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Trimellitic Anhydride-Induced Cellular Infiltration into Guinea Pig Lung Varies with Age but Not Gender

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Abstract

Background—In humans the incidence of asthma changes with age and gender. Immature guinea pigs have been used to model the allergic response to the occupational allergen trimellitic anhydride (TMA) where exposure to adults is paramount. We hypothesized that the TMA-induced allergic response in immature guinea pigs was similar to mature animals, regardless of gender.

Methods—Sexually immature and mature female and male guinea pigs were sensitized intradermally with TMA. Three weeks after sensitization they were challenged intratracheally with TMA conjugated to guinea pig serum albumin (TMA-GPSA) or GPSA as a control. Twenty-four hours later cell infiltration into the lung was determined. TMA-specific IgG₁ and IgG₂ were measured in plasma and the complement activation product C3a was measured in the bronchoalveolar lavage fluid.

Results—In control animals, numbers of eosinophils and neutrophils varied with age and gender. The TMA-GPSA-induced cellular infiltration was similar in all age/gender groups. However, neutrophils in the lung tissue increased only in immature animals. IgG antibodies differed between groups but did not account for differences in cell infiltration. C3a correlated with the extent of cell infiltration in all groups except mature females.

Conclusions—TMA-induced neutrophilia differs with age. TMA-induced changes in eosinophils and macrophages did not vary with age or gender. The relationship between complement activation and inflammation in mature females differs from that in the other groups, suggesting mediators of the response may change with age and gender. Effects of age and gender need to be considered in animal models of the allergic response.

Keywords

Asthma, occupational; Inflammation; Trimellitic anhydride; Acid anhydrides; Eosinophils; Complement; C3a

Introduction

Trimellitic anhydride (TMA), used in the paints and plastics industries, is one of many low-molecular-weight chemicals known to cause occupational asthma. Previous studies by us [1] and others [2–4] have characterized a guinea pig model of TMA-induced asthma. Guinea pigs sensitized by intradermal injection of TMA in corn oil and challenged by intratracheal instillation of TMA conjugated to guinea pig serum albumin (TMA-GPSA) respond with

immediate bronchoconstriction, microvascular leakage of proteins and infiltration of inflammatory cells into the lung, including significant eosinophilia. These and other studies of TMA-induced respiratory disease in the guinea pig have used Hartley or Dunkin-Hartley guinea pigs weighing approximately 300 g (estimated age of 21–28 days) at the time of sensitization [see 5–10]. Guinea pigs this size are not yet sexually mature, but have been used to study effects of an occupational allergen where exposure to adults is of primary concern. Certainly in humans the incidence of asthma varies with age and gender [11–13]. Arakawa et al. [2] studying the effects of TMA in guinea pigs speculated that differences in airway resistance and plasma exudation observed over time were due to maturation of the animals. However, the effects of age and gender on the guinea pig allergic response induced by TMA and other low-molecular-weight chemicals have received little consideration.

The effects of age and gender on bronchoconstriction and vascular permeability induced by inflammatory mediators and the antigen ovalbumin have been studied. However, consistent patterns of the effects have not emerged. The effects of leukotriene D₄ [14] and histamine [15] in contracting isolated respiratory tissues, and leukotriene D₄ and a thromboxane A₂ mimetic in inducing changes in lung resistance [16] are more pronounced in immature guinea pigs than in mature animals. By contrast, ovalbumin- and histamine-induced bronchoconstriction are more pronounced in mature guinea pigs [17,18]. Histamine-induced plasma exudation in the lung is more pronounced in immature guinea pigs than in mature animals [18], whereas ovalbumin- or eicosanoid-induced plasma exudation is more pronounced in mature guinea pigs [16,17]. Bradykinin, neurokinin A and substance P are more effective at increasing airway resistance and extravasation of Evans blue dye in mature guinea pigs compared to immature animals [19,20]. With regard to gender differences, Duncan and Douglas [14] reported that the potency of leukotriene D₄ in contracting respiratory tissue decreased with maturation to a greater extent in female than male animals. Although these studies provide some understanding of the effects of age and gender with respect to changes in bronchoconstriction and vascular permeability in the guinea pig, the effects of age and gender on antigen-induced cell infiltration are relatively unexplored.

In the present study we hypothesize that TMA-induced cellular infiltration into the guinea pig lung is similar in animals of both genders, whether sensitized to TMA when sexually immature, as has historically been done, or sensitized after reaching sexual maturity. To test this hypothesis, TMA-induced cellular infiltration into the lung and bronchoalveolar lavage fluid (BAL) was examined 24 h after intratracheal instillation of TMA-GPSA in both female and male guinea pigs sensitized to TMA when either sexually immature or after reaching sexual maturity. Red blood cells, an indicator of lung injury, and total protein in the BAL were also measured. To determine if differences in cell infiltration could be accounted for by differences in sensitization, TMA-specific IgG₁ and IgG₂ antibodies in plasma were measured. Previously we had shown that activation of the complement system played a role in TMA-induced cellular infiltration in immature guinea pigs [1], and that the concentration of the complement activation product C3a correlated with the extent of TMA-induced cell infiltration in immature female guinea pigs [21]. Therefore, C3a was measured to determine if differences in cell infiltration might be attributed to differences in mediator release.

Materials and Methods

TMA Sensitization and Challenge

All animal studies were approved by the University of Minnesota Institutional Animal Care and Use Committee and were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the US National Institutes of Health. Four groups of Hartley guinea pigs (n = 71, Charles River Laboratories, Portage, Mich., USA) were studied: female and male animals that were either sexually immature or sexually mature

at the time of sensitization (table 1). Guinea pigs were sensitized with approximately 1 mg TMA/kg body weight by intradermal injection of 0.3% TMA (w/v in corn oil) as shown in table 1. The asthmatic response to TMA was elicited 20–22 days after sensitization. Because bronchoconstriction following TMA-GPSA instillation can cause up to 50% mortality in sensitized guinea pigs, the animals were given 6.1 mg/kg i.p. of the H1 antagonist pyrilamine 30 min prior to challenge to reduce the potentially fatal immediate bronchoconstriction. Guinea pigs were anesthetized with ketamine and xylazine, then challenged by intratracheal instillation with either GPSA (control) or TMA-GPSA. Volumes of GPSA or TMA-GPSA (dissolved in water) used for intratracheal instillation (table 1) correspond to approximately 9 mg protein/kg of body weight. This dose of TMA-GPSA consistently elicits in immature female guinea pigs an immediate bronchoconstriction as well as the characteristic eosinophilia associated with asthma. The TMA-GPSA conjugate had 18–21 mol TMA/mol GPSA. Our previous studies have shown that the peak inflammatory response to TMA-GPSA occurs 24 h after challenge [21]. Therefore, 24 h after intratracheal instillation with either GPSA or TMA-GPSA, the guinea pigs were anesthetized with pentobarbital, the lungs were lavaged with six 5-ml aliquots of phosphate-buffered saline (PBS) and were removed for analysis. The BAL was centrifuged to sediment the cells and the BAL cell pellet was resuspended in 1.0 ml PBS. The BAL supernatant was used for measurements of protein and C3a as previously described [21]. Within each gender, 10–13 immature animals and 5–7 mature animals were subjected to each treatment. Details of this guinea pig model of the TMA-GPSA-induced allergic response in the lung as well as conjugation of TMA to GPSA appear in Fraser et al. [1].

Cell Infiltration

White blood cells in an aliquot of the resuspended BAL cell pellet were counted by standard methods in a hemacytometer and total white blood cells in the BAL were calculated. Differential counts were done on cytopsin preparations of BAL cells (3×10^4 cells) stained with a modified Wright's stain (Diff Quik, American Scientific Products, McGraw Park, Ill., USA). Two hundred cells were counted and identified as eosinophils, neutrophils or macrophages. Lung lobes were processed as previously described [1] for the measurement of eosinophil peroxidase (EPO) and myeloperoxidase (MPO) activity as an estimate of the number of eosinophils and neutrophils, respectively. EPO activity is expressed as the change in OD per minute per gram dry lung, and MPO activity is expressed as enzyme units per gram dry lung.

Red Blood Cells and Total Protein in the BAL

Red blood cells (RBC) in the cell pellet were lysed by freeze-thawing and the absorbance of hemoglobin (OD₄₁₂) determined as an indicator of lung injury [1]. Total protein in the BAL supernatant was measured by the Lowry method [22].

TMA-Specific Antibody in Plasma

TMA-specific guinea pig IgG₁ and IgG₂ antibodies were measured by ELISA, based on the methods of Kawabata et al. [23] as we have previously described [24]. Concentrations of TMA-specific IgG₁ and IgG₂ antibodies in the unknown plasma samples are expressed in terms of relative concentration compared to that of a reference antibody which is defined as 1. Our previous studies were unable to detect TMA-specific IgE in guinea pigs sensitized by the regimen used in this study (unpubl. data).

C3a in the BAL

C3a was used as an indicator of complement system activation. C3a in the BAL supernatant was measured by Western blot based on the methods of Maeno et al. [25] as we have previously described [21]. C3a concentration is expressed as YAC equivalents. A YAC equivalent is

defined as the microliters of a standard pool of yeast-activated complement (YAC) having an equivalent amount of C3a as that of the BAL sample.

Statistical Methods

Prior to statistical analyses, data were normalized to guinea pig size by dividing the measured value by the dry weight of the guinea pig lung, then log-transformed to equalize variances. To determine if the numbers of resident inflammatory cells or concentration of C3a in the BAL supernatant varied with gender or maturity, differences between GPSA-instilled control groups were tested by ANOVA with two-tailed single degree of freedom contrasts (fig. 1–3, 5, table 2). The comparisons made were immature female to immature male, mature female to mature male, immature female to mature female and immature male to mature male. To determine if the TMA-GPSA-induced inflammatory response or concentration of C3a in the BAL varied with gender or maturity, ANOVA with one-tailed single degree of freedom contrasts was used to test for differences between GPSA control and TMA-GPSA-challenged animals within each of the four age/gender groups (fig. 1–3, 5; table 2).

The concentration of TMA-specific antibody in the plasma prior to challenge was log-transformed to equalize variances but not normalized to guinea pig size. Differences between the four groups of animals were tested by ANOVA with two-tailed single degree of freedom contrasts (fig. 4, table 2). The comparisons made were immature female to immature male, mature female to mature male, immature female to mature female and immature male to mature male. Correlation analyses (Pearson product-moment correlation) were used to determine if differences in the inflammatory response might be due to differences in TMA-specific IgG₁ or IgG₂.

To determine if the inflammatory response was related to the extent of complement system activation, correlation analyses (Pearson product-moment correlation) were used to test for linear relationships between numbers of inflammatory cells and concentrations of C3a within each age/gender group (table 3).

Statistical significance for all tests was defined as $p < 0.05$. Statistical analyses were done using JMP software (SAS Institute, Cary, N.C., USA). Data shown in the figures represent geometric means ± 1 SE for each treatment.

Results

The effect of gender and sexual maturity on the TMA-GPSA-induced allergic response was examined in four groups of guinea pigs as shown in table 1. Immature animals are of similar weight (275–300 g) as those used in prior studies of TMA-induced allergy by us and others. The dose of TMA used for sensitization was based on the mean weight of the appropriate experimental group and ranged in individual animals from 0.9 to 1.3 mg TMA/kg body weight. The challenge dose of TMA-GPSA or control GPSA was based on the mean weight of the appropriate experimental group and ranged in individual animals from 7.2 to 10.4 mg protein/kg body weight. At the time of challenge immature females had become sexually mature whereas immature males had not. The volume of BAL fluid recovered ranged from 23 to 28 ml and did not significantly differ between experimental groups as determined by ANOVA.

Do the Resident Inflammatory Cells Vary with Gender and Maturity?

ANOVA with single degree of freedom contrasts was used to determine if numbers of resident inflammatory cells significantly differed between GPSA-instilled control groups. The population of resident inflammatory cells in lungs of GPSA-instilled groups (fig. 1–3, table 2) was similar in immature females and immature males. However, differences between mature

females and mature males were evident. Mature female guinea pigs had significantly more eosinophils in the BAL and greater EPO activity in the lung tissue than did mature males. Mature males by contrast had significantly greater MPO activity in the lung tissue than did mature females. Comparing immature and mature animals, mature females had significantly more eosinophils in the BAL and greater EPO activity in the lung tissue than did immature females, whereas mature males had significantly fewer eosinophils in the BAL and less EPO activity in the lung tissue than did immature males. Differences between the GPSA control groups were not detected for numbers of neutrophils or macrophages in the BAL (fig. 2, 3). In addition, the number of RBC and the amount of protein in the BAL did not differ between the GPSA control groups (table 2).

Does the TMA-GPSA-Induced Inflammatory Response Vary with Gender and Maturity?

Because resident inflammatory cells varied in the different groups, ANOVA with single degree of freedom contrasts was used to determine, within each age/gender group, if numbers of inflammatory cells in TMA-GPSA-challenged animals were significantly increased relative to GPSA controls. Numbers of eosinophils in the BAL and EPO activity in the lung tissue (fig. 1) increased in all groups after TMA-GPSA instillation relative to the appropriate GPSA control, although the change in eosinophils in the BAL of mature female animals was not quite significant ($p = 0.07$) at the $p = 0.05$ level. Numbers of neutrophils in the BAL (fig. 2) increased in all groups, although in mature males the change was not quite significant ($p = 0.08$). The most striking difference between immature and mature animals was the TMA-GPSA-induced effect on MPO activity in the lung tissue (fig. 2). MPO activity in immature animals increased after TMA-GPSA challenge, while in mature animals the change was not significant. Numbers of macrophages in the BAL (fig. 3) increased in all groups, though in immature males the change was not quite significant ($p = 0.10$). Numbers of RBC in the BAL (table 2) significantly increased after TMA-GPSA challenge in all groups relative to the appropriate GPSA control. Significant TMA-GPSA-induced increases in protein (table 2) were observed in immature females and mature males, but not in immature males ($p = 0.22$) or mature females ($p = 0.12$).

Are Differences in the Inflammatory Response due to Differences in Sensitization?

To determine if the level of sensitization might account for differences in cell infiltration, concentrations of TMA-specific IgG₁ and IgG₂ antibodies were measured in plasma collected just prior to challenge. Prechallenge plasma samples were not obtained from all animals. Significant differences in antibody concentrations between the groups were detected, with the mature animals generally having more antibody than the corresponding immature animals, and immature males having more antibody than immature females (fig. 4). These data suggest that the level of circulating antibody was not an important determinant of the magnitude of the response. For example, animals with the highest concentrations of antibody (mature males or females) did not have a corresponding large increase in neutrophils with TMA-GPSA challenge (fig. 2) when compared to the immature animals with significantly less antibody. Further, correlation analysis (data not shown) also indicated that the concentrations of TMA-specific IgG₁ or IgG₂ did not significantly correlate with any measure of the response.

Does the Relationship between Complement System Activation and the Inflammatory Response Vary with Gender and Maturity?

The concentration of C3a in the BAL (fig. 5) of GPSA control animals tended to increase with age. Mature females had significantly more C3a in the BAL than did immature females, whereas the concentrations of C3a in the BAL of immature and mature males were not significantly different. TMA-GPSA challenge tended to increase the concentration of C3a in the BAL relative to the appropriate GPSA control group. C3a significantly increased in

immature animals of both genders, and was not quite significant in mature males ($p = 0.06$) at the $p = 0.05$ level. The change in mature females was clearly not significant.

The relationship between the extent of complement activation as determined by C3a in the BAL and the numbers of inflammatory cells in the lung is shown in table 3. Increasing concentrations of C3a correlated with increasing numbers of eosinophils, neutrophils, macrophages and red blood cells in the BAL within each age/gender group except for mature females. Protein in the BAL significantly correlated with C3a concentration for all groups. Thus, mature females clearly differ from the other groups in that C3a does not correlate with cellular infiltration.

Discussion

Immature guinea pigs have been used as a model of occupational asthma to elucidate the mechanisms of TMA-induced allergy in the lung. However, little consideration has been given to the potential effects of animal age and gender even though it is known that the incidence of asthma within the general human population changes with age and gender at about the time of puberty. In this study we demonstrated that the numbers of inflammatory cells residing in the lungs of control animals varied with age and gender. Despite these age and gender differences, the data overall suggest that the influx of eosinophils and macrophages into the lung in response to TMA-GPSA challenge was similar in immature and mature guinea pigs regardless of gender. However, neutrophil infiltration (MPO activity) into the lung tissue clearly varied with age.

Lung eosinophilia is a hallmark of asthma. In this study, the TMA-GPSA-induced eosinophilia in the guinea pig was not distinctly affected by either gender or sexual maturity at the time of sensitization. In contrast to our observations, Barrett et al. [26] investigating the effects of gender in a mouse model of ovalbumin-induced asthma reported that the increase in eosinophils in the BAL after challenge was 2 times greater in male animals than in females. Neutrophilia in human asthma is observed only in some circumstances [27]. Although the role for neutrophils in allergic asthma is not clear, they seem to be associated with severe cases of the disease or exacerbation of the asthmatic response [28–30]. Warner et al. [31] reported that the numbers of neutrophils in the BAL of infant wheezers and children with asthma were increased compared to normal children. In our study neutrophils increased after TMA-GPSA challenge in the airways regardless of age and gender. Neutrophils also increased in the lung tissue of immature guinea pigs after challenge, but not in mature animals. Differences between immature and mature animals with respect to TMA-GPSA-induced neutrophilia in the lung tissue may be attributed to the effects of aging. However, the lack of a measurable increase in numbers of neutrophils in the lung tissue of mature guinea pigs after TMA-GPSA challenge may have been due to a difference in time course of neutrophilia. Our measurements were made 24 h after challenge. Previous experiments using immature female guinea pigs showed that numbers of neutrophils after TMA-GPSA challenge were significantly elevated relative to GPSA controls at 24 h but not at either 6 or 48 h [21]. Time-course studies using mature guinea pigs would help to establish if neutrophilia occurs in older animals at a different time after TMA-GPSA challenge. Distinct differences due to age and gender with respect to numbers of macrophages, red blood cells and amount of protein in the BAL were not observed.

The numbers of resident inflammatory cells in the lungs of GPSA control animals changed with age, and the changes were distinctly different between females and males. As female guinea pigs aged, numbers of eosinophils increased and numbers of neutrophils remained about the same. In contrast, as male animals aged, numbers of eosinophils decreased and numbers of neutrophils increased. The changes in numbers of cells in the lung with age are not due to the size of the animals as the data were normalized to the dry weight of the lung prior to statistical analyses. Lung dry weight correlates well with guinea pig size ($r = 0.88$) and analysis

of the data normalized to weight of the whole animal at the time of challenge yielded statistically identical results. Changes with age and gender of the numbers of inflammatory cells or the ratio of cell types residing in the lung may affect the amount and proportions of mediators of the allergic response released after allergen stimulation. In guinea pigs of various ages, Arakawa et al. [2] observed differences in airway resistance and plasma exudation after TMA challenge. They suggested that the differences were due not only to physical size, but also to changes in the number and location of allergen-responding cells and altered responsiveness to mediators.

In the present study we measured both IgG₁ and IgG₂ antibodies because our previous studies using passive sensitization techniques demonstrated that both IgG subclasses play an important role in TMA-induced bronchoconstriction and cellular infiltration in the guinea pig [24]. IgE in guinea pigs is also believed to mediate allergic reactions in the lung, though it is typically at low concentrations or below the level of detection. IgE was not measured in this study because our previous studies in guinea pigs using the same sensitization regimen were unable to detect TMA-specific IgE (unpubl. data).

Correlation of antibody titer with an allergic response is suggestive of an antibody-mediated event. In humans, however, attempts to correlate antibody titers with TMA-induced airway syndromes have been inconclusive [32,33]. In the current study we demonstrated that the ability of guinea pigs to produce TMA-specific antibody varied with age and gender. Differences in the amount of antibody may have caused the observed differences in TMA-induced cellular infiltration. However, concentrations of TMA-specific IgG₁ and IgG₂ did not significantly correlate with the response. The data overall suggest that the amount of specific IgG antibody alone does not account for differences in the TMA-GPSA-induced response seen with age and gender. Even though specific antibody concentrations increased with age, the TMA-GPSA-induced change in inflammatory cells was not greater in mature animals. For example, in mature animals with relatively high concentrations of specific antibody, MPO activity did not increase after TMA-GPSA challenge, whereas in immature animals with lower levels of antibody significant changes in MPO activity were observed.

Studies by others have attempted to relate specific antibody to the immediate-onset allergic response in the lung, i.e. bronchoconstriction and increased microvascular permeability. Arakawa et al. [2] observed that TMA-induced plasma extravasation in the guinea pig correlated with TMA-specific IgG₁. Zhang et al. [34], studying airway responses to hexahydrophthalic anhydride (HHPA) in the guinea pig, demonstrated that changes in plasma extravasation and lung resistance following challenge were related to the dose of HHPA used for sensitization and levels of specific IgG₁ measured in the serum. In contrast, other studies in the guinea pig have found that serum concentrations of IgG₁ or IgE do not correlate with TMA-induced bronchoconstriction [2–4] or airway microvascular leakage [4].

Although several studies have examined the relationship between specific antibody and the immediate bronchoconstriction and microvascular permeability, few have related antibody titer to the delayed-onset response. In a rat model of TMA-induced lung injury, Zeiss et al. [35] demonstrated that the TMA-induced histopathological changes in the lung over a period of 10 days occurred in parallel with development of specific antibody, and antibody levels in the BAL and serum correlated with lung injury. In contrast, the present study in guinea pigs demonstrated no significant correlation between antibody levels and the delayed-onset cellular infiltration. This indicates that the relationship between either IgG₁ or IgG₂ and TMA-induced cellular infiltration is not simply linear. The interplay between more than one antibody class may be important in determining the magnitude of the response as demonstrated by our previous studies using passively sensitized guinea pigs. In these studies the magnitude of TMA-specific IgG₁-mediated eosinophil infiltration was significantly augmented in the presence of

TMA-specific IgG₂, whereas eosinophilia did not occur in animals sensitized with IgG₂ alone [24].

The complement system plays a role in the TMA-GPSA-induced allergic response in immature guinea pigs [1]. In guinea pigs depleted of complement by cobra venom factor, the TMA-GPSA-induced leakage of RBC and infiltration of eosinophils and mononuclear cells into the BAL are significantly reduced. We previously demonstrated that in immature female guinea pigs, the magnitude of the TMA-GPSA-induced influx of inflammatory cells correlates with the extent of complement activation [21]. In the present study we also observed significant correlations between complement activation and cellular infiltration in immature female as well as in immature and mature male animals. Notably different is the lack of a significant correlation of complement activation with the numbers of inflammatory cells in mature female guinea pigs. Correlations of C3a with cellular infiltration suggest a common link between the two. However, the relationship between complement activation and cellular infiltration in mature females differs from that in the other groups, suggesting mediators of the response may change with age and gender.

Eosinophils in the lung are a hallmark of asthma. TMA-induced eosinophilia demonstrated in this study was not greatly affected by age or gender even though resident populations of eosinophils measured in the control animals were different. Although the role that neutrophils play in asthma is as yet unknown, our data clearly suggest that age be considered with respect to the potential of neutrophils to affect the asthmatic response. Similarly, the relationship between mediators released by complement system activation and cellular infiltration changes with age and gender. These data suggest that the mediators involved in the inflammatory response of mature females differ from the other groups of animals. Although TMA-induced cellular infiltration in immature female and male guinea pigs was similar to that in mature animals, differences do exist. This suggests that age and gender should more often be considered in animal models of the allergic response, not only with respect to TMA but when studying the mechanisms and effects of other allergens as well.

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Fig. 1. Eosinophils in the BAL and EPO activity in the lung tissue 24 h after instillation with GPSA (control) or TMA-GPSA. EPO activity is used as an estimate of the numbers of eosinophils in the lung. Data are geometric means $\pm$ 1 SE. † p < 0.05 comparing GPSA control animals; * p < 0.05 comparing GPSA control to TMA-GPSA-challenged animals within an age/gender group.

Fig. 2.

Neutrophils in the BAL and MPO activity in the lung tissue 24 h after instillation with GPSA (control) or TMA-GPSA. MPO activity is used as an estimate of the number of neutrophils in the lung. Data are geometric means $\pm$ 1 SE. $\dagger$ $p < 0.05$ comparing GPSA control animals; * $p < 0.05$ comparing GPSA control to TMA-GPSA-challenged animals within an age/gender group.

Fig. 3. Macrophages in the BAL measured 24 h after instillation with GPSA (control) or TMA-GPSA. Data are geometric means $\pm$ 1 SE. * $p < 0.05$ comparing GPSA control to TMA-GPSA-challenged animals within an age/gender group.

Fig. 4. Concentration of TMA-specific IgG₁ and IgG₂ antibodies in plasma collected just prior to challenge. Data are geometric means $\pm$ 1 SE of between 9 and 23 animals. † $p < 0.05$ comparing age/gender groups.

Fig. 5.

C3a in the BAL 24 h after instillation with GPSA (control) or TMA-GPSA. YAC eq are the microliters of a standard pool of yeast-activated complement that would give a signal equivalent to that of the BAL sample in the Western blot. Data are geometric means $\pm$ 1 SE. † p < 0.05 comparing GPSA control animals; * p < 0.05 comparing GPSA control to TMA-GPSA-challenged animals within an age/gender group.

Table 1

Experimental groups of animals

Group	n	Sensitization		Challenge			
		weight, g	age ^a , days	dose ^b , µl	weight, g	age ^a , days	dose ^c , µl
Immature females	24	274 ± 5	26	100	451 ± 9	46–48	40
Immature males	23	300 ± 3	30	100	454 ± 6	50	40
Mature females	12	435 ± 3	48	150	565 ± 9	68	50
Mature males	12	587 ± 5	62	200	725 ± 11	82	65

Weight is given as mean ± SE.

^aDetermined by the number of days between the date of birth (estimated by Charles River Laboratories using the weight of the animal prior to shipping) and the date of treatment. Females become sexually mature at 35–42 days and males at 56–70 days.

^bVolume of 0.3% TMA used for intradermal sensitization.

^cVolume of GPSA or TMA-GPSA used for intratracheal instillation.

Table 2

RBC and total protein in the BAL 24 h after instillation with GPSA (control) or TMA-GPSA

Group	Challenge	RBC (OD ₄₁₂) ^a	Protein, mg
Immature females	GPSA ^b	0.19 (0.15–0.23)	2.53 (2.21–2.90)
	TMA-GPSA	0.63 (0.37–1.06) *	3.69 (2.94–4.63) *
Immature males	GPSA	0.11 (0.06–0.21)	2.49 (1.89–3.28)
	TMA-GPSA	0.35 (0.17–0.71) *	3.18 (2.59–3.90)
Mature females	GPSA	0.23 (0.14–0.38)	3.38 (2.23–5.11)
	TMA-GPSA	0.86 (0.32–2.26) *	4.70 (3.59–6.16)
Mature males	GPSA	0.34 (0.15–0.75)	3.05 (2.53–3.66)
	TMA-GPSA	0.91 (0.37–2.28) *	4.15 (3.04–5.67) *

Values represent geometric mean (95% confidence interval).

* p < 0.05 comparing GPSA control to TMA-GPSA-challenged animals within an age/gender group.

^a Absorbance of hemoglobin at 412 nm is an indicator of the number of RBC.^b The number of RBC and the amount of protein in the BAL did not differ between the GPSA control groups.

Table 3

Correlation of C3a concentration in the BAL with the numbers of inflammatory cells 24 h after intratracheal instillation of GPSA or TMA-GPSA

	Correlation coefficients			
	immature		mature	
	females	males	females	males
<i>Cell infiltration into airspace</i>				
Eosinophils	0.60 [*]	0.60 [*]	0.02	0.87 [*]
Neutrophils	0.76 [*]	0.71 [*]	0.56	0.65 [*]
Macrophages	0.66 [*]	0.77 [*]	0.12	0.64 [*]
<i>Cell infiltration into the lung</i>				
Lung EPO	0.24	0.27	0.45	0.60 [*]
Lung MPO	0.10	0.48 [*]	0.34	−0.11
RBC in BAL	0.62 [*]	0.90 [*]	0.19	0.58 [*]
Protein in BAL	0.77 [*]	0.90 [*]	0.80 [*]	0.86 [*]

Values are Pearson correlation coefficients (r). Data were log-transformed before analysis.

^{*}
p < 0.05.