunogenicity of these materials.

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