

Serological responses induced in mice by immunogenic proteins and by protein respiratory allergens

J. Hilton^{*a}, R.J. Dearman^a, D.A. Basketter^b, I. Kimber^a

^a*Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire SK10 4TJ, UK*

^b*Unilever Environmental Safety Laboratory, Colworth House, Sharnbrook, Bedfordshire MK44 1LQ, UK*

(Received 11 June 1993; revision received 7 September 1993; accepted 27 September 1993)

Abstract

It is known that a variety of materials, including both low molecular weight chemicals and proteins, is able to induce occupational respiratory allergy. We have shown previously that exposure of mice to chemical respiratory sensitizers results in both a marked increase in the serum concentration of IgE and the appearance of specific IgE antibody. In the present study we have examined the characteristics of immune responses induced in mice following intraperitoneal exposure to 3 protein respiratory allergens, ovalbumin (OVA), a lipase from *Aspergillus oryzae* (LP) and an amylase from *Bacillus subtilis* (AM) and to a fourth protein, bovine serum albumin (BSA), which is considered usually not to cause respiratory sensitization. Under conditions where all proteins provoked IgG antibody responses, only OVA, LP and AM elicited specific IgE antibody. As judged by passive cutaneous anaphylaxis (PCA) assay, BSA failed to induce an IgE response. In contrast to chemical respiratory sensitizers, the protein allergens examined here failed to cause a substantial increase in the serum concentration of IgE; OVA and AM induced no increase in serum IgE and LP only a comparatively modest increase relative to control values. In conclusion, these data demonstrate that while protein respiratory allergens are able to provoke specific IgE antibody, they fail to cause a marked increase in the concentration of this immunoglobulin in the sera of treated mice. It would appear, therefore, that the mouse IgE test, which seeks to evaluate chemical respiratory sensitization potential as a function of induced changes in the concentration of serum IgE, will be inappropriate for the identification of protein respiratory allergens. Nevertheless, identification of protein allergens may be possible by exploiting the observations reported here that such proteins induce in mice specific IgE antibody responses.

Keywords: Protein respiratory allergens; IgE concentration; IgG antibody

* Corresponding author, Fax: 0625 590249.

1. Introduction

Occupational respiratory allergy is known to be caused by a variety of materials and is associated frequently with the presence of IgE antibody [1,2]. Chemicals known to cause respiratory hypersensitivity in susceptible individuals include certain acid anhydrides [3–5] and diisocyanates [6–8], platinum salts [9] and some reactive dyes [10–12]. In addition, occupational respiratory allergy has been associated with certain proteins and in particular with proteolytic enzymes [13,14].

There is a need to evaluate the ability of proteins and chemicals to induce respiratory hypersensitivity. However, there are, as yet, no well-validated or widely applied methods available for the routine prospective identification of respiratory allergens. Progress to date has focused largely upon studies in the guinea pig and several methods for the induction and elicitation of respiratory hypersensitivity in this species have been described. Karol and her colleagues have been instrumental in developing methods for the investigation of respiratory sensitization to chemicals in guinea pigs. It has been shown that animals sensitized previously by inhalation exposure to chemical or chemical conjugates will exhibit changes in respiratory rate and breathing pattern following exposure to atmospheres containing the same chemical in the form of a hapten-protein conjugate [15–18]. More recently it has been shown also that guinea pigs sensitized by intradermal or subcutaneous injection will mount pulmonary reactions following subsequent inhalation exposure to free chemical [19,20]. Although such methods continue to be of great value for the investigation of the elicitation of respiratory hypersensitivity reactions, they are costly and time-consuming to perform and do not lend themselves readily to the predictive evaluation of respiratory sensitization potential in the context of routine toxicology.

We have described previously an alternative approach to investigation of the sensitization potential of chemicals. This method, the mouse IgE test, is based upon a series of observations made recently, that only those chemical allergens which are known or suspected to cause respiratory hypersensitivity in man provoke a substantial increase in the serum concentration of IgE in mice [21].

The purpose of the present study was to investigate the utility of this approach for evaluation of the respiratory sensitization potential of proteins. Specifically, we have examined the characteristics of immune responses induced in mice by 3 protein respiratory allergens; ovalbumin, a lipase from *Aspergillus oryzae* and an amylase from *Bacillus subtilis*. The respiratory sensitization potential of ovalbumin has been established previously in both guinea pigs and mice [22–25]. The lipase and amylase tested are used for a variety of industrial applications and both have been implicated in the induction of respiratory hypersensitivity [26,27]. Responses to these 3 protein allergens have been compared with those elicited in mice by bovine serum albumin, an immunogenic protein which is not associated usually with the induction of respiratory allergy.

2. Materials and methods

2.1. Animals

Young adult (8–12 weeks old) female BALB/c strain mice (Barriered Animal Breeding Unit, Alderley Park, Cheshire, UK) were used throughout these studies.

2.2. Test materials

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

2.3. Sensitization for antibody

Groups of mice ($n = 10$) received 250 μl of 1% test material in phosphate buffered saline (PBS) via intraperitoneal (i.p.) injection. Seven days later treatment was repeated. Fourteen days following the initiation of exposure, mice were exsanguinated by cardiac puncture and serum prepared and stored at -20°C until analysis. Control animals were treated in the same way with PBS alone.

2.4. Enzyme-linked immunosorbent assays (ELISA)

(i) *Measurement of serum IgE concentration.* Serum IgE was measured using a sandwich ELISA. Plastic microtitre plates were coated with 1.0 $\mu\text{g/ml}$ rat monoclonal anti-mouse IgE antibody (kindly provided by Dr F.D. Finkelman, Uniformed Services University of the Health Sciences, Bethesda, MD) in 0.1 M carbonate buffer (pH 9.6) by overnight incubation at 4°C . The plates were then blocked by treatment for 30 min at 37°C with 5% normal goat serum (NGS) in PBS. Individual test serum samples and a mouse monoclonal IgE anti-dinitrophenol DNP (clone SPE-7; ICN Immunobiologicals, Bucks, UK), diluted to various extents in 0.5% NGS in PBS, were added to triplicate wells and the plates incubated for 3 h at 4°C . There followed an incubation for 3 h at 4°C with a 1:2000 dilution of goat anti-mouse IgE conjugated with horseradish peroxidase (Nordic Immunology, Tilberg, The Netherlands). Enzyme substrate (*o*-phenylenediamine and urea hydrogen peroxide) was added and the reaction terminated after 5 min by 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, Ayrshire, UK). The concentration of IgE in test serum samples was derived from a standard curve for monoclonal mouse IgE. Results are expressed as serum IgE concentration ($\mu\text{g/ml}$).

(ii) *Measurement of protein-specific IgG antibody.* IgG antibodies were detected by ELISA. Plastic microtitre plates were coated with 100 $\mu\text{g/ml}$ 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 5% NGS in PBS. Doubling dilutions of mouse serum samples (pooled for each experimental group) diluted in 0.5% NGS in PBS were added to consecutive wells and incubated for 3 h at 4°C . The plates were then incubated for 3 h at 4°C with peroxidase-labelled goat anti-mouse IgG (Nordic Immunology, Tilberg, The Netherlands) diluted 1:8000 with 0.5% NGS in PBS. Substrate (as described above) was added and the reaction stopped by addition of 0.5 M citric acid after 15 min. Between each incubation the plates were washed with PBS containing 0.05% Tween 20. Substrate conversion was measured as described previously. Antibody titres are expressed as optical density at 450 nm.

2.5. Measurement of protein-specific IgE antibody

The presence in the serum of IgE antibodies was detected by passive cutaneous

anaphylaxis (PCA) assay. Pooled serum samples were diluted serially in saline and injected (30 μ l) into the dermis of naïve recipient mouse ears. Two days later 0.25 mg of BSA or OVA or 0.125 mg of AM or LP together with Evans Blue dye (1.25 mg) in 250 μ l saline were injected intravenously. Thirty minutes following challenge mice were killed and the diameter of the cutaneous reaction measured. Antibody titre was recorded as the highest dilution of pooled serum resulting in a measurable (> 3 mm) blue lesion in the skin.

3. Results

3.1. Specific IgG antibody responses

Mice received 250 μ l of 1% BSA or OVA in PBS via i.p. injection and a repeat exposure 7 days later. Mice were exsanguinated 14 days following initiation of exposure and the presence of serum IgG antibody measured by ELISA. Representative results from one of 3 replicate experiments are illustrated in Fig. 1. BSA and OVA each induced a strong specific IgG antibody response, with significant substrate con-

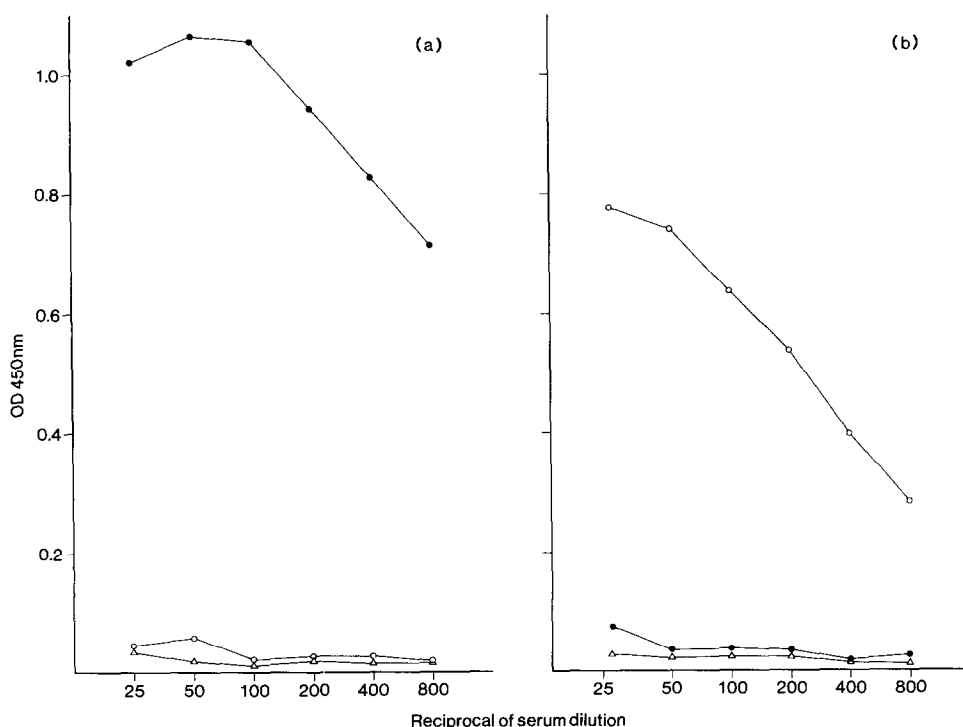


Fig. 1. IgG antibody production following exposure to BSA or OVA. Groups of mice ($n = 10$) received 250 μ l of 1% BSA (●) or 1% OVA (○) in PBS via i.p. injection, with repeat treatment 7 days later. Fourteen days following the initiation of exposure, treated mice and naïve controls (Δ) were exsanguinated and pooled sera tested for IgG anti-BSA (a) or anti-OVA (b) antibody by ELISA.

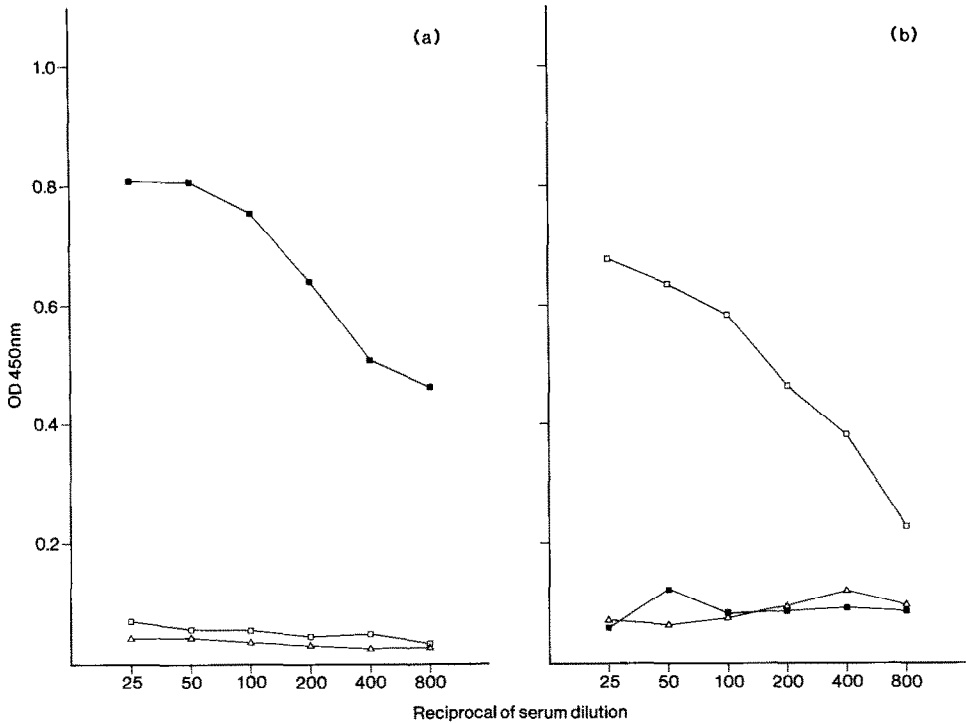


Fig. 2. IgG antibody production following exposure to AM or LP. Groups of mice ($n = 10$) received 250 μ l of 1% AM (■) or 1% LP (□) in PBS via i.p. injection, with repeat treatment 7 days later. Fourteen days following the initiation of exposure, treated mice and naïve controls (Δ) were exsanguinated and pooled sera tested for IgG anti-AM (a) or anti-LP (b) antibody by ELISA.

Table 1

Specific IgE antibody responses following exposure to BSA, OVA, AM or LP

Immunizing antigen	Protein used for PCA intravenous challenge	
	BSA	OVA
BSA	—	—
OVA	—	1/8
	AM	LP
AM	1/8	—
LP	—	1/16

Groups of mice ($n = 10$) received 250 μ l of 1% protein in PBS via i.p. injection, with the treatment repeated 7 days later. Fourteen days after the initiation of exposure, mice were exsanguinated. Serial dilutions of pooled sera were analyzed for the presence of specific IgE antibody by PCA assay using i.v. challenge with the relevant protein. Cross-reactivity was tested by intravenous challenge with unrelated protein. Antibody titre is recorded as the highest dilution of pooled serum resulting in a positive PCA reaction. — represents a negative PCA reaction with neat serum.

version detected even at serum dilutions of 1:800. The data indicate that, under the conditions of exposure used, BSA is at least as immunogenic as OVA. No cross-reactivity between the proteins was detected, with equivalent levels of background staining observed with BSA antisera and OVA substrate, and vice versa, to those recorded with naïve mouse serum.

Serum IgG antibody responses were measured also following i.p. administration of the protein respiratory allergens AM and LP. Both proteins provoked substantial IgG antibody production with similar profiles to those elicited by BSA and OVA. The antibody responses were specific; no cross-reactivity was observed between the 2 enzymes. Representative results from one of 3 replicate experiments are shown in Fig. 2.

3.2. *IgE antibody responses*

Experiments were performed to examine whether, under conditions of exposure which resulted in strong IgG antibody responses, these proteins induced also the production of specific IgE antibody. Mice received 250 μ l of 1% protein in PBS via i.p. injection and the same treatment 7 days later. Mice were exsanguinated 14 days following the initiation of exposure, and pooled serum samples analyzed for specific IgE production by PCA assay. Representative results from one of 3 replicate experiments are recorded in Table 1.

Undiluted serum from mice exposed to BSA did not elicit a PCA reaction. In contrast, exposure to OVA, AM and LP each caused the appearance of specific IgE antibody. Thus, serum from OVA- or AM-treated mice and LP-treated mice, at dilutions of 1:8 and 1:16 or less, respectively, provoked cutaneous responses in naïve recipients. Comparable PCA titres have been observed following topical exposure of mice to chemical respiratory allergens such as phthalic anhydride [28]. In common with IgG antibody, IgE responses were protein-specific, with no cross-reactivity detected between OVA and BSA or between the 2 enzymes (Table 1).

3.3. *Serum IgE concentration*

Finally, experiments were conducted to determine whether the differential capacity of these materials to elicit specific IgE antibody production was associated with a variable ability to provoke changes in total serum concentration of IgE. Mice were immunized as described above and bled 14 days after the initiation of exposure. The concentration of IgE in individual serum samples was measured by ELISA. Over an 18-month period, we have found that in naïve BALB/c mice, the serum concentration of IgE varies between 0.085 and 0.320 μ g/ml; values in broad agreement with previous studies in this strain [29]. In the present study control mice treated with PBS alone were found to have a mean serum IgE concentration of 0.125 μ g/ml. Under conditions where BSA stimulated specific IgG, but not IgE, antibody production, the concentration of serum IgE (mean $\pm$ S.E. = 0.120 $\pm$ 0.011 μ g/ml) did not vary from this normal range. Despite provoking specific IgE antibody responses, exposure to OVA (mean $\pm$ S.E. = 0.131 $\pm$ 0.019 μ g/ml) and AM (mean $\pm$ S.E. = 0.149 $\pm$ 0.015 μ g/ml) both failed to cause an increase in serum IgE concentrations relative to control values. Compared with the normal range of serum IgE,

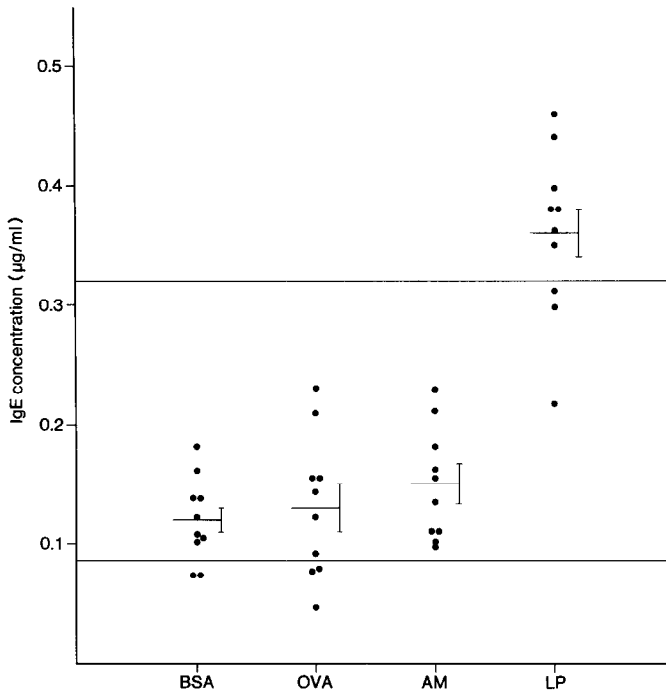


Fig. 3. Serum IgE concentration following exposure to BSA, OVA, AM and LP. Groups of mice ($n = 10$) received $250 \mu\text{l}$ of 1% protein in PBS via i.p. injection, with repeat treatment 7 days later. Fourteen days following the initiation of exposure, mice were exsanguinated and the concentration of IgE in individual sera was measured by sandwich ELISA. Results are recorded as the IgE concentration in individual serum samples together with the mean $\pm$ S.E. ($\mu\text{g/ml}$) for each experimental group. The range of serum IgE concentrations for naïve BALB/c mice recorded over an 18-month period is represented by the horizontal lines.

intraperitoneal administration of LP resulted in slightly elevated serum IgE concentrations in 7 out of 10 animals. These levels, however, (mean $\pm$ S.E. = $0.359 \pm 0.022 \mu\text{g/ml}$) are markedly lower than those elicited following topical exposure to chemical respiratory allergens such as the acid anhydride phthalic anhydride or the isocyanate diphenylmethane-4,4'-diisocyanate, where mean concentrations of approximately $0.8\text{--}1.5 \mu\text{g/ml}$ were recorded [21]. Toxicity limited investigation of the capacity of the enzymes LP and AM to cause more profound effects on serum IgE concentration at higher exposure concentrations.

4. Discussion

There is a need for accurate test methods which permit the routine prospective identification of respiratory sensitizing potential. Approaches to date have focused

largely upon the guinea pig and the elicitation, by inhalation challenge or intratracheal instillation, of pulmonary reactions in previously sensitized animals [18–20,30]. We have chosen to adopt an alternative approach, the mouse IgE test, in which respiratory sensitization potential is measured as a function of induced changes in the serum concentration of IgE following topical exposure of mice to the test chemical [21]. This method is based upon previous investigations in which it was found that known chemical respiratory allergens provoke in mice a substantial increase in the serum concentration of IgE. Contact allergens, which are known or suspected not to induce respiratory hypersensitivity, do not [21]. An objective of the present study was to determine whether the mouse IgE test, and measurement of induced changes in serum IgE concentration, could be used also for the identification of proteins which have the potential to cause respiratory allergy.

Under conditions of exposure where all 4 proteins examined were clearly immunogenic, resulting in strong IgG antibody responses, specific IgE antibody production was elicited only by those proteins known to have a significant potential to cause respiratory allergy. Bovine serum albumin has been implicated as a cause of food allergy, particularly to cow's milk. However, although BSA is used widely by the research community, we are aware of only a single report of occupational asthma associated with exposure to this protein [31]. While it is not possible, therefore, to conclude that BSA is without the capacity to cause respiratory allergy, it may have only a limited potential to do so. Despite the induction of specific IgE antibody by OVA, AM and LP, however, substantial increases in serum IgE concentration were not observed. The dose levels and frequency of administration of these materials were limited by the enzymatic activity of LP and AM and by the ability of OVA to cause anaphylactic reactions.

It is possible, therefore, that more chronic exposure or exposure to higher concentrations of protein would stimulate a significant increase in serum IgE levels. Mitigating against this, however, is the fact that under conditions where chemical and protein respiratory allergens show striking differences in their ability to influence the concentration of total serum IgE, similar titres of specific IgE antibody were provoked [28]. The data indicate therefore that protein and chemical respiratory sensitizers exhibit a differential capacity to induce increases in serum IgE concentration.

There is now evidence for a functional heterogeneity among murine T helper (T_H) cells [32,33] and for the ability of their secreted cytokine products to exert reciprocal antagonistic effects on IgE production both in vitro and in vivo [33,34]. Thus, the initiation and maintenance of IgE responses in mice is dependent on interleukin 4 (IL-4), a product of T_{H2} cells [35,36], whilst interferon γ (IFN- γ), secreted by T_{H1} cells, inhibits IgE production [37]. In man also there is evidence for a functional heterogeneity among T_H cells [38] and for reciprocal regulation of IgE synthesis by IL-4 and IFN- γ [39,40]. We have reported previously that chemicals with the potential to cause respiratory hypersensitivity stimulate in mice immune responses consistent with the preferential activation of T_{H2} cells [28,41–44]. This conclusion derived primarily from analysis of the isotype distribution of the IgG antibody response and the induction of both hapten-specific IgE antibody and an

increase in the serum concentration of IgE following topical exposure of mice to a variety of sensitizing chemicals.

The stimulation by OVA, AM and LP of specific IgE antibody is consistent with the ability of these proteins to induce respiratory allergy and is, in addition, suggestive of the preferential elicitation of T_{H2} -type responses. However, unlike chemical respiratory allergens, these proteins appear not to cause an increase in serum IgE concentration. Although the reasons for this difference are at present unclear, it might be that chemical, but not protein, respiratory allergens act as adjuvants for IgE production. Adjuvanticity may, in turn, reflect differences in the way protein and chemical allergens are handled, processed and presented by the immune system and/or in the kinetics of responses induced by these classes of sensitizer. Clearly the availability of IL-4 will be a critical factor in determining the magnitude of changes in IgE concentration.

In conclusion, the data reported indicate that, in its present form, the mouse IgE test is inappropriate for the identification of protein respiratory allergens which fail to provoke changes in serum IgE concentration. One option is of course to use guinea pig inhalation models for analysis of the potential allergenicity of proteins and there exist precedents for this [45,46]. It is tempting to suggest, however, that it may be possible also to exploit the fact that, on the basis of the limited evidence presented here, protein respiratory allergens have the capacity to induce IgE responses in mice. Such an approach would provide a more realistic and cost-effective method for the toxicological evaluation of the respiratory sensitizing potential of proteins. A more rigorous examination of this phenomenon with a wider range of proteins will be required before firm recommendations can be made.

References

- 1 Chan-Yeung, M. and Lam, S. (1986) Occupational asthma — state of the art. *Am. Rev. Respir. Dis.* 133, 686–703.
- 2 Butcher, B.T. and Salvaggio, J.E. (1986) Occupational asthma. *J. Allergy Clin. Immunol.* 78, 547–556.
- 3 Bernstein, D.I., Patterson, R. and Zeiss, C.R. (1982) Clinical and immunologic evaluation of trimellitic anhydride and phthalic anhydride-exposed workers using a questionnaire and comparative analysis of enzyme-linked immunosorbent and radioimmunoassay studies. *J. Allergy Clin. Immunol.* 69, 311–318.
- 4 Howe, W., Venables, K.M., Topping, M.D., Dally, M.B., Hawkins, R., Law, J.S. and Taylor, A.T. (1983) Tetrachlorophthalic anhydride asthma: evidence for specific IgE antibody. *J. Allergy Clin. Immunol.* 71, 5–11.
- 5 Moller, D.R., Gallagher, J.S., Bernstein, D.I., Wilcox, T.G., Burroughs, H.E. and Bernstein, I.L. (1985) Detection of IgE-mediated respiratory sensitization in workers exposed to hexahydrophthalic anhydride. *J. Allergy Clin. Immunol.* 76, 663–672.
- 6 Zeiss, C.R., Kanellakes, T.M., Bellone, J.D., Levitz, D., Pruzansky, J.J. and Patterson, R. (1980) Immunoglobulin-E mediated asthma and hypersensitivity pneumonitis with precipitating anti-hapten antibodies due to diphenylmethane diisocyanate (MDI) exposure. *J. Allergy Clin. Immunol.* 65, 346–352.
- 7 Zammit-Tabona, M., Sherkin, M., Kijek, K., Chan, H. and Chan-Yeung, M. (1983) Asthma caused by diphenylmethane diisocyanate in foundry workers. Clinical, bronchial provocation and immunologic studies. *Am. Rev. Respir. Dis.* 128, 226–230.

- 8 Keskinen, H., Tupasela, O., Tiikkainen, U. and Nordman, H. (1988) Experience of specific IgE in asthma due to diisocyanates. *Clin. Allergy* 18, 597–604.
- 9 Biagini, R.E., Bernstein, I.L., Gallagher, J.S., Moorman, W.J., Brooks, S. and Gann, P.H. (1985) The diversity of reaginic immune responses to platinum and palladium salts. *J. Allergy Clin. Immunol.* 76, 794–802.
- 10 Alanko, K., Keskinen, H., Bjorksten, F. and Ojanen, S. (1978) Immediate-type hypersensitivity to reactive dyes. *Clin. Allergy* 8, 25–31.
- 11 Docker, A., Wattie, J.M., Topping, M.D., Luczynska, C.M., Newman Taylor, A.J., Pickering, C.A.C., Thomas, P. and Gompertz, D. (1987) Clinical and immunological investigations of respiratory disease in workers using reactive dyes. *Br. J. Ind. Med.* 44, 534–541.
- 12 Topping, M.D., Forster, H.W., Ide, C.W., Kennedy, F.M., Leach, A.M. and Sorkin, S. (1989) Respiratory allergy and specific immunoglobulin E and immunoglobulin G antibodies to reactive dyes in the wool industry. *J. Occup. Med.* 31, 857–862.
- 13 Pepys, J., Wells, I.D., D'Souza, M.F. and Greenberg, M. (1973) Clinical and immunological responses to enzymes of *Bacillus subtilis* in factory workers and consumers. *Clin. Allergy* 3, 143–160.
- 14 Liss, G.H., Kominsky, J.R., Gallagher, J.S., Melius, J., Brooks, S.M. and Bernstein, I.L. (1984) Failure of enzyme encapsulation to prevent sensitization of workers in the dry bleach industry. *J. Allergy Clin. Immunol.* 73, 348–355.
- 15 Karol, M.H., Ioset, H.H., Riley, E.J. and Alarie, Y.C. (1978) Hapten-specific respiratory hypersensitivity in guinea pigs. *Am. Ind. Hyg. Assoc.* 39, 546–556.
- 16 Karol, M.H., Dixon, C., Brady, M. and Alarie, Y. (1980) Immunologic sensitization and pulmonary hypersensitivity by repeated inhalation exposure to aromatic isocyanates. *Toxicol. Appl. Pharmacol.* 53, 260–270.
- 17 Karol, M.H. (1983) Concentration-dependent immunologic response to toluene diisocyanate following inhalation exposure. *Toxicol. Appl. Pharmacol.* 68, 229–241.
- 18 Karol, M.H., Stadler, J. and Magreni, C. (1985) Immunotoxicologic evaluation of the respiratory system: animal models for immediate- and delayed-onset pulmonary hypersensitivity. *Fundam. Appl. Toxicol.* 5, 459–472.
- 19 Botham, P.A., Rattray, N.J., Woodcock, D.R., Walsh, S.T. and Hext, P.M. (1989) The induction of respiratory allergy in guinea-pigs following intradermal injection of trimellitic anhydride: a comparison with the response to 2,4-dinitrochlorobenzene. *Toxicol. Lett.* 47, 25–39.
- 20 Pauluhn, J. and Eben, A. (1991) Validation of a non-invasive technique to assess immediate or delayed onset of airway hypersensitivity in guinea-pigs. *J. Appl. Toxicol.* 11, 423–431.
- 21 Dearman, R.J., Basketter, D.A. and Kimber, I. (1992) Variable effects of chemical allergens on serum IgE concentration in mice. Preliminary evaluation of a novel approach to the identification of respiratory sensitizers. *J. Appl. Toxicol.* 12, 317–323.
- 22 Barton, H.J., Bond, A., Boot, J.R., Brandon, D.R. and O'Brien, A. (1991) The relationship between development of lung inflammation and changes in bone marrow populations in guinea-pigs following inhaled antigen challenge. *Int. Arch. Allergy Appl. Immunol.* 96, 1–11.
- 23 Compton, M.R., Seale, J.P. and Shaw, J. (1987) Effects of aerosol antigen challenge in vivo on responsiveness of guinea-pig tracheal and parenchymal strips in vitro to contractile agonists. *Int. Arch. Allergy. Appl. Immunol.* 84, 40–44.
- 24 Ito, K., Ishii, A., Yamashita, N., Miyamoto, T. and Watanabe, N. (1988) Comparison of guinea pig IgE antibodies estimated by ELISA with those estimated by passive cutaneous anaphylaxis. *Int. Arch. Allergy Appl. Immunol.* 87, 424–429.
- 25 Renz, H., Smith, H.R., Hensen, J.E., Bradley, S.R., Irvine, C.G. and Gelfand, E.W. (1992) Aerosolized antigen exposure without adjuvant causes increased IgE production and increased airway responsiveness in the mouse. *J. Allergy Clin. Immunol.* 89, 1127–1138.
- 26 Losada, E., Hinojosa, M., Quirce, S., Sanchez-Cano, M. and Monea, I. (1992) Occupational asthma caused by alpha-amylase inhalation: clinical and immunologic findings and bronchial response patterns. *J. Allergy Clin. Immunol.* 89, 118–125.
- 27 Bossert, J., Fuchs, E., Wahl, R. and Maasch, H.J. (1988) Occupational sensitisation by inhalation of enzymes diaphorase and lipase. *Allergologie* 11, 179–181.
- 28 Dearman, R.J. and Kimber, I. (1992) Divergent immune responses to respiratory and contact

- chemical allergens: antibody elicited by phthalic anhydride and oxazolone. *Clin. Exp. Allergy* 22, 241–250.
- 29 Azuma, M., Hirano, T., Miyajima, H., Watanabe, N., Yagita, H., Enomoto, S., Furusawa, S., Ovary, Z., Kinashi, T., Honjo, T. and Okumura, K. (1987) Regulation of murine IgE production in SJA/9 and nude mice. Potentiation of IgE production by recombinant interleukin 4. *J. Immunol.* 139, 2538–2544.
- 30 Sarlo, K. and Clark, E.D. (1992) A tier approach for evaluating respiratory allergenicity of low molecular weight chemicals. *Fundam. Appl. Toxicol.* 18, 107–114.
- 31 Joliat, T.L. and Weber, R.W. (1991) Occupational asthma and rhinoconjunctivitis from inhalation of crystalline bovine serum albumin powder. *Ann. Allergy* 66, 301–304.
- 32 Mosmann, T.R., Cherwinski, H., Bond, M.W., Giedlin, M.A. and Coffman, R.L. (1986) Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.* 136, 2348–2357.
- 33 Mosmann, T.R. and Coffman, R.L. (1989) Heterogeneity of cytokine secretion patterns and functions of helper T cells. *Adv. Immunol.* 46, 111–147.
- 34 Coffman, R.L., Seymour, B.W.P., Lebman, D.A., Hiraki, D.D., Christiansen, J.A., Schrader, B., Cherwinski, H.M., Savelkohl, H.F.J., Finkelman, F.D., Bond, M.W. and Mosmann, T.R. (1988) The role of helper T cell products in mouse B cell differentiation and isotype regulation. *Immunol. Rev.* 102, 5–28.
- 35 Finkelman, F.D., Katona, I.M., Urban Jr., J.F., Snapper, C.M., Ohara, J. and Paul, W.E. (1986) Suppression of in vivo polyclonal IgE response by monoclonal antibody to the lymphokine B cell stimulatory factor 1. *Proc. Natl. Acad. Sci. USA* 83, 9675–9678.
- 36 Finkelman, F.D., Katona, I.M., Urban Jr., J.F., Ohara, J., Tung, A.S., Sample, J.G. and Paul, W.E. (1988) IL-4 is required to generate and sustain in vivo IgE responses. *J. Immunol.* 141, 2335–2341.
- 37 Finkelman, F.D., Katona, I.M., Mosmann, T.R. and Coffman, R.L. (1988) IFN- γ regulates the isotypes of Ig secreted during in vivo humoral immune responses. *J. Immunol.* 140, 1022–1027.
- 38 Romagnani, S. (1991) Human T_{H1} and T_{H2} subsets: doubt no more. *Immunol. Today* 12, 256–257.
- 39 Pene, J., Rousset, F., Briere, F., Chretien, I., Paliard, X., Banchereau, J., Spits, H. and De Vries, J.E. (1988) IgE production by normal human B cells induced by alloreactive T cell clones is mediated by IL-4 and suppressed by IFN- γ . *J. Immunol.* 141, 1218–1224.
- 40 Chretien, I., Pene, J., Briere, F., De Waal Malefijt, R., Rousset, F. and De Vries, J.E. (1990) Regulation of human IgE synthesis. I. Human IgE synthesis in vitro is determined by the reciprocal antagonistic effects of interleukin 4 and interferon γ . *Eur. J. Immunol.* 20, 243–251.
- 41 Dearman, R.J. and Kimber, I. (1991) Differential stimulation of immune function by respiratory and contact chemical allergens. *Immunology* 72, 563–570.
- 42 Dearman, R.J., Spence, L.M. and Kimber, I. (1992) Characterization of murine immune responses to allergenic diisocyanates. *Toxicol. Appl. Pharmacol.* 112, 190–197.
- 43 Dearman, R.J., Basketter, D.A., Coleman, J.W. and Kimber, I. (1992) The cellular and molecular basis for divergent allergic responses to chemicals. *Chem-Biol. Interact.* 84, 1–10.
- 44 Dearman, R.J. and Kimber, I. (1992) The mechanism and evaluation of chemically induced allergy. *Toxicol. Lett.* 64/65, 79–84.
- 45 Thorne, P.S., Hillebrand, J., Magreni, C., Riley, E.J. and Karol, M.H. (1986) Experimental sensitization to subtilisin. *Toxicol. Appl. Pharmacol.* 86, 112–123.
- 46 Griffiths-Johnson, D.A. and Karol, M.H. (1991) Validation of a non-invasive technique to assess development of airway hyperreactivity in an animal model of immunologic pulmonary hypersensitivity. *Toxicology* 65, 283–294.