

Specific immunological and bronchopulmonary responses following intradermal sensitization to free trimellitic anhydride in guinea pigs

J. P. HAYES*†, R. DANIEL*, R. D. TEE*, P. J. BARNES†, K. F. CHUNG† and A. J. NEWMAN TAYLOR*

*Department of Occupational and Environmental Medicine and †Department of Thoracic Medicine, Royal Brompton and National Heart Hospitals, National Heart and Lung Institute, London, U.K.

Summary

We have developed a guinea pig model of trimellitic anhydride-induced airway hypersensitivity responses. In one group of guinea pigs, injected intradermally with 0.1 ml 30% trimellitic anhydride (TMA), we examined the specificity of the bronchopulmonary response to TMA comparing the effect of intravenous TMA conjugated to guinea pig serum albumin (GPSA) with a control hapten (procion dye) protein conjugate (PD-GPSA). A significant increase in pulmonary inflation pressure (PIP) was provoked in sensitized animals following intravenous injection with TMA-GPSA (20%; 0–400, median; range) as compared to intravenous injection of PD-GPSA. In the second group we compared three different methods of sensitization: single injection of 0.1 ml of 0.3% TMA; four injections of 0.1 ml of 0.1% TMA; and a single high dose injection of 30% TMA. Following intravenous TMA-GPSA guinea pigs sensitized with a single injection 0.3% TMA had an increase in PIP of 395%; 220–600, while those given four repeat injections of 0.1% TMA had an increase in PIP of 343%; 315–490. These results were significantly higher than the increase in PIP (160%; 0–220) which occurred in guinea pigs sensitized with a single dose of 30% TMA. Four of 11 guinea pigs given low dose injections of TMA had bronchopulmonary responses to inhaled TMA-GPSA. All sensitized guinea pigs had specific IgG1 antibodies demonstrated by enzyme linked immunosorbent assay (ELISA) and confirmed by ELISA inhibition. Four guinea pigs sensitized by low dose injections of TMA had IgE antibodies demonstrated by passive cutaneous anaphylaxis.

We conclude that intradermal sensitization to free TMA induces a specific immune and airway hypersensitivity response to TMA-GPSA. Single low dose injection of TMA is the most effective method of sensitization. Intradermal sensitization to free TMA may be a valuable method of sensitization for the developing of an animal model of occupational asthma caused by low molecular weight chemicals.

Clinical and Experimental Allergy, Vol. 22, pp. 694–700. Submitted 18 July 1991; revised 4 December 1991; accepted 9 December 1991.

Introduction

Trimellitic anhydride (TMA) causes both occupational

asthma [1] and pulmonary haemorrhage with haemolytic anaemia in man [2]. It is a mucosal irritant and may induce a polyclonal antibody response in man. Occupational asthma caused by acid anhydrides is associated with a specific IgE or IgG4 antibody response in the majority of patients [3,4]. Studies in a variety of animal species [5,6] have shown a systemic and pulmonary immune response following intratracheal installation of TMA. Pulmonary haemorrhage also follows sensitization

Correspondence: Dr J. P. Hayes, Department of Occupational and Environmental Medicine, National Heart and Lung Institute, Manresa Road, London SW3 6LR, U.K.

Reprints: Dr A. J. Newman Taylor, Department of Occupational and Environmental Medicine, National Heart and Lung Institute, Manresa Road, London SW3 6LR, U.K.

Table 1. Protocol for sensitization with trimellitic anhydride (TMA)

Study A	
Single dose	0.1 ml 30% TMA (<i>n</i> = 14)
Single dose	0.1 ml corn oil (<i>n</i> = 6)
Study B	
Single dose	0.1 ml 30% TMA (<i>n</i> = 5)
Single dose	0.1 ml 0.3% TMA (<i>n</i> = 5)
Four doses	0.1 ml 0.1% TMA (<i>n</i> = 6)
Single dose	0.1 ml corn oil (<i>n</i> = 5)

by inhalation in rats and this response has recently been suggested to have an immunological basis [7]. To avoid pulmonary haemorrhage, sensitization by a route other than inhalation would seem appropriate in the development of an animal model of TMA induced asthma. Botham *et al.* [8] recently found an immune response in guinea pigs after intradermal sensitization and observed that subsequent exposure to TMA dust inhaled in high concentration, provoked changes in respiratory rate, which they used as an index of abnormal pulmonary function. In this study we have further examined the effectiveness of intradermal sensitization with free TMA and have measured bronchopulmonary responses to subsequent inhalation and intravenous injection of trimellitic anhydride conjugated to guinea pig albumin (TMA-GPSA), comparing three different schedules of TMA dosage as a means of sensitizing guinea pigs to this chemical.

Materials and methods

Sensitization

We undertook two series of experiments on male Dunkin-Hartley guinea pigs (300–350 g) (Table 1). In the first series of experiments (group A), 14 guinea pigs were given single dose intradermal injections of 0.1 ml of 30% (w/v) TMA suspended in corn oil and as a control group, 0.1 ml corn oil alone was injected into six guinea pigs. In the second series of experiments (group B), we compared this single high dose intradermal sensitization schedule with two different schedules of low dose intradermal sensitization injected either as a single low dose of 0.1 ml of 0.3% (w/v) TMA or as four doses of 0.1 ml of 0.1% (w/v) TMA equally spaced over a two week period.

Conjugates

TMA was conjugated to guinea pig serum albumin (GPSA) as follows. Four aliquots of 250 mg GPSA

(Sigma, Poole, U.K.) were mixed with 50 ml of 9% NaHCO₃ on ice and 250 mg of ground TMA dust (Aldrich, Gillingham, U.K.) was added and allowed to stand for 10 min. Each aliquot was stirred separately for 1 hr. The aliquots were pooled and spun for 10 min at 600 *g* (MSE Centaur 2). The supernatant was separated and dialysed with 0.02 M NH₄HCO₃ for 48 hr with six changes. This was further dialysed with distilled water for 24 hr and lyophilized. The degree of substitution was assessed by ultraviolet spectrophotometry. Briefly 1.0 mg TMA-GPSA was added to 1 ml of 0.1 M borate buffer at pH 9.3. A 0.03 M solution of 2,4,6-trinitrobenzenesulfonic acid (TNBS; Aldrich, Gillingham, U.K.) in borate buffer was prepared. Then 25 μ l of TNBS solution was added to 1 ml of the TMA-GPSA solution and incubated for 20 min at room temperature. The sample was read on the spectrophotometer at an optical density of 420 nm. The substitution ratio for TMA-GPSA was 21:1. In order to examine the specificity of the response to intravenous TMA-GPSA a control hapten conjugate was prepared. Procione dye, a dichlorotriazine reactive dye, was conjugated as follows; 50 mg GPSA was added to 2.5 ml of 50 mM carbonate buffer to give a 2% GPSA solution. To this 2.5 mg procione yellow YMX4R (Sigma, Poole, U.K.) was added and mixed for 1 hr at room temperature. The resultant solution was dialysed against phosphate buffered saline (PBS) overnight with two changes and lyophilized. The substitution ratio (analysed as described above) was 5:1.

ELISA for assessment of IgG1 antibodies

Nunc Immunoplates (Maxisorb F96, A/S Nunc, DK-4000 Roskilde, Denmark) were coated with 5 μ g/ml TMA-GPSA in coating buffer and incubated overnight at 4°C. On the next day the plates were washed four times in PBS/Tween. Serum was diluted to 1:50 solution and plates were coated with PBS/Tween. Serum was added in doubling dilutions across the plate and incubated for 3 hr at 27°C. The plates were again washed four times with PBS/Tween and 1:2500 of rabbit anti-guinea pig IgG1 (ICN Biomedicals Ltd, High Wycombe, Bucks, U.K.) was added and incubated for 2 hr at 27°C. Again the plates were washed with PBS/Tween. Anti-rabbit IgG-peroxidase (ICN Biomedicals Ltd, High Wycombe, Bucks, U.K.) was added at 1:5000 dilution and incubated at 27°C for 2 hr. Finally the plates were washed in PBS/Tween and chromogen (*O*-phenylenediamine 8 mg in 23.5 ml of citrate/phosphate buffer) was added. The reaction was stopped after 6 min with 1 M H₂SO₄ and light absorbance read at an optical density of 492 nm (Dynatech MR 700, Dynatech, Billingham, U.K.). The titres of IgG1 were calculated from the last dilution that was double the control sera.

ELISA inhibition

Plates were coated with 5.0 µg/ml of TMA-GPSA and kept overnight at 4°C. Stock solutions were made of TMA-GPSA and GPSA at 1 mg/ml in PBS/Tween and diluted serially. Similarly a stock solution of TMA (5 mg/ml) was made and diluted. Positive serum was diluted to 1:4000. The plates were washed four times in PBS/Tween and 50 µl of TMA-GPSA, GPSA or TMA solutions in increasing concentrations was added to different wells. To each well 50 µl of positive serum was added and the plates were incubated overnight at 27°C. Rabbit anti-guinea-pig IgG was added and the procedure complemented as for the ELISA described above.

Passive cutaneous anaphylaxis

In group B passive cutaneous anaphylaxis (PCA) was performed to detect IgE antibodies. Serum from sensitized guinea pigs was injected into the flanks of naive guinea pigs at a dilution of 1:2 in saline. Six days later TMA-GPSA (2.5 mg), together with Evans Blue dye (5 mg) was injected intravenously. The animals were killed and the cutaneous responses read on the outer surface of the skin 30 min after the intravenous injection. A blue lesion of greater than 5 mm in diameter was considered positive.

Measurement of bronchopulmonary response

On days 21–28 guinea pigs were anaesthetized (urethane 10 mg/kg), tracheostomized and ventilated at a tidal volume of 10 ml/kg and a respiratory rate of 60/min on an animal ventilator (Harvard, Lectromed, Leeds, U.K.). Cannulae were inserted into the carotid artery and jugular vein. Pulmonary inflation pressure (PIP) was measured via a sidearm tube attached at the origin of the outlet tube by a pressure transducer (Sensym 744, Farnell, Letchworth, U.K.) connected to a 2-channel recorder (Lectromed, Leeds, U.K.). Blood pressure was measured via a pressure transducer attached to the carotid artery cannulae. Changes in PIP were expressed as percentage change from the baseline.

Challenges

On days 21–28 group A animals were challenged with intravenous injections (1.5 mg/kg) of PD-GPSA followed 15 min later or on return to baseline with TMA-GPSA (1.5 mg/kg). On days 21–28 group B animals were challenged with 80 breaths of 2.5% TMA-GPSA via DeVilbiss nebuliser (output 10 µl/min) followed 15 min later or on return to baseline with an intravenous injection TMA-GPSA (1.5 mg/kg). Blood samples were

taken from all animals for assessment of IgG1 levels. At the end of the experiment the guinea pigs were sacrificed and the lungs eviscerated for histological examination. The tissue was sectioned (3 µm thick) and stained with haematoxylin & eosin and examined blindly.

Data analysis

In study A, Wilcoxon rank test was used to compare changes in bronchopulmonary response. Immune responses were compared using Mann-Whitney *U*-test. In study B, airway and immune responses were compared using Kruskal-Wallis non-parametric analysis of variance. A *P* value of less than 0.05 was considered significant.

Materials

Trimellitic anhydride, sodium bicarbonate, ammonium bicarbonate 2,4,6-dinitrobenzenesulfonic acid, urethane (Aldrich, Gillingham, U.K.), guinea pig serum albumin, procion yellow YMX4R, *O*-phenylenediamine, Evans Blue dye (Sigma, Poole, U.K.), rabbit anti-guinea pig IgG1, anti rabbit IgG-peroxidase (ICN Biomedicals Ltd, High Wycombe, Bucks, U.K.), Nunc immunoplates (Maxisorb F96, A/S Nunc, DK-4000 Roskilde, Denmark).

Results

All guinea pigs given intradermal injections of TMA in corn oil developed specific IgG1 antibodies to TMA-GPSA (Fig. 1). No IgG1 antibodies were detected in the sera of the control animals. ELISA inhibition confirmed the specificity of the immune response to TMA-GPSA (Fig. 2). Guinea pigs injected with a single dose of 30% TMA or four injections of 0.1% TMA had significantly higher IgG1 antibody responses ($P < 0.01$) than those guinea pigs injected with a single dose of 0.3% TMA but the antibody titre did not correlate with the change in PIP observed following injection of TMA-GPSA. Four guinea pigs given low dose injections of TMA had positive PCA responses indicating the presence of IgE antibodies to TMA-GPSA.

The group A animals who were sensitized to TMA showed no significant increase in PIP on intravenous challenge to PD-GPSA (0% of baseline, median; range 0–70%). Intravenous injection of TMA-GPSA provoked an immediate increase in PIP in seven of the 14 group A animals (20% of baseline; 0–400%; $P < 0.05$, Fig 3). Intravenous injection of PD-GPSA and TMA-GPSA provoked no changes in PIP in the control group of animals.

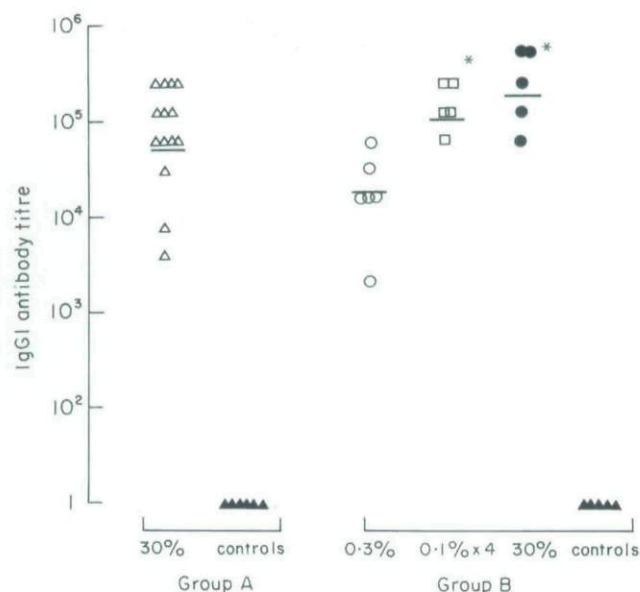


Fig. 1. IgG1 antibody titres for group A and group B. Group A guinea pigs were given 0.1 ml of 30% TMA. In study B guinea pigs given a single high dose (30%) or four repeat low dose (0.1%) intradermal injections of TMA had significantly higher levels of IgG1 antibody titres compared to those that received a single low dose injection of TMA (0.3%) (* $P < 0.01$). Horizontal bars indicate median values.

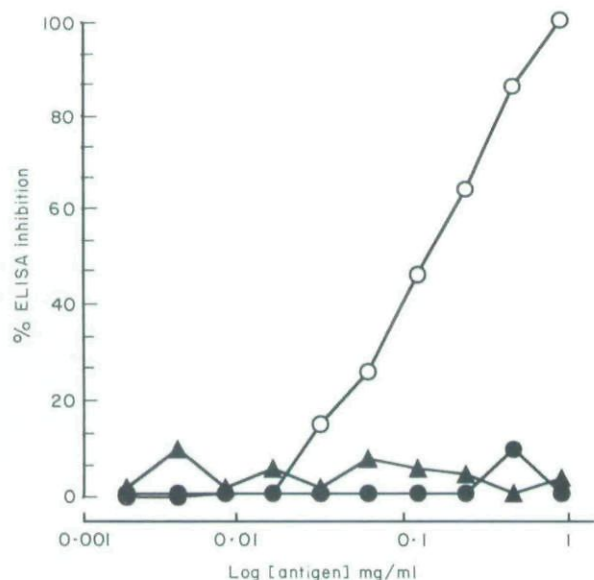


Fig. 2. ELISA inhibition comparing the effects of increasing concentrations of TMA-GPSA (○), TMACI (▲) and GPSA (●) in inhibiting the ELISA reaction at a serum dilution of 1:4000.

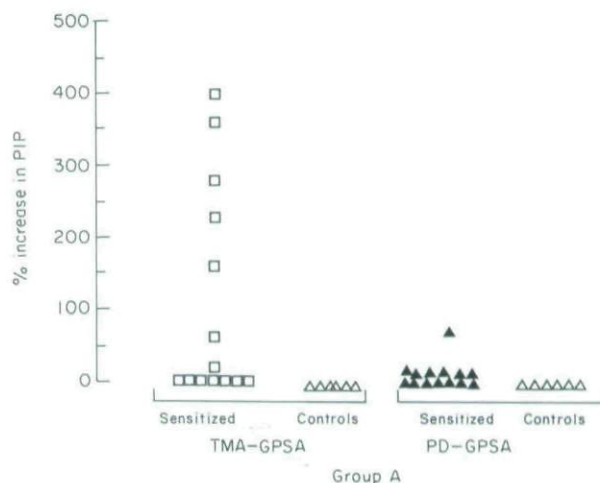


Fig. 3. Per cent increase in pulmonary inflation pressure (PIP) after intravenous injections of PD-GPSA (control conjugate) and TMA-GPSA in guinea pigs sensitized with intradermal injections of 30% TMA in corn oil and in control guinea pigs (group A). Sensitized guinea pigs showed a significant increase in PIP after challenge with TMA-GPSA ($P < 0.05$).

In group B, inhalation of 2.5% TMA-GPSA provoked an increase in PIP in two of five guinea pigs sensitized with single injections of 0.3% TMA (80% and 220%) and two of six animals sensitized with repeated injections of 0.1% TMA (100% and 275%). This was not significantly different from the control group. In guinea pigs injected with a single dose of 30% TMA, inhalation of TMA-GPSA provoked no change in PIP. TMA-GPSA nebulized poorly with an output of 10 μ l/min approximately.

Guinea pigs sensitized with 30% TMA, 0.3% TMA and 0.1% TMA showed a significant increase in PIP following intravenous TMA-GPSA compared to controls (Fig. 4). Guinea pigs sensitized with a single dose of 0.3% TMA or repeated doses of 0.1% TMA had greater maximal responses in PIP ($P < 0.01$) after intravenous injection of TMA-GPSA when compared to those guinea pigs sensitized with a single dose of 30% TMA. These responses were also of significantly greater duration ($P < 0.01$). The control group showed no change in PIP after inhalation or intravenous injection of TMA-GPSA. There was no difference in the size or duration of response to intravenous TMA-GPSA in guinea pigs sensitized with 0.3% TMA or 0.1% TMA. Changes in bronchopulmonary response occurred within 1 min of the intravenous injection of TMA-GPSA and a maximum response was reached rapidly. This was followed by a spontaneous decline in pulmonary inflation pressure. These responses were accompanied by a fall in blood pressure in most guinea pigs occurring immediately after injection of the

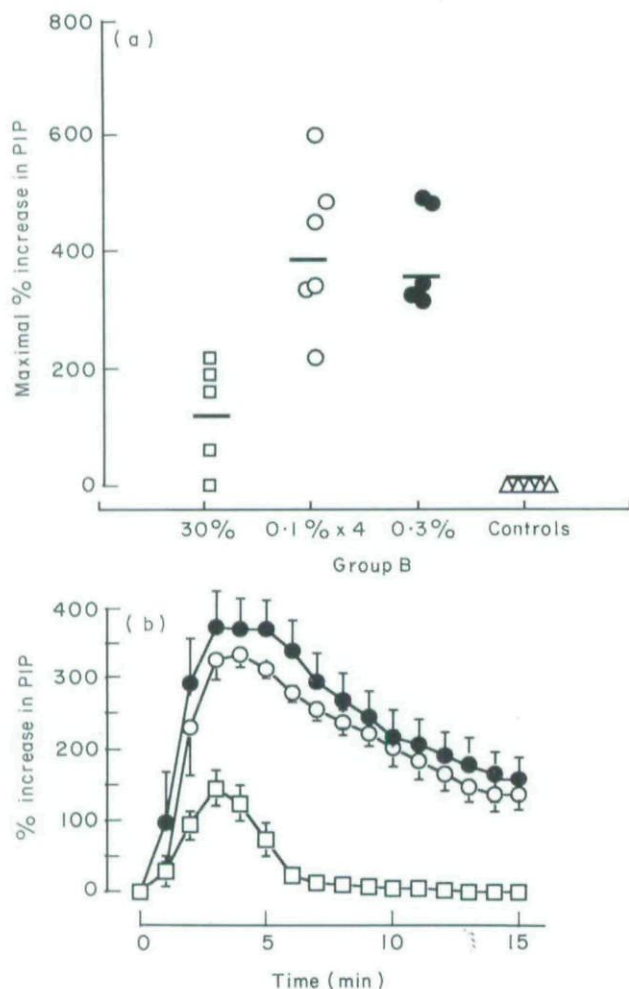


Fig. 4. (a) Per cent maximal increase in pulmonary inflation pressure following intravenous TMA-GPSA in guinea pigs sensitized by single injections with 0.1 ml of 30% TMA, 0.1 ml of 0.3% TMA and four injections with 0.1 ml of 0.1% TMA. *Indicates the subgroups with significantly greater maximal responses ($P < 0.01$) compared to the subgroup receiving 30% TMA. Horizontal bars indicate median values. (b) The time course of bronchopulmonary response following intravenous TMA-GPSA in guinea pigs sensitized by single injections with 0.1 ml of 30% TMA ($\square$), 0.1 ml of 0.3% TMA ($\bullet$) and four injections of 0.1 ml of 0.1% TMA ($\circ$).

antigen which returned to baseline within 1 min. There was no evidence of pulmonary oedema or haemorrhage on histological examination.

Discussion

In these experiments we have found that intradermal sensitization with free TMA in corn oil can induce a specific IgG1 immune response in guinea pigs. This response is specific for the hapten conjugate TMA-GPSA

and was induced by all three dosage schedules examined. We have also shown that intradermal injection of TMA may induce an IgE antibody response as determined by PCA. Challenge with inhaled and intravenous TMA-GPSA can provoke bronchopulmonary responses which in the case of intravenous TMA-GPSA are hapten specific. These responses are rapid in onset, with evidence of spontaneous recovery. This, in conjunction with the absence of morphological changes indicate that the response reflects changes in the intrathoracic airways. We found no relationship between the level of IgG1 antibody titres or the presence of IgE antibodies and the bronchopulmonary response to either inhaled or intravenous TMA-GPSA. It is likely that IgG1 antibodies reflect exposure to TMA and while IgE antibodies seem more likely to be associated with greater bronchopulmonary responses, the response, at least in this model, may not be dependent on the presence of IgE antibodies. There are two other possible explanations, namely PCA measurements may not be sensitive enough to detect low levels of IgE antibody, much of which may be cell bound or the response may be more closely associated with other IgG antibodies which were not measured here. We found, as in previous studies of sensitization with ovalbumin in guinea pigs [9], that low dose intradermal sensitization with TMA is a more effective method of sensitization as determined by the greater magnitude and duration of the bronchopulmonary response observed after intravenous challenge with TMA-GPSA. Single or repeat low dose sensitization also seemed more likely to result in bronchopulmonary responses to inhaled TMA-GPSA. A greater number of responses to inhaled TMA-GPSA might have been seen with increased concentration of antigen as TMA-GPSA in solution nebulized poorly in comparison to normal saline. In contrast to sensitization of guinea pigs [10] with ovalbumin, we observed no advantage in giving repeat low dose injections of TMA. This may be the consequence of slow release of TMA suspended in corn oil from the site of injection. In study A, only 50% of the guinea pigs sensitized responded to intravenous challenge with TMA-GPSA, an effect that may reflect the dose of TMA used to sensitize the guinea pigs as high dose sensitization has been previously noted to be less effective [9].

Previous work with TMA in animals has focused on methods of sensitization in different species and on the immunological basis for pulmonary haemorrhage-haemolytic anaemia syndrome. More recently Botham *et al.* has shown that inhaled free TMA provoked changes in respiratory rate in animals after high dose intradermal sensitization [8]. Our paper to our knowledge, is the first to report an animal model of airway hypersensitivity responses to acid anhydrides. Previous studies have

shown that different animals can be sensitized to various haptens including isocyanates, plicatic acid and complex salts of platinum by parenteral or inhalation routes. The success of parenteral sensitization has depended upon conjugation of the hapten to serum albumin of another species. Bernstein *et al.* [11] provoked significant changes in pulmonary conductance in the airways of guinea pigs passively sensitized by antisera to hexamethylene diisocyanate (HDI) conjugated to human serum albumin and challenged with HDI conjugated to human transferrin 48 hr later. Van Oostdam *et al.* [12] have shown specific homocytrophic antibody responses to parenteral plicatic acid conjugated to ovalbumin in rabbits and guinea pigs and Chan *et al.* [13] using the same method of sensitization were able to induce airway hypersensitivity responses in rabbits challenged with intravenous plicatic acid conjugated to human serum albumin. There have been several reports of animal models of sensitization by inhalation of free haptens with subsequent airway responses on further exposure to the sensitizing agent. Karol [14] has reported changes in respiratory rate provoked by inhaled toluene diisocyanate (TDI) in guinea pigs sensitized previously to inhaled TDI. The exact nature of this response remains unclear but has been correlated with an IgE immune response. Biagini sensitized cyanomologous monkeys to inhaled hexachloroplatinate and was able to demonstrate increased bronchial hyperresponsiveness to methacholine and hexachloroplatinate in monkeys exposed to ozone and platinum but not to hexachloroplatinate alone [15].

In man trimellitic anhydride has been reported to cause several patterns of pulmonary and systemic responses including asthma-rhinitis, late respiratory systemic syndrome and pulmonary haemorrhage haemolytic anaemia [16]. Occupational asthma due to acid anhydrides has been associated with an IgE or IgG4 antibody response. There is often persistence of respiratory symptoms and increased bronchial hyperresponsiveness in spite of complete avoidance of further exposure, which in occupational asthma due to tetrachlorophthalic anhydride has been associated with continued IgE secretion [17]. Occupational asthma due to low molecular weight compounds differs from allergic asthma in that atopic individuals do not appear to be at greater risk of developing asthma than those who are non-atopic. However, cigarette smoking has been associated with an increased risk of developing specific IgE antibodies to tetrachlorophthalic anhydride. [18] and of developing occupational asthma suggesting that it and possibly other environmental factors may have an important role in the development of asthma by inhaled low molecular weight chemicals.

We have been able to induce both an immunological response to an intradermal injection of TMA and subse-

quently to provoke changes in airway function by inhalation and intravenous injection of TMA-GPSA in the guinea pig. We have optimized our method of sensitization, which may allow further studies of the effects of inhaled conjugate on airway responses. This model should allow examination of the pathophysiological mechanisms of asthma caused by acid anhydrides and possibly other low molecular weight chemicals.

Acknowledgments

We thank Drs P. Botham and P. Hext of the Toxicology Unit, ICI Alderley, U.K. for useful discussion during the initial stages of this study.

References

- 1 Fawcett JW, Newman Taylor AJ, Pepys J. Asthma due to inhaled chemical agents-epoxy resin systems containing phthalic anhydride, trimellitic anhydride and triethylene tetramine. *Clin Allergy* 1977; 7:1-14.
- 2 Zeiss CR, Patterson R, Pruzansky JJ, Miller MM, Rosenberg M, Levitz D. Trimellitic-anhydride induced airway syndromes: clinical and immunologic syndromes. *J Allergy Clin Immunol* 1977; 60:96-103.
- 3 Topping MD, Venables KM, Luczynska CM, Howe W, Newman Taylor AJ. Specificity of the human IgE response to inhaled acid anhydrides. *J Allergy Clin Immunol* 1986; 77:834-42.
- 4 Nielson J, Welinder H, Shutz A, Skerfving S. Specific serum antibodies against phthalic anhydride in occupationally exposed subjects. *J Allergy Clin Immunol* 1988; 82:126-33.
- 5 Sale SR, Patterson R, Zeiss CR, Fiore M, Harris KE, Yawn D. Immune response of dogs and rabbits to intrabronchial trimellitic anhydride. *Int Archs Allergy appl Immunol* 1982; 67:329-34.
- 6 Patterson R, Roberts M, Harris KE, Levitz D, Zeiss CR. Pulmonary and systemic immune responses of rhesus monkeys to intrabronchial administration of trimellitic anhydride. *Clin Immun Immunopath* 1979; 15:357-66.
- 7 Leach CL, Hatoum N, Rataczak HV, Zeiss CR, Garvin PJ. Evidence of immunologic control of lung injury induced by trimellitic anhydride. *Am Rev Respir Dis* 1988; 137:186-91.
- 8 Botham PA, Rattray NJ, Woodcock DR, Walsh ST, Hext PM. The induction of respiratory allergy in guinea pigs following intradermal injection of trimellitic anhydride: a comparison with the response to 2,4-dinitrochlorobenzene. *Toxicol Letters* 1989; 47:25-39.
- 9 Andersson P. Antigen-induced bronchial anaphylaxis in actively sensitised guinea pigs. Pattern of response in relation to immunisation regimen. *Allergy* 1980; 35:65-71.
- 10 Andersson P. Antigen induced bronchial anaphylaxis in actively sensitised guinea pigs. The effect of booster injection and cyclophosphamide treatment. *Int Arch Allergy Appl Immunol* 1981; 64:249-58.
- 11 Bernstein IL, Splansky GL, Chen SE, Vinegar A. The guinea

- pig model of diisocyanate sensitization ii. Physiologic studies. *J Allergy Clin Immunol* 1982;70:393-8.
- 12 Van Oostdam J, Moreno R, Chan H, Pare PD, Schellenberg RR, Yeung M. Animal models of western red cedar asthma. *Am Rev Respir Dis* 1985; 31:supplA9.
- 13 Chan H, Kam ST, Van Oostdam J, Moreno R, Pare PD, Chan-Yeung M. A rabbit model of hypersensitivity to plicatic acid, the agent responsible for red cedar asthma. *J Allergy Clin Immunol* 1987; 79:762-7.
- 14 Karol MH. Concentration dependent immunologic response to toluene diisocyanate (TDI) following inhalation exposure. *Toxicol Appl Pharmacol* 1983; 68:229-41.
- 15 Biagini RE, Moorman WJ, Lewis TR, Bernstein IL. Ozone enhancement of platinum asthma in a primate model. *Am Rev Respir Dis* 1986; 134:719-25.
- 16 Venables KM. Low molecular weight chemicals, hypersensitivity, and direct toxicity: the acid anhydrides. *Br J Ind Med* 1989; 46:222-32.
- 17 Venables KM, Topping MD, Nunn AJ, Howe W, Newman Taylor AJ. Immunologic and functional consequences of chemical (tetrachlorophthalic anhydride)-induced asthma after four years of avoidance of exposure. *J Allergy Clin Immunol* 1987; 80:212-18.
- 18 Venables KM, Topping MD, Howe W, Luczynska CM, Hawkins R, Newman Taylor AJ. Interaction of smoking and atopy in producing specific IgE antibody against a hapten protein conjugate. *Br Med J* 1985;290:201-4.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.