

Airway allergy to trimellitic anhydride in guinea pigs: Different time courses of IgG₁ titer and airway responses to allergen challenge

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Background: Trimellitic anhydride (TMA) is a low molecular weight chemical that may cause occupational asthma in human beings. The objectives of this study were to determine the time course of immune and airway responses to TMA in guinea pigs and to relate the immunologic response to the immediate responses in lung resistance (R_L) and plasma exudation induced by allergen challenge.

Methods: We studied the effects of time course after sensitization on airway response to TMA in guinea pigs actively sensitized to free TMA, given by intradermal injection (0.1 ml of 0.3% TMA in corn oil). During weeks 1, 2, 3, 5, and 8 after sensitization, anesthetized animals were challenged with TMA conjugated to guinea pig serum albumin (TMA-GPSA), instilled via the airway route. Nonsensitized animals were challenged with the same amount of conjugate 4 weeks after intradermal injection of corn oil only. In the same animal, we measured both R_L to monitor airflow obstruction and extravasation of Evans blue dye (20 mg/kg) to quantify airway plasma exudation.

Results: Instillation of TMA-GPSA (0.5%; 50 μ l) into the tracheal lumen caused a significant increase in R_L , reaching a maximum at 2.5 minutes after the instillation in the 1-week group (9.0 ± 5.9 cm H₂O/ml/sec) and between 5 and 6 minutes in the 2-, 3-, 5-, and 8-week groups (9.4 ± 4.8 , 12.7 ± 5.5 , 3.7 ± 1.1 , and 1.7 ± 0.2 cm H₂O/ml/sec, respectively). The maximal increase in R_L after the challenge in nonsensitized animals was 0.39 ± 0.05 cm H₂O/ml/sec. TMA-GPSA also produced significant extravasation of Evans blue dye at all airway levels in the sensitized groups, and the amount of dye in the peripheral airways was significantly greater than that in the trachea. Furthermore, the level of Evans blue dye in airway tissue increased with the time after sensitization, up to the latest time point tested (8 weeks). Specific IgG₁ antibodies to TMA-GPSA demonstrated by ELISA were detected in all animals in the 3-, 5-, and 8-week groups, with maximal levels 5 weeks after sensitization. Specific IgG₁ titers to TMA-GPSA significantly correlated with the level of Evans blue dye induced by challenge with TMA-GPSA but not with the increase in R_L .

Conclusions: Intradermal sensitization to free TMA induces specific airway allergy for a long period after sensitization. Specific IgG₁ antibodies to allergen may influence allergen-induced plasma exudation rather than the airflow obstruction in this animal model of TMA-induced asthma. (*J ALLERGY CLIN IMMUNOL* 1993;92:425-34.)

Key words: Occupational asthma, vascular permeability, sensitization, plasma exudation, maturation

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Abbreviations used

- GPSA: Guinea pig serum albumin
 R_L : Lung resistance
 R_s : Spearman's rank correlation coefficient
TMA: Trimellitic anhydride

Trimellitic anhydride (TMA) is a reactive chemical of low molecular weight used widely in alkyl and epoxy resins. TMA may cause both occupational asthma^{1, 2} and pulmonary hemorrhage with hemolytic anemia in human beings.^{3, 4} Occupational asthma caused by acid anhydrides is associated with a specific IgE antibody response in a majority of patients.⁵ Recently, a guinea pig model of airway allergy to TMA was developed. In guinea pigs previously sensitized to free TMA, airway challenge with TMA-guinea pig serum albumin (GPSA) produces both immediate airflow obstruction and immediate extravasation of plasma into the airways.^{6, 7} In previous studies the animals were challenged with TMA-GPSA 3 weeks after intradermal sensitization.^{6, 7} This time point was chosen on the basis of previous studies in which ovalbumin was used as the allergen and in which the maximal response in airflow obstruction was found 3 weeks after intraperitoneal sensitization.⁸ However, the time course of immune and airway responses to TMA after intradermal sensitization to free TMA is unknown. To our knowledge there are no previous studies of the time course of allergen-induced plasma exudation in airways after sensitization.

The aims of this study were therefore first to study the time course of immune response and airway responses to TMA and second to relate the immunologic response to the immediate response in lung resistance (R_L) and that in plasma exudation induced by allergen challenge. To do this, we challenged sensitized guinea pigs with TMA-GPSA at 1, 2, 3, 5, and 8 weeks after intradermal sensitization. In the same animals airflow obstruction was monitored by measurement of R_L , and plasma exudation into the airways was monitored with Evans blue dye as the albumin marker.

METHODS**Sensitization**

Thirty-one pathogen-free male Dunkin Hartley guinea pigs (which weighed 300 to 350 gm) were ac-

tively sensitized by two intradermal injections of 0.1 ml of 0.3% (wt/vol) TMA suspended in corn oil. Six control animals were given two intradermal injections of 0.1 ml of corn oil alone. The injections were given 24 hours apart.

Conjugate

TMA was conjugated to GPSA with a method described previously.⁹ 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 minutes. Each aliquot was stirred separately for 1 hour. The aliquots were pooled and spun for 10 minutes at 600 g (MSE Centaur 2, Centaur West, Inc., Sparks, Nev.). The supernatant was separated and dialyzed with 0.02 M NH_4HCO_3 at pH 7.4 for 48 hours with six changes. This supernatant was further dialyzed with distilled water for 24 hours and lyophilized.

Challenge with TMA-GPSA

Animals were anesthetized with ketamine (50 mg given intraperitoneally) and xylazine (4 to 6 mg given intramuscularly). The guinea pigs were placed on a heated blanket (Harvard model 50-7061, Harvard Apparatus Ltd., Edenbridge, Kent, England), which maintained body temperature at about 37° C. A polyethylene catheter was inserted into the carotid artery of each animal 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 in length with a 2.7 mm inner diameter) was inserted into the lumen of the cervical trachea through a tracheostomy, secured with a suture, and connected to a constant volume mechanical ventilator (Harvard model 50-1718, Harvard Apparatus Ltd.). A tidal volume of 10 ml/kg and a frequency of 60 breaths/min were used. The ventilatory circuit had a total volume of 18 ml. Airflow was measured with a pneumotachograph (model F1L; GM Instruments Ltd., Kilwinning, U.K.) connected to a transducer (model FCO44; ± 20 mm H_2O ; Furness Controls Ltd., Bexhill, Sussex, England). Transpulmonary pressure was measured with a pressure transducer (model FCO44; ± 1000 mm H_2O ; Furness Controls Ltd.) 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 analog preamplifier (Kungsbacka Mät & Reglerteknik AB, Kungsbacka, Sweden). All signals were digitalized with a 12-bit analog digital board (National Instruments, Austin, Texas) connected to a Macintosh II computer (Apple Computer Inc., Cupertino, Calif.) and analyzed with software (LabView, National Instruments, Austin, Texas), which was programmed to instantaneously calculate R_L by the method of von Neergaard and Wirz.¹⁰

Mean blood pressure was also monitored throughout the experiments.

Protocol

During weeks 1, 2, 3, 5, and 8 after intradermal sensitization of free TMA in corn oil, and after 4 weeks after intradermal injection of corn oil alone, animals were challenged with instillation of TMA-GPSA suspension via the airway route.

After preparation, blood samples (1.0 ml) were taken from all animals and centrifuged; the serum samples were frozen at -25°C , for later assessment of IgG₁ levels. Animals were then pretreated with propranolol (1 mg/kg given intravenously) to block the bronchodilator effects of catecholamines and with suxamethonium (succinylcholine) (5 mg given intravenously), to avoid artefacts induced by spontaneous breathing. Ten minutes later, Evans blue dye (20 mg/kg) was given intravenously over 1 minute. After another minute, TMA-GPSA was instilled into the airways. Airway instillation was performed by flushing 50 μl of 0.5% TMA-GPSA with 1 ml of air through a needle, directly into the tracheal lumen via the tracheal cannula. R_L and mean systemic blood pressure were monitored every 15 seconds for 1 minute and subsequently every 30 seconds for another 5 minutes after instillation of TMA-GPSA.

Determination of plasma extravasation

Six minutes after instillation of TMA-GPSA, the animals were disconnected from the ventilator, and the thoracic cavities were opened. A catheter was inserted into the aorta of each animal through the left ventricle, and each animal was 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 solution to eliminate dye present in the pulmonary circulation. The trachea and lungs were dissected out en bloc, the parenchyma was carefully scraped, and extraneous tissue was 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 tissue samples were dried, with the use of a freeze dryer (MicroModulyo, Edwards High Vacuum International, West Sussex, England), for 24 hours. All tissue samples were then weighed dry, and Evans blue dye was extracted in 2 ml of formamide in a 40°C water bath for 24 hours. Absorption at 620 nm was measured in a spectrophotometer (PU8670 VIS/NIR, Philips, Cambridge, England). The extracted Evans blue dye was quantified by interpolation on a standard curve of dye concentrations in the range of 0 to 10 $\mu\text{g/ml}$ and expressed as nanograms per milligram of dry tissue. Evans blue dye

extravasation has been previously shown to highly correlate with the extravasation of radiolabeled albumin in guinea pig airways.¹¹

ELISA for assessment of specific IgG₁ antibodies

Microtiter plates (Maxisorp F 96 A/S Nunc, Life Technologies, Glasgow, Scotland) were coated with 2 $\mu\text{g/ml}$ TMA-GPSA in 0.05 mol/L $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ buffer, pH 9.6, and incubated overnight at 4°C . After four washes in phosphate-buffered saline-Tween, guinea pig serum was added to the plates at doubling dilutions that ranged from 1:100 to 1:102,400 and incubated for 3 hours at 27°C . After another four washes, the plates were incubated with 1:5,000 rabbit anti-guinea pig IgG₁ (64-320-1, ICN Biomedicals Ltd., High Wycombe, Bucks, England), followed by four additional washes with 1:10,000 peroxidase-labeled goat anti-rabbit IgG (61-202-3, ICN Biomedicals Ltd.); both incubations lasted for 2 hours at 27°C . After four final washes, o-phenylenediamine substrate in phosphate-citrate buffer with urea hydrogen peroxide was added for 10 minutes. The reaction was stopped with 3 mol/L sulfuric acid, and the plate was read at an optical density of 490 nm. IgG₁ titers were calculated by interpolation on a dilution curve, with doubling serum dilutions on the x axis, and ELISA reading at 490 nm on the y axis. An ELISA reading above 0.2 at a dilution of 1:100 or more (above the 95% confidence interval for control animals at a dilution of 1:100) was considered to be positive.

Drugs and reagents

The following drugs and reagents were used: ketamine hydrochloride (Parke-Davis S.A., Barcelona, Spain); xylazine chloride (Bayer Sverige AB, Göteborg, Sweden); propranolol hydrochloride (ICI, Macclesfield, Cheshire, England); suxamethonium chloride (KabiVitum, Stockholm, Sverige, Sweden); Evans blue dye, and trimellitic anhydride (Aldrich Chemical Co., Milwaukee, Wis.); guinea pig serum albumin, urea hydrogen peroxide, and o-phenylenediamine substrate (Sigma Chemical Co., Poole, England).

Data analysis

Data are reported as means $\pm$ SEM. Nonparametric 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 determine significant difference between individual groups. A *p* value of less than 0.05 was considered significant. Spearman's rank correlation coefficient (R_s) was used to analyze the relationship between variables. Data were analyzed by a Macintosh computer with a standard statistical package (StatView II).

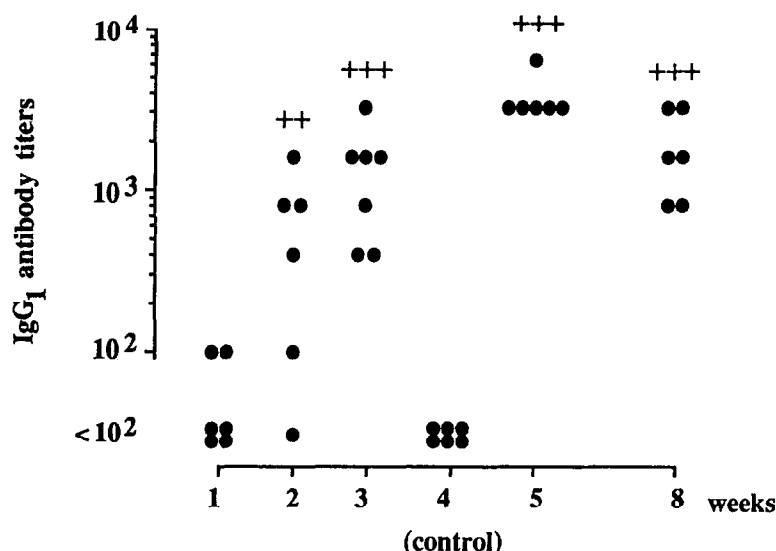


FIG. 1. Specific IgG₁ titers to TMA-GPSA for guinea pigs in the control group (given two intradermal injections of 0.1 ml corn oil and challenged with TMA-GPSA 4 weeks later) and in all sensitized groups (challenged with TMA-GPSA at 1, 2, 3, 5, or 8 weeks after sensitization with two intradermal injections of 0.1 ml of 0.3% TMA in corn oil). All animals in the 3-, 5-, and 8-week groups had specific IgG₁ antibodies to TMA-GPSA, with maximal levels of IgG₁ 5 weeks after sensitization. ++ $p < 0.01$, +++ $p < 0.005$ compared with the control group.

RESULTS

Time course of anti-TMA-GPSA IgG₁ titers

All animals in the 3-, 5-, and 8-week groups had specific IgG₁ antibodies to TMA-GPSA, with maximal levels of IgG₁ at 5 weeks after sensitization (Fig. 1). Only two of the six animals in the 1-week group and five of the six animals in the 2-week group had specific IgG₁ antibodies.

Time course of R_L responses to TMA-GPSA

Baseline R_L and peak R_L values after TMA-GPSA challenge in the sensitized and nonsensitized control guinea pigs are shown in Fig. 2. There was an inverse correlation between the baseline R_L and the weight of animals ($R_s = -0.55$, $p < 0.005$), indicating growth and larger airways in older animals. Therefore the 1-week group had significantly higher baseline R_L values compared with the 5- and 8-week groups. The peak R_L values (cm H₂O/ml/sec) to TMA-GPSA in sensitized guinea pigs were 9.0 ± 5.9 , 9.4 ± 4.8 , 12.7 ± 5.5 , 3.7 ± 1.1 , and 1.7 ± 0.2 cm H₂O/ml/sec at 1, 2, 3, 5, and 8 weeks after sensitization, respectively; all of these values were significantly higher than those observed after TMA-GPSA in the nonsensitized control group (0.39 ± 0.05 cm H₂O/ml/sec; $p < 0.005$ for the 1-, 2-, 3-, and 8-week groups and $p < 0.005$ for the

5-week group). The peak R_L values in animals were significantly higher at 2 and 3 weeks after sensitization compared with those in animals at 5 and 8 weeks ($p < 0.05$).

The individual immediate R_L changes after instilled TMA-GPSA for animals in the 1-, 3-, and 8-week groups are shown in Fig. 3. The response of R_L to TMA-GPSA was slowly progressing, peaking at 2.5 minutes in the 1-week group and at between 5 and 6 minutes in the 2-, 3-, 5-, and 8-week groups. An obvious progressive increase in R_L was observed in three of six animals at 1 week and in five of six animals at 3 weeks, but the response was very discrete in all animals at 8 weeks.

Time course of Evans blue dye extravasation to TMA-GPSA

Compared with instillation of TMA-GPSA in the nonsensitized control group, instillation of TMA-GPSA produced a significantly increased amount of Evans blue dye in the trachea for all sensitized groups except the 3-week group, in the main bronchi for every group except the 1- and 2-week groups, in the proximal intrapulmonary airway for all groups, and in the distal intrapulmonary airway for all groups except the 1-week group (Fig. 4). In addition, the amount of dye in

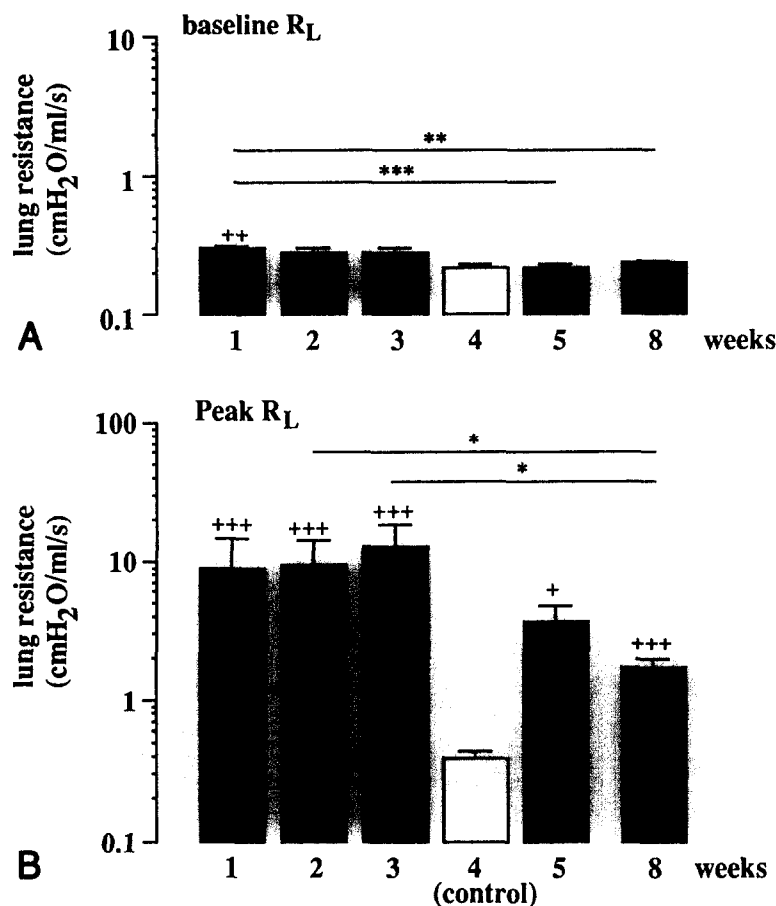


FIG. 2. A, Mean baseline R_L values in the control group and in all sensitized groups. Data are expressed as means $\pm$ SEM. There were no significant differences in baseline R_L values between any of the groups, except for the 1-week group, which had significantly higher baseline R_L values compared with the control group and the 5- and 8-week groups. B, Peak R_L values after the challenge in the control group and in all sensitized groups. Data are expressed as means $\pm$ SEM. Instillation of TMA-GPSA into the tracheal lumen caused a significant increase in R_L in all sensitized groups, compared with the control group. Peak R_L values were significantly higher in the 2- and 3-week groups compared with the 8-week group. $^+p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.005$ compared with the control group. $*p < 0.05$, $**p < 0.01$, $***p < 0.005$ when baseline R_L or peak R_L values after the challenge were compared among groups.

the peripheral airway was significantly greater than that in the central airway in all groups (Fig. 4). Also, the TMA-GPSA challenge produced a significantly higher degree of extravasation of Evans blue dye at 5 and 8 weeks, compared with that at 2 and 3 weeks (Fig. 4).

Relationship between antibody titers and airway responses

There was no correlation between the titers of specific IgG₁ antibodies to TMA-GPSA and the changes in R_L observed after TMA-GPSA challenge ($R_s = 0.162$). However, there was a significant correlation between the antibody titers and the TMA-GPSA-induced extravasation of Evans

blue dye at all airway levels ($R_s = 0.393$, $p < 0.05$ in trachea; $R_s = 0.559$, $p < 0.005$ in main bronchi; $R_s = 0.55$, $p < 0.005$ in proximal intrapulmonary airway; $R_s = 0.605$, $p < 0.001$ in distal intrapulmonary airway).

Blood pressure responses

There were no significant differences in baseline mean systemic blood pressure between the sensitized animals at different weeks after TMA sensitization and nonsensitized control animals (Table I). However, baseline mean systemic blood pressure was significantly higher at 3, 5, and 8 weeks after sensitization compared with that observed at 1 and 2 weeks after sensitization (Table

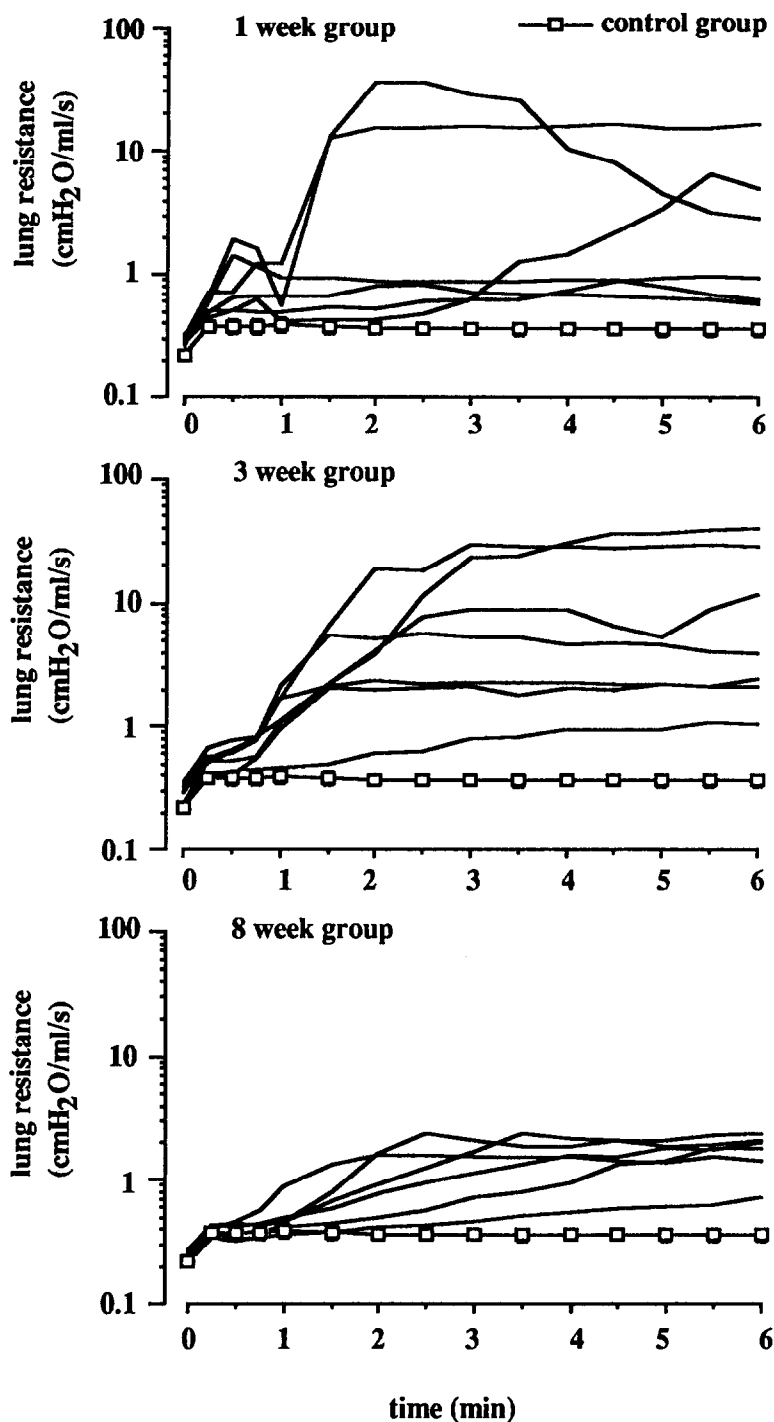


FIG. 3. The individual immediate R_L changes after challenge with instilled TMA-GPSA for animals in the 1-, 3-, and 8-week groups. Instilled TMA-GPSA induced a slowly progressing increase in R_L in three of the six animals in the 1-week group and in all of the animals in the 3- and 8-week groups.

I). TMA-GPSA induced a significant fall in mean systemic blood pressure in all groups except the 1-week group after challenge, compared with controls (Table I).

DISCUSSION

We have found that in guinea pigs sensitized with free TMA in corn oil, specific IgG₁ antibody titers to TMA-GPSA increased over time and

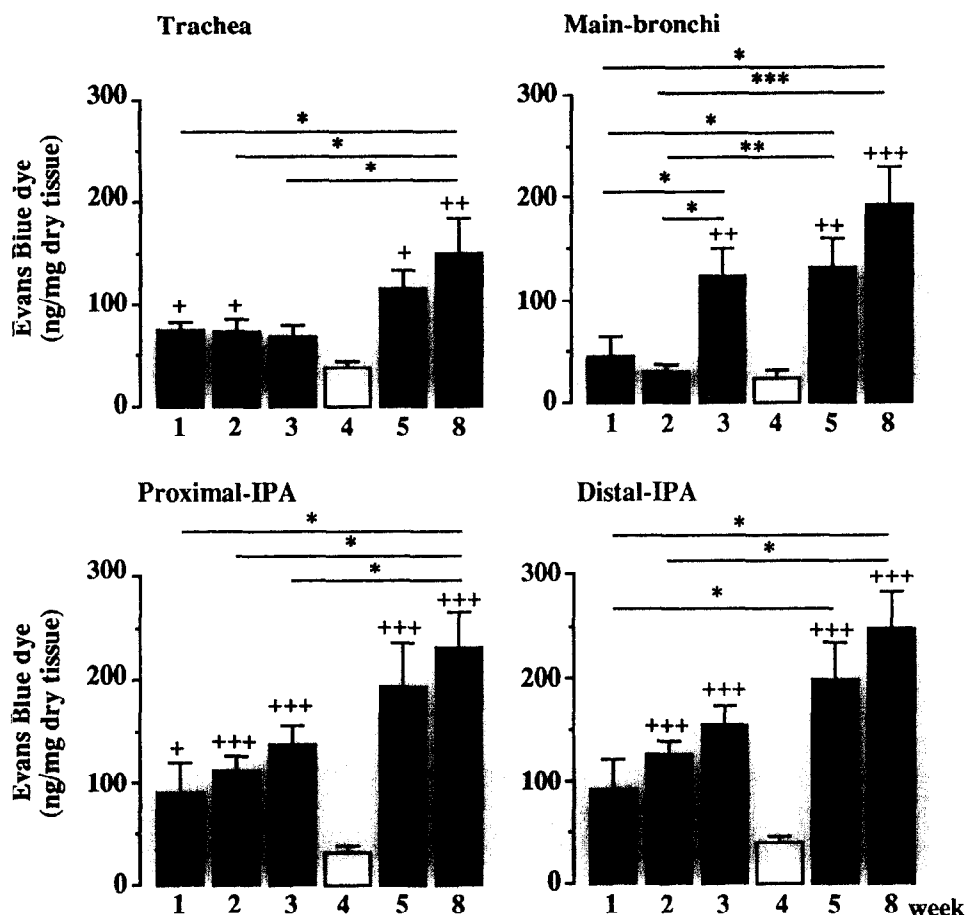


FIG. 4. Extravasation of Evans blue dye after the challenge in the control group and in all sensitized groups in trachea, main bronchi, and proximal and distal intrapulmonary airways. Data are expressed as means $\pm$ SEM. The extravasation of Evans blue dye induced by TMA-GPSA instilled into the tracheal lumen was significantly enhanced at all airway levels of all sensitized groups compared with the control group. IPA, Intrapulmonary airway. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ compared with the control group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ when extravasation of Evans blue dye after the challenge was compared among groups.

were sustained for up to 8 weeks, with maximal levels at 5 weeks after sensitization. Challenge with intratracheal TMA-GPSA produced a significant increase in R_L , with a maximal response 2 and 3 weeks after sensitization. Airway plasma exudation at different airway levels induced by TMA-GPSA increased at later time points after sensitization (observations up to 8 weeks after sensitization), even though the R_L response subsided with time. Plasma exudation was more pronounced in peripheral airways than in central airways. The specific IgG₁ titers significantly correlated with the TMA-GPSA-induced degree of plasma exudation but not with the induced airflow obstruction.

In previous models of airway allergy in experimental animals, the allergen was given intraperi-

toneally together with adjuvant, the latter being required for successful and reproducible sensitization.⁸ In the present study we administered free TMA, suspended in corn oil, by intracutaneous injections to induce sensitization in guinea pigs, without any adjuvant added during sensitization. All sensitized animals had specific IgG₁ antibodies to TMA-GPSA at 3 weeks and later after sensitization. We found that the titers were sustained over time, up to 8 weeks after sensitization, with a maximal level of antibody titers in the 5-week group. Furthermore, 15 of the 18 sensitized animals responded with a clear increase in R_L between 2 and 5 weeks after sensitization. It is unclear whether this high reproducibility is due to the route of sensitization or to the high reactivity of the TMA molecule. Perhaps the close relation

TABLE I. Baseline data of weight and mean systemic blood pressure and percent change in mean systemic blood pressure after instillation of TMA-GPSA for animals in each group

	Group					
	1-week (n = 6)	2-week (n = 6)	3-week (n = 7)	4-week (control) (n = 6)	5-week (n = 6)	8-week (n = 6)
Body weight	345 ± 7	388 ± 8	420 ± 11	543 ± 9	603 ± 29	803 ± 26
Blood pressure (baseline) (mm Hg)	36.3 ± 3.0	43.0 ± 1.8	47.7 ± 1.0*†	45.8 ± 3.4	54.0 ± 4.7*†	51.2 ± 1.6‡§
Percent change	-49.6 ± 10.7	-62.1 ± 8.9	-65.3 ± 8.6¶	-21.7 ± 3.8	-58.0 ± 8.4¶	-48.3 ± 6.1

Values are expressed as means ± SEM.

**p* < 0.01 compared with the 1-week group.

†*p* < 0.05 compared with the 2-week group.

‡*p* < 0.005 compared with the 1-week group.

§*p* < 0.005 compared with the 2-week group.

||*p* < 0.01 compared with the control group.

¶*p* < 0.05 compared with the control group.

to dermally localized antigen-presenting cells (Langerhans cells) is an important factor for the strong production of antibodies and allergic airway responses.¹² Botham et al.¹³ found that sensitization by inhalation of TMA produced specific homocytotropic antibodies in guinea pigs, but later challenge with atmosphere that contained TMA failed to induce change in respiratory rate. The same investigators⁶ found that animals sensitized to free TMA intracutaneously at a high dose (30%; 0.1 ml) responded by increased respiratory rate to an atmosphere that contained TMA (17 of 24 sensitized guinea pigs) or to nebulized TMA-GPSA (18 of 57 sensitized guinea pigs). In the present study a low dose for sensitization was used (0.3% TMA; 100 µl, twice), which may have produced a different antibody profile. Also, at challenge, allergen was administered directly into the trachea, guaranteeing that it reached the lower airways.

In ovalbumin-induced airway allergy in guinea pigs, the bronchial anaphylactic reactivity reaches a peak at 2 to 3 weeks after sensitization, with a subsequent decrease in reactivity in animals given relatively high doses of allergen (100 µg ovalbumin with aluminum hydroxide, intraperitoneally). However, in animals given much lower doses of allergen (1 µg ovalbumin with aluminum hydroxide, intraperitoneally), a high degree of reactivity persisted for 70 days.⁸ It was suggested that small quantities of antigen produced IgE antibodies.⁸ In the present study animals were actively sensitized by two intradermal injections of 0.1 ml of 0.3% (wt/vol) TMA suspended in corn oil. Thus a total amount of 600 µg TMA was given to each animal.

It is not clear whether this amount of TMA is a high or low dose of allergen, but in a preliminary study we found very little airway responsiveness to TMA-GPSA challenge in animals sensitized with a dose of 6 µg free TMA (unpublished observation).

We found that the relative contribution of airflow obstruction and airway plasma exudation in the airway responses to allergen varied at different times after sensitization. Thus airway plasma exudation in response to a fixed amount of TMA-GPSA increased with time after sensitization up to the latest time point tested (8 weeks), whereas the maximal response in airflow obstruction was observed at 3 weeks. The mechanisms behind the discrepancy in different airway responses to allergen are probably complex but may be explained by changes in either immunologic or physiologic mechanisms over time. First, the maturation of the animals during the course of the study must be considered. Animals challenged at 8 weeks after sensitization were larger and had significantly lower baseline R_L values, which correlated with the weight of the animals. Therefore geometric factors may explain the smaller increase in R_L values in the 8 week group. Also, we have previously shown that the responsiveness to different agonistic mediators in the airways changes over time in guinea pigs.¹⁴ Immature guinea pigs produce more plasma exudation in response to fixed dose of histamine, and adult guinea pigs produce more airflow obstruction.¹⁴ Because of the different profile of the histamine and allergen responses with maturation, it is unlikely that changes in the bronchial reactivity of smooth

muscle to allergen-induced histamine release are responsible for the decreased responsiveness at 5 and 8 weeks. However, altered responsiveness with growth or age to a fixed amount of other allergic mediators, such as leukotrienes, platelet activating factor, or thromboxane A_2 , may be responsible for the different levels of airflow obstruction and plasma exudation at different times after sensitization. Second, the number and the exact location in the microenvironment of allergen-responding cells may change for several weeks and months after sensitization. Thus mediator-releasing cells in the vicinity of the airway smooth muscle may produce mainly airflow obstruction through contraction of this muscle, and cells in the vicinity of the microvasculature may produce airway plasma exudation. Third, the correlation between specific IgG₁ titers and airway plasma exudation may imply that the specific IgG₁ antibodies to TMA mainly influence allergen-induced airway plasma exudation, whereas the induced airflow obstruction may be mediated via activation of inflammatory cells by other antibodies, possibly IgE, although this antibody was not quantified in the present study. However, in a previous study only 4 of 11 sensitized animals had positive passive cutaneous anaphylaxis, indicating the presence of specific IgE antibodies to TMA.⁹ It is difficult to separate IgG₁ from IgE with passive cutaneous anaphylaxis testing in the guinea pig, and there are as yet no good methods of determining guinea pig IgE levels. In fact, it is possible that airway allergic responses in guinea pigs are driven by IgG₁ and not by IgE as in human beings. Lastly, the extent of the airway response after allergen challenge may depend on the degree of bronchial reactivity in addition to the level of antibody titers.¹⁵

We found that instillation of TMA-GPSA into the airways caused a significant fall in systemic mean blood pressure, suggesting that mediators released after allergic response are involved in the decrease in blood pressure. We have previously found that in this model of airway allergy to TMA, pyrilamine (histamine H₁-receptor antagonist) attenuated the TMA-GPSA-induced decrease in blood pressure.¹⁶

Acid anhydrides are commonly used in the plastic industry and have been suggested to produce asthma in some exposed workers. In human beings anhydride asthma is associated with either an IgE or an IgG₄ antibody response.^{17, 18} In our animal model we can demonstrate several features of occupational asthma. We have sensitized

guinea pigs to free TMA by intradermal injection and induced an immune response similar to that seen in human beings. Exposure of sensitive individuals to anhydride dust may cause a reduction of airway calibre, as observed in this study with sensitized guinea pigs, showing acute bronchoconstriction with concomitant airway plasma exudation after intratracheal challenge with TMA-GPSA. Furthermore, anhydride challenge in sensitive individuals produces bronchial hyperresponsiveness,¹⁹ which has also been shown with an accompanying eosinophilic inflammatory exudation in this guinea pig model of anhydride allergic asthma.²⁰

This study shows that TMA is a strong sensitizer in guinea pigs when administered by intracutaneous injection. Another finding in this study in guinea pigs is that the contribution of plasma exudation to the immediate allergic response seems to increase gradually after sensitization, at least up to 8 weeks. We conclude that the guinea pig model of TMA-induced airway allergy in several aspects mimics occupational anhydride asthma in human beings and may therefore prove to be a helpful tool in elucidating mechanisms behind this disease.

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