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Airway Responses following Intradermal Sensitization to Different Types of Allergens: Ovalbumin, Trimellitic Anhydride and *Dermatophagoides farinae*

Abstract

Sensitization of guinea pigs by intradermal injections of the occupational allergen trimellitic anhydride (TMA) in oily vehicle has been shown to be very reproducible. We studied the effect of intradermal sensitization with ovalbumin (OA) in oily vehicle on immune and airway responses in guinea pigs. We also compared airway responses to trimellitic anhydride or *Dermatophagoides farinae* (Df; mite) with those to OA in guinea pigs intradermally sensitized to respective allergens. Three to four weeks after sensitization, the animals were challenged with intratracheal instillation of these allergens. Intradermal injections with OA developed dose-dependently specific IgG1 antibodies to OA demonstrated by ELISA. In animals sensitized with different doses of OA in corn oil vehicle, a challenge with OA induced a reversely dose-dependent airflow obstruction and airway plasma exudation. In contrast, animals sensitized with OA in saline vehicle had dose-dependent airway responses to OA. Challenge with OA caused an immediate peak and subsequently persistent airflow obstruction, whereas this response to either TMA guinea pig serum albumin or Df was slowly progressive in animals sensitized to respective allergens. The animals sensitized to TMA or Df may show a different profile of airway responses following the challenge compared to OA. Intradermal sensitization may be a valuable method of sensitization for the development of an animal model of airway allergy to different types of allergens, including chemicals or mites.

Key Words

Occupational asthma
Airway response
Ovalbumin
Vascular permeability
IgG1
Sensitization intradermal
House dust mite

Introduction

A guinea pig model of airway allergy to ovalbumin (OA) was employed to understand further the pathophysiological mechanisms of asthma, and contribute to the development and preclinical use of novel drugs in the search for new

treatments [1]. This model mimics allergic bronchial asthma, because of the similar capacity of allergen challenge to induce airflow obstruction. Although OA is one important food allergen causing atopic disease, especially in younger children, it is an uncommon allergen in bronchial asthma.

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Table 1. Baseline data of weight, R_L and mean systemic blood pressure

	OA in saline		OA in corn oil				TMA in corn oil			Df in corn oil		
	3 μ g	300 μ g	0 μ g	3 μ g	30 μ g	300 μ g	0 μ g	3 μ g	300 μ g	0 μ g	30 μ g	300 μ g
Body weight, g	634 $\pm$ 24 (n = 5)	652 $\pm$ 25 (n = 6)	585 $\pm$ 20 (n = 4)	652 $\pm$ 10 (n = 6)	642 $\pm$ 14 (n = 6)	662 $\pm$ 15 (n = 6)	620 $\pm$ 12 (n = 6)	558 $\pm$ 9 (n = 6)	538 $\pm$ 11 (n = 5)	520 $\pm$ 25 (n = 5)	550 $\pm$ 4 (n = 5)	558 $\pm$ 14 (n = 5)
R_L , cm H ₂ O/ml/s	0.26 $\pm$ 0.02	0.27 $\pm$ 0.01	0.28 $\pm$ 0.03	0.25 $\pm$ 0.01	0.27 $\pm$ 0.01	0.25 $\pm$ 0.01	0.26 $\pm$ 0.01	0.25 $\pm$ 0.01	0.26 $\pm$ 0.01	0.29 $\pm$ 0.03	0.26 $\pm$ 0.01	0.26 $\pm$ 0.01
Blood pressure, mm Hg	46 $\pm$ 2	41 $\pm$ 2	48 $\pm$ 7	43 $\pm$ 2	41 $\pm$ 3	46 $\pm$ 3	49 $\pm$ 2	48 $\pm$ 2	44 $\pm$ 1	42 $\pm$ 5	49 $\pm$ 2	45 $\pm$ 3

Recently, guinea pig models of airway allergy to other allergens have been developed. One allergen is trimellitic anhydride (TMA) [2–4], which is a reactive chemical of low molecular weight and may cause occupational asthma [5]. The other is *Dermatophagoides farinae* (Df) [6], which is house dust mite and one of the most important inhaled allergens inducing asthma and allergic rhinitis with specific IgE antibodies [7]. However, we do not know whether guinea pigs sensitized do different types of allergens may have different profiles of airway responses to the respective allergens compared to the OA model.

Several different procedure have been utilized to sensitize guinea pigs to OA; intraperitoneal injection of OA mixed with the killed *Bordetella pertussis* organisms, intraperitoneal injection of OA mixed in aluminium hydroxide, subcutaneous injection of OA in Freund's complete adjuvant and aerosol of OA [8–11]. In the Df model, the sensitization was performed by intraperitoneal injection of Df mixed in Al(OH)₃ [6]. We have previously demonstrated that sensitization of guinea pigs with TMA is highly reproducible [4, 12]. In these previous studies, we administered free TMA in corn oil vehicle by intradermal injection to guinea pigs without any adjuvant added during sensitization. It is unclear whether this high reproducibility is due to the route of sensitization or to the oily vehicle during sensitization.

The aim of this study was firstly to evaluate the effects of intradermal injections of OA suspended in corn oil vehicle at the sensitization on immune and airway responses in guinea pigs compared with those of the animals sensitized with OA diluted in saline vehicle. Secondly, we compared airway responses following intradermal sensitization with OA, TMA or Df suspended in corn oil in guinea pigs.

Materials and Methods

Sensitization Protocol

Male Dunkin Hartley guinea pigs (300–350 g) were divided into five groups (n = 5–6 in each group) in order to study the effects of two intradermal injections of OA (3, 30 or 300 μ g) in corn oil (0.1 ml) at the sensitization on immune and airway responses compared with animals sensitized with OA (3 or 300 μ g) in saline (0.1 ml; table 1). In another series of experiments, four groups of animals (n = 5–6 in each group) were sensitized separately by two intradermal injections of free TMA (3 or 300 μ g) or Df (30 or 300 μ g) in corn oil (0.1 ml). None of these antigens were soluble in corn oil, and they were therefore suspended in corn oil. Control groups consisted of animals which were injected intradermally with corn oil alone and challenged with OA, TMA-guinea pig serum albumin (TMA-GPSA) or Df separately (n = 4–6 in each group; table 1). The doses of allergens at the sensitization or the challenge were chosen from our previous study of the TMA model [3, 4, 12, 19].

Measurement of Lung Function

Animals were anesthetized with ketamine (50 mg i.p.) and xylazine (4–6 mg i.m.). Guinea pigs were placed on a heated blanket (Harvard model 50-7061, Harvard Apparatus, Edenbridge, UK), which maintained body temperature at about 37°C. A polyethylene catheter was inserted into the carotid artery to monitor blood pressure with a pressure transducer (model P23XL, Viggo-Spectramed, Helsingborg, Sweden). The right external jugular vein was cannulated for administration of drugs and Evans blue dye. A tracheal cannula (10 mm length and 2.7 mm inner diameter) was inserted into the lumen of the cervical trachea through a tracheostomy, and was secured with a suture and connected to a constant volume mechanical ventilator (Harvard model 50-1718, Harvard Apparatus). A tidal volume of 10 ml/kg and a frequency of 60 breaths/min was used. The ventilatory circuit had a total volume of 18 ml. Airflow was measured with a pneumotachograph (Model FIL; Mercury Electronics, Glasgow, UK) connected to a transducer (Model FCO44; $\pm$ 20 mm H₂O; Furness Controls, Bexhill, Sussex, UK). Transpulmonary pressure was measured with a pressure transducer (Model FCO44; $\pm$ 1,000 mm H₂O; Furness Controls), with one side attached to a catheter inserted into the right pleural cavity and the other side connected to a side port of the intratracheal cannula. The signal from the blood pressure transducer was amplified with an analogue preamplifier (Kungsbacka mät & reglerteknik, Sweden). All signals were digitalized with a 12-bit analogue digital board (National

Instruments, Austin, Tex., USA) connected to a Macintosh II computer (Apple computer, Cupertino, Calif., USA) and analyzed with a software (Lab View, National Instruments™, Austin, Tex., USA), which was programmed to instantaneously calculate lung resistance (R_L) with the method of von Neergaard and Wirz [13]. Mean blood pressure was also monitored throughout the experiments.

TMA-GPSA Conjugate

TMA was conjugated to GPSA using a method described previously [3]. Briefly, four aliquots of 250 mg GPSA were mixed with 50 ml of 9% NaHCO_3 on ice and 250 mg of ground TMA dust was added over 10 min. Each aliquot was stirred separately for 1 h. The aliquots were pooled and spun for 10 min at 600 g (MSE Centaur 2). The supernatant was separated and dialyzed with 0.02 M NH_4HCO_3 for 48 h with six changes. This was further dialyzed with distilled water for 24 h and lyophilized. The degree of substitution was assessed by UV 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) in borate buffer was prepared. Then 25 μl 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. TMA-GPSA was viscous and not freely soluble in saline. Therefore, the TMA-GPSA suspension was used.

Challenge with OA, TMA-GPSA or Df

After the preparation, blood samples (1.0 ml) were taken from all animals sensitized to OA, centrifuged and the sera were frozen at -25°C for later assessment of IgG1 levels. Animals were then pretreated with propranolol (1 mg/kg i.v.) to block the bronchodilator effects of catecholamines, and with suxamethonium (5 mg i.v.), to avoid artifacts induced by spontaneous breathing. Ten minutes later, Evans blue dye (20 mg/kg) was given intravenously over 1 min. Another minute later, OA, TMA-GPSA or Df was instilled into the airways. Airway instillation was performed by flushing 50 μl of OA (0.3 mg/ml), TMA-GPSA (5 mg/ml) or Df (5 mg/ml) in saline with 1 ml of air through a needle, directly into the tracheal lumen via the tracheal cannula. Lung resistance and mean systemic blood pressure were monitored every 15 s over 1 min, and subsequently every 30 s for another 5 min after instillation of these allergens.

Determination of the Plasma Extravasation

Six minutes after instillation of OA, TMA-GPSA or Df, the animals were disconnected from the ventilator and the thoracic cavity was opened. A catheter was inserted into the aorta through the left ventricle, and the animals were perfused with 50 ml of 0.9% NaCl to remove dye within the bronchial circulation. After perfusion through this route another catheter was inserted into the pulmonary artery, and the pulmonary circulation was then perfused with 20 ml of saline to eliminate dye present in the pulmonary circulation. The trachea and lungs were dissected out en bloc, and the parenchyma carefully scraped off and extraneous tissue removed. The trachea, main bronchi and intrapulmonary airways were separated from each other, and intrapulmonary airways were then divided into two lengthwise equal portions, arbitrarily named proximal and distal. All airway tissues were dried, using a freeze dryer (MicroModulyo, Edwards High Vacuum International, West Sussex, UK) for 24 h. All dry tissues were then weighed, and Evans blue dye was extracted in 2 ml of formamide in a 40°C water bath for 24 h. Absorption at 620 nm was measured in a spectrophotometer (PU8670 VIS/NIR, Philips, Cambridge, UK). The extracted

Evans blue dye was quantified by interpolation on a standard curve of dye concentrations in the range of 0–10 $\mu\text{g}/\text{ml}$, and expressed as ng/mg dry tissue. Evans blue dye extravasation has been previously shown to highly correlate with the extravasation of radiolabelled albumin in guinea pig airways [14].

ELISA for Assessment of Specific IgG1-Antibodies [12]

Microtiter plates (Sero-Well, Bibby Sterlin, Stone Staffs, UK) were coated with 5 $\mu\text{g}/\text{ml}$ OA in 0.05 M $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ buffer pH 9.6 and incubated overnight at 4°C . After 4 washes in phosphate-buffered saline/Tween, guinea pig serum was added to the plates at 1:100 and incubated overnight at 4°C . After another 4 washes, the plates were incubated with 1:5,000 rabbit antiguinea pig IgG1 (NI 501140, AB Kemila-Preparat, Stockholm, Sweden), followed after a further 4 washes with 1:10,000 peroxidase-labelled goat antirabbit IgG (NI 670260, AB Kemila-Preparat), both incubations being for 2 h at 27°C . After 4 final washes, 2',2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic-acid (ABTS) substrate in ABTS buffer with hydrogen peroxide (10 μl of 30% H_2O_2 in 100 ml substrate buffer) was added 10 min before measurement. The reaction was stopped with 4 M sulfuric acid and the plate read at an optical density of 405 nm. The values were expressed as absorbance at a serum dilution of 1:100.

Drugs

The following drugs were used: ketamine hydrochloride (Park-Davis, Barcelona, Spain), xylazine chloride (Bayer Sverige, Göteborg, Sweden), propranolol hydrochloride (ICI, Macclesfield Cheshire, UK), suxamethonium chloride (Kabi Vitum, Stockholm, Sweden), Evans blue dye, trimellitic anhydride (Aldrich Chemical Co., Milwaukee, Wisc., USA), guinea pig serum albumin, egg albumin, ABTS (sigma Chemical Co., Poole, UK). The Df extract was kindly donated by Dr Roland Einarsson.

Data Analysis

Data are reported as means $\pm$ SEM. Non parametric analysis of variance (Kruskal-Wallis method) was used to determine any significant variance among groups. If a significant variance was found, a Mann-Whitney U test was used to test for significant differences between individual groups. A p value less than 0.05 was considered significant. Data were analyzed by a Macintosh computer using a standard statistical package (StatView II).

Results

Specific IgG1 Antibodies to OA

Guinea pigs sensitized with higher doses of OA in either saline or corn oil vehicle developed significantly higher levels of IgG1 antibodies to OA demonstrated by enzyme-linked immunoassay (ELISA) compared with animals sensitized with lower doses of OA (fig. 1a).

Peak R_L and Extravasation of Evans Blue Dye after Challenge with OA

There were no significant differences in baseline R_L in any of the groups studied (table 1). In animals sensitized with different doses of OA in corn oil vehicle, an intratra-

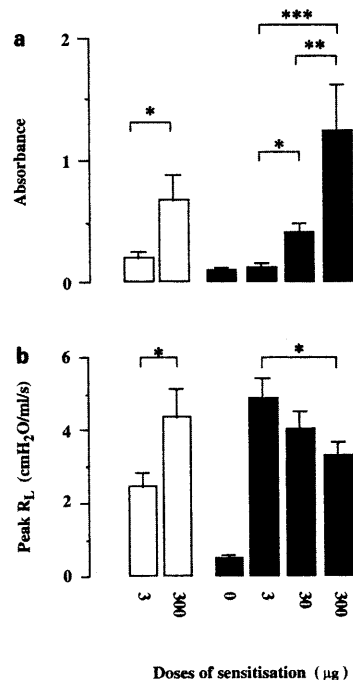


Fig. 1. Specific IgG1 antibodies to OA (a) and peak R_L after challenge with OA (b) 3–4 weeks after intradermal sensitization with different doses of OA in saline vehicle (□) or corn oil vehicle (■) in guinea pigs. Data are shown as mean $\pm$ SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$.

cheal challenge with OA induced a reversely dose-dependent increase in R_L (fig. 1b). The peak R_L was significantly higher in animals sensitized with 3 µg OA than 300 µg OA suspended in corn oil. In contrast, the peak R_L was significantly higher in animals sensitized with 300 µg OA than 3 µg OA in saline vehicle (fig. 1b). As observed with the peak R_L , a reversely dose-dependent extravasation of Evans blue dye after OA challenge was observed at all airway levels in animals sensitized with different doses of OA in corn oil vehicle (fig. 2). In contrast, extravasation of Evans blue dye was larger in animals sensitized with 300 µg OA than with 3 µg OA in saline vehicle.

Time Course of R_L after Challenge with OA, TMA-GPSA or Df

There were no differences in baseline R_L between any of the groups studied (table 1). The time courses of R_L after intratracheal challenge with OA, TMA-GPSA or Df in animals sensitized with different doses of the respective allergen in corn oil vehicle are illustrated in figure 3. The animals after challenge with OA, TMA-GPSA or Df following

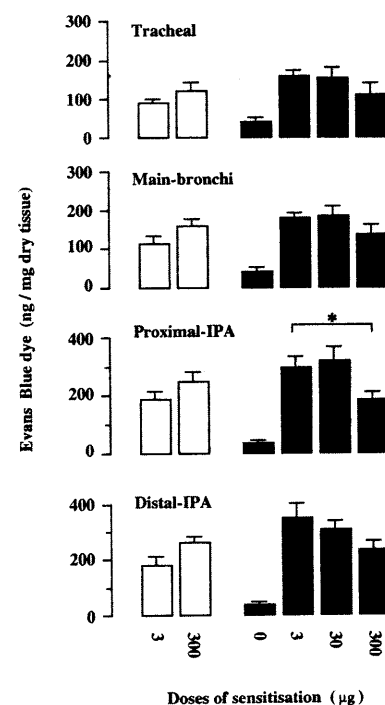


Fig. 2. Extravasation of Evans blue dye at different airway levels after challenge with OA 3–4 weeks after intradermal sensitization with different doses of OA in saline vehicle (□) or corn oil vehicle (■) in guinea pigs. IPA = Intrapulmonary airways. Data are shown as mean $\pm$ SEM. * $p < 0.05$.

intradermal injections with corn oil alone had a very small increase in R_L due to instillation of 50 µl volume. Intratracheal instillation of OA induced a rapidly progressing increase in R_L in all groups of animals sensitized to OA. In contrast, instilled TMA-GPSA or Df caused a slowly progressing increase in R_L in animals sensitized with 300 µg TMA or Df (fig. 3). Although the different profiles in the time course of R_L after the challenge was observed, the maximal levels of increase in R_L were similar in animals sensitized with the same dose (300 µg) of these allergens.

Extravasation of Evans Blue Dye after Challenge with TMA-GPSA or Df

There were no significant differences in Evans blue dye content in airway tissues among animals after challenge with OA, TMA-GPSA or Df following intradermal injections with corn oil alone [data not shown]. In animals sensitized with 300 µg of either TMA or Df in corn oil vehicle, the challenge with respective allergens produced a significant extravasation of Evans blue dye at all airway levels (table 2). However, TMA-GPSA or Df did not produce a

Fig. 3. The time course of R_L changes after challenge with OA, TMA-GPSA or Df in animals sensitized with different doses ($\blacktriangle = 3 \mu\text{g}$; $\triangle = 30 \mu\text{g}$; $\bullet = 300 \mu\text{g}$) of the respective allergen in corn oil vehicle. Control animals were intradermally injected with corn oil alone and challenged with the respective allergen 3–4 weeks later (no symbol). Data are shown as mean $\pm$ SEM.

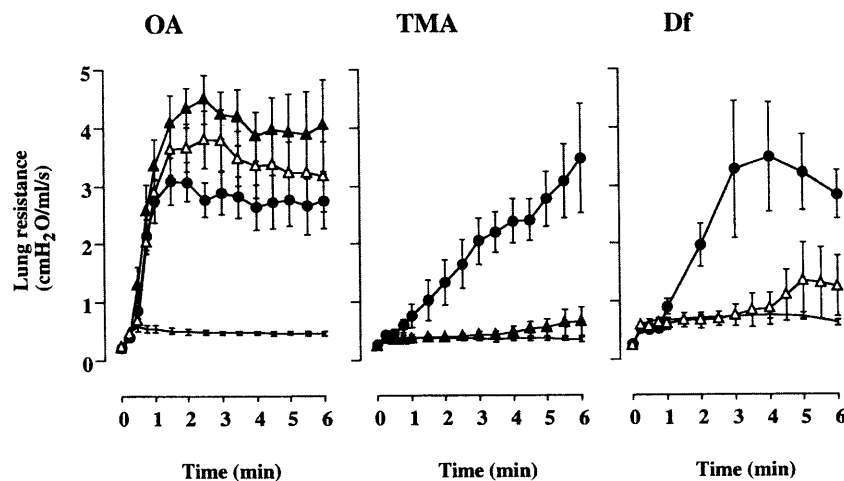


Table 2. Extravasation of Evans blue dye after challenge with intratracheal instillation of TMA-GPSA or Df in guinea pigs sensitized with the respective allergens

Group	Doses of sensitization μg	Amount of Evans blue dye ng/kg dry tissue			
		trachea	main bronchi	proximal IPA	distal IPA
TMA	3 (6)	50 $\pm$ 8	49 $\pm$ 15	46 $\pm$ 7	59 $\pm$ 7
	300 (5)	87 $\pm$ 12	99 $\pm$ 5**	179 $\pm$ 13**	179 $\pm$ 18**
Df	30 (5)	80 $\pm$ 16	64 $\pm$ 17	67 $\pm$ 22	76 $\pm$ 21
	300 (5)	119 $\pm$ 17**	192 $\pm$ 18**	184 $\pm$ 42**	245 $\pm$ 55**

Values are expressed as mean $\pm$ SEM. Figures in parentheses represent number.

** $p < 0.01$, vs. control animals which were injected with corn oil alone and challenged with the respective allergen. IPA = Intrapulmonary airways.

significant extravasation of Evans blue dye in animals sensitized with a lower dose of TMA or Df (table 2).

Discussion

We found that in guinea pigs sensitized with intradermal injections of OA, TMA or Df in corn oil vehicle, specific airway responses to respective allergens can be produced 3–4 weeks later. In the case of OA, specific IgG1 antibodies to OA were produced in a dose-dependent manner. However, airway responses to OA were more pronounced in animals sensitized with the lowest dose of OA in corn oil vehicle, whereas this was not the case in animals sensitized with OA in saline vehicle. Intratracheal challenge with OA caused rapidly progressing airflow obstruction and pronounced airway plasma exudation. In contrast, a slowly progressing air-

flow obstruction was observed in animals sensitized with either TMA or Df.

In the present study, guinea pigs sensitized with higher doses of OA developed significantly larger amount of specific IgG1 antibodies to OA, even when either corn oil or saline was used as the vehicle for OA during sensitization. Interestingly, in animals sensitized with different doses of OA in corn oil vehicle, intratracheal challenge with OA induced a reversely dose-dependent increase in R_L . Therefore, the lower dose of sensitization most likely produces specific antibodies from a different group of immunoglobulins. It is possible that the airway response following airway challenge after low dose sensitization is due to IgE antibodies, although this antibody was not quantified in the present study. However, it is difficult to separate IgG1 from IgE with passive cutaneous anaphylaxis testing in the guinea pigs, and there are as yet no good methods of determining guinea

pig IgE levels. Because high levels of IgG1 were associated with lower airway responses to allergen challenge, it is possible that a specific IgG1 is protective in the guinea pigs. Alternatively, it is possible that a specific IgG1 inhibits the production of other allergen-specific immunoglobulins. Importantly, it seems that the amount of IgG1 antibodies reflects exposure to allergens, but does not relate to the airway responses after allergen challenge.

In the present study, we found greater airflow obstruction and larger airway plasma exudation after the challenge in animals sensitized with 3 µg OA in corn oil vehicle compared to animals sensitized with higher doses of OA in corn oil. These data suggest that intradermal sensitization with low doses of OA is the most effective method of sensitization to produce the specific airway responses at a later time. Also, these data confirm the study of Andersson [9], who found that intraperitoneal sensitization with low amounts of OA (1 µg) produced the most pronounced bronchoconstriction, compared to guinea pigs given much higher doses of allergen (100 µg). Furthermore, experimentally induced antibody formation in animals greatly depends on the doses of antigen [15, 16]. Therefore, our data taken together with the previous studies suggest that small quantities of antigen may produce a larger amount of antibodies associated with airway responses.

The animals sensitized with OA in corn oil vehicle had slightly more pronounced responses in the airways compared to animals sensitized with OA in saline, at least with the lowest sensitization dose. Thus, corn oil seemed to enhance the development of allergy, but the exact mechanism behind this finding is unclear. It is possible that the oil slowly releases the antigen from the site of injection, leading to a prolonged low dose exposure to antigen-presenting cells in the skin (Langerhans cells). Injection of corn oil alone intradermally produces a very small inflammatory response, compared to animals given TMA suspended corn oil [Mölne and Lötval, unpubl. observation]. In contrast to the inverse dose relationship when the vehicle is oil, a larger amount of allergen seemed to potentiate the reactivity to allergen challenge when saline is used as the vehicle for OA during sensitization. Again, it is possible that OA in saline is more rapidly eliminated from the site of injection, compared to OA in oil, shortening the time of OA being available for the immunization to be triggered. The route of sensitization and whether the antigen remains in the microenvironment may determine how the antigen interacts with antigen-presenting cells, and B and T lymphocytes [17].

Animals sensitized with intradermal TMA (300 µg) responded with both airflow obstruction and airway plasma exudation after intratracheal challenge with TMA-GPSA.

However, much lower doses of sensitization (3 µg TMA) failed to induce airway responses to TMA-GPSA. Hayes et al. [3] found that pulmonary inflation pressure after challenge with intravenous TMA-GPSA was higher in animals sensitized with a single injection 300 µg TMA compared with animals given a single high dose injection of 30 mg TMA, showing that an even higher dose for sensitization is less effective. Thus, 300 µg TMA may be an appropriate dose to sensitize guinea pigs and to induce specific airway responses. During sensitization, TMA was only given in oil suspension, because the anhydride molecule is destroyed in water. At the challenge, we gave TMA-GPSA to the sensitized guinea pigs, because the introduction of TMA dust into the tracheal lumen would be technically difficult and because the reactivity of TMA might result in direct airway damage [18]. Furthermore, the antibody response to TMA is directed against the protein conjugate [19], and thus it would be more appropriate to examine the effect of challenge with TMA-GPSA on airway responses.

We found that in all animals sensitized with 300 µg Df in corn oil, a challenge with Df produced both airflow obstruction and airway plasma exudation. A previous study demonstrated that the positive rate of airway response to Df was only 20–60% in animals sensitized with an intraperitoneal injection of Df (10–1,000 µg) mixed in Al(OH)₃, even though much higher doses (2–8 mg/ml) were given as an inhalation challenge [6].

In the present study, intratracheal instillation of OA induced a rapidly progressing increase in R_L and pronounced extravasation of Evans blue dye at all airway levels in all animals sensitized to OA. In contrast, instilled TMA-GPSA or Df caused a slowly progressing increase in R_L in animals sensitized with free TMA or Df in corn oil. The mechanisms behind the discrepancy in the time course of airway responses to different types of allergens is unknown, but may be explained in part by the physiochemical differences between these allergens. TMA is a low molecular weight chemical (200 molecular weight), but at the challenge we used TMA conjugated to guinea pig serum albumin, whose molecular weight is approximately 70 kD. On the other hand, Df contains multiple proteins, and two major allergens (*Der f*I and *Der f*II) in Df have recently been demonstrated [20, 21] with molecular weights of 24 and 14 kD, respectively. Thus, TMA-GPSA and Df may be complicated and larger allergens, whereas OA is a single pure protein, whose molecular weight is 45 kD. Therefore, there may be differences in diffusion and interaction to target cells, such as mast cells, between these different types of allergens.

Acid anhydrides are commonly used in the plastic industry and have been suggested to produce asthma in some ex-

posed workers [5]. On the other hand, house dust mite (Df) is the most common inhaled allergen associated with asthma [7]. In man, both anhydride and Df asthma are associated with either an IgE or IgG4 antibody response [22]. Guinea pigs sensitized to TMA or Df show several features of asthma, including airflow obstruction and exudation of plasma in the airways. Exposure of sensitive individuals to these allergens may cause a reduction of airway caliber, as observed in the sensitized guinea pigs.

This study shows that these three allergens are strong sensitizers in guinea pigs when administered by intracutaneous injections. In addition, low doses of allergen are enough for the development of sensitization when suspended in corn oil. It is possible that skin exposure to allergens

may be important for the induction of allergy, and may subsequently lead to allergic asthma, in sensitive individuals. We conclude that the guinea pig model of allergen-induced acute airway responses in several aspects mimics asthma in man, and should therefore lead to the examination of several pathophysiological mechanisms of this disease.

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