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ORIGINAL ARTICLE Experimental Models of Allergic Disease

Changes in asthma-like responses after extended removal from exposure to trimellitic anhydride in the Brown Norway rat model

X. D. Zhang, A. F. Hubbs and P. D. Siegel

National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Morgantown, WV, USA

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Summary

Background Organic acid anhydride-induced occupational asthma is considered to be IgE-mediated. Airway and skin exposure are the two main routes of sensitization in the work place. Recently we developed an allergic asthmatic Brown Norway rat model sensitized by dermal exposure to trimellitic anhydride (TMA) using an occlusion patch application.

Objectives The objectives of this study were (1) to develop a model of non-occluded dermal exposure leading to allergic sensitization and (2) to examine the effect of extended removal from exposure on persistence of both specific IgE and TMA aerosol-induced airway responses in this model.

Methods TMA powder (4 or 40 mg) was applied, unoccluded, to the skin of rats for 4 h, once/week for 4 weeks. Rats were given a 10-min aerosol challenge to 40 mg/m^3 TMA 2 weeks after the last dermal exposure (day 35). Another group was challenged on day 35 and again 18–24 months later. Respiratory enhanced pause (Penh), pulmonary histopathology and inflammation and specific IgE titres were measured.

Results Rats produced dose-dependent specific IgE titres after exposure and developed early-phase (EAR) and late-phase airway responses (LAR) after airway challenge to TMA aerosol as well as airway eosinophilic inflammation. Specific airway responses were still manifested after a second TMA airway challenge given 18–24 months following the initial airway challenge. While persistent, airway inflammation, specific IgE and EAR were significantly attenuated following the second TMA challenge. LAR remained robust at 18–24 months and was not significantly different from the response on day 35.

Conclusions These results demonstrate the persistence of chemical sensitization and further suggest that IgE is not essential for LAR.

Keywords Brown Norway rat, IgE, persistent occupational asthma, skin/dermal exposure, trimellitic anhydride

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Correspondence:
Paul D. Siegel, National Institute for Occupational Safety and Health, 1095 Willowdale Road, Morgantown, WV 26505, USA.
E-mail: Psiegel@cdc.gov

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Introduction

Organic acid anhydrides (OAA), such as trimellitic anhydride (TMA), phthalic anhydride, tetrachloride phthalic anhydride and hexahydrophthalic anhydride, are low-molecular-weight chemical asthmagens. They can cause specific IgE-mediated allergy and asthma among workers following exposure [1–4]. TMA is a highly reactive chemical that is widely used to make epoxy and alkyd resins, plasticizers, high-temperature polymers and surfactants.

Several animal models of OAA sensitization and asthma have been established using TMA, phthalic anhydride or hexahydrophthalic anhydride following airway or skin exposure [5–8]. OAA sensitization through the dermal route was first demonstrated in animals using invasive techniques (i.e. intradermal injection with OAA suspended in plant oil or liquid paraffin) [6, 9, 10]. Non-invasive methods using topical application of a mixture of TMA, acetone and plant oil have been used to produce sensitization [11–14].

Recently, we developed a non-invasive sensitization method in Brown Norway rats by applying dry TMA powder to the dorsum with occlusion using surgical tape [15, 16]. The rats developed TMA-specific IgE titres as well as asthma-like early-phase (EAR) and late-phase airway

responses (LAR) after challenge with TMA aerosol. In the present study it was hypothesized that dry TMA powder applied to non-occluded skin would produce a long-lasting immune sensitization and TMA airway responsiveness. To test this hypothesis sensitizing exposures were modified by the application of dry TMA powder without occlusion. Allergic asthma-like responses after initial TMA aerosol challenge were documented, along with that from a second TMA airway challenge given 18–24 months later.

Material and Methods

Animals

Female, inbred, 2–3-month-old Brown Norway rats were purchased from Charles River Laboratories (Wilmington, MA, USA). Animals were kept in an Association for Assessment and Accreditation of Laboratory Animal Care accredited facility, fed Teklad 2918 rat chow and water, *ad libitum*, provided HEPA-filtered air and kept in a room with a 12-h light : dark cycle. Rats were acclimated in the facility for 1 week before use. Aged Brown Norway rats were obtained from the National Institute for Aging, Bethesda, MD for use as aged histological and non-sensitized, TMA-challenged controls. Animals were free of viral pathogens, parasites, mycoplasms, Helicobacter and CAR bacillus.

Chemicals

TMA was purchased from Acros Organics (Fair Lawn, NJ, USA). Pentobarbital was purchased from Jones Pharma (Saint Louis, MO, USA). Sheep anti-rat IgE (Epsilon chain, Cat. No. 64-352) and horse radish peroxidase (HRP) conjugated donkey anti-sheep IgG (Cat. No. 67541) were from ICN Biomedicals Inc. (Costa Mesa, CA, USA). Rat serum albumin (RSA, fraction V powder), horse serum, phosphate-buffered saline (PBS, pH 7.4), PBS with Tween-20 (PBS–Tween, pH 7.4), 3,3′,5,5′-tetramethylbenzidine (TMB), were purchased from Sigma Chemical Company (Saint Louis, MO, USA).

Dry trimellitic anhydride powder preparation

TMA flakes were ground in a water-cooled analytical mill (IKA Works Inc., Wilmington, NC, USA). The fine powder was collected and stored in the presence of desiccant. Greater than 90% of the particles had diameters $< 4\,\mu m$ as determined by a Coulter® Multisizer II (Coulter Corporation, Hialeah, FL, USA) using particles suspended in Isoton® (Coulter® balanced electrolyte solution, Coulter Corporation).

Direct skin exposure to dry trimellitic anhydride powder

The method for dermal sensitization to TMA was modified from that previously reported [15, 16]. Brown Norway rats were anaesthetized [pentobarbital, 40–50 mg/kg, intraperitoneally (i.p.)] and a patch of fur on the rat's dorsum was carefully clipped with scissors to reduce skin irritation and trauma. TMA powder was applied directly on to the dorsum of anaesthetized Brown Norway rats for 4 h and then the area was rinsed with water.

Dose–response study. The dose–response study was performed on the younger rats only (2–3 month old at the onset). The experimental groups for this study were:

Group 1a: Rats ($n = 6$) were given sham dermal exposure (0 mg of TMA) as controls on days 0, 7, 14 and 21. Sera were collected from the tail vein on days 0, 7, 14, 21, 28 and 35.

Group 1b: Dry TMA powder was applied to the clipped area ($0.5\,cm \times 0.5\,cm$) at the dose of 4 mg on days 0, 7, 14 and 21. Sera were collected on days 0, 7, 14, 21, 28 and 35 ($n = 8$).

Group 1c: Dry TMA powder was applied to the clipped area ($2\,cm \times 1.5\,cm$; area of dermal exposure to TMA increases with dose) at the dose of 40 mg on days 0, 7, 14 and 21. Sera were collected on days 0, 7, 14, 21, 28 and 35 ($n = 8$).

All dose–response study rats were challenged to 10 min, 40 mg/m^3 TMA aerosol using a nose-only exposure chamber on day 35 and enhanced pause (Penh) monitored as described subsequently.

Sensitization and airway responsiveness persistence study. Group 2a: Normal 20-month-old rats ($n = 8$) were used as controls for Group 2b and given a single 10 min, 40 mg/m^3 TMA aerosol inhalation challenge. These rats were naïve with respect to TMA before the single aerosol challenge. Sera were collected 24 h after challenge.

Group 2b: A separate group of rats were sensitized by dermal application of 40 mg TMA by the same method described in Group 1c of the dose–response study and sera were collected on day 35 following a 10 min, 40 mg/m^3 TMA aerosol challenge. A second 10 min, 40 mg/m^3 TMA aerosol challenge was given at 18–24 months. Sera were collected 24 h after challenge. Four rats (of 14) were lost before the second challenge due to age-related causes.

Preparation of trimellitic anhydride–rat serum albumin conjugate for specific IgE enzyme-linked immunosorbent assay

RSA was dissolved (3 mg/mL) in half saturated sodium borate buffer (pH 9.4), TMA was dissolved in acetone (10 mg/100–200 μL) and slowly added drop-wise to the RSA solution while stirring. The final TMA : RSA molar ratio was 60 : 1. The pH of the solution was maintained at 9.4 by titration with 2 M sodium hydroxide (NaOH) during the addition of TMA. The TMA–RSA solution was stirred for 30 min, and then dialyzed against distilled water.

TMA–RSA was lyophilized and stored, desiccated, at −20 °C until use.

Trimellitic anhydride-specific IgE

Rat serum-specific IgE against TMA was analysed by ELISA. Microtitration plate wells (ICN Biomedicals Inc., Horsham, PA, USA) were coated with TMA–RSA (100 μL, 0.15 mg/mL) in carbonate buffer (1.59 g Na_2CO_3, 2.93 g $NaHCO_3$ in 1 L water, pH 9.6) and incubated overnight at 4 °C. The contents from each well were removed and plates were washed twice with PBS–Tween-20. This wash was repeated following each subsequent addition/incubation procedure. Non-specific binding was blocked by incubation with 200 μL of 5% heat-inactivated horse serum in carbonate buffer for 2 h at room temperature. Plates were stored at −20 °C until use. Rat sera were diluted in PBS. Diluted sera (100 μL) were added to the wells and incubated for 1 h at 37 °C. Sheep anti-rat IgE (100 μL, dilution 1 : 5000) was added to the wells and incubated for 1 h at 37 °C. HRP-donkey anti-sheep IgG (100 μL, dilution 1 : 10 000) was added to the wells and incubated for 1 h at 37 °C. Plates were developed using TMB (100 μL/well) in the dark, at room temperature, for 30 min. Optical density (OD) was read at 630 nm with a photometer (ELX808 Microplate reader, Bio-Tek Instruments Inc., Winooski, VT, USA). Quantitative analyses expressed as 'relative unit' were performed for the sera with positive specific IgE: (i) Aliquots from all positive sera were pooled and used as a standard serum pool/reference sera. The standard pool was serially diluted to develop a standard curve to which each sample was referenced. A relative unit value of 100 for IgE was assigned to the reference sera. The standard pool was run on each plate used for antibody analysis. (ii) Individual serum samples were diluted at 1 : 50, 1 : 500 and 1 : 1000 for ELISA analyses to obtain an OD values within the log-linear range of the standard curve.

Trimellitic anhydride aerosol generation

TMA aerosol was generated using the Wright Dust Feed Mechanism (Messrs. L. Adams Ltd, London, UK) packed with ground TMA powder. The mean aerodynamic particle diameter was 2.77 ($\sigma_g = 0.94$) μm.

Airway challenge with trimellitic anhydride aerosol

Specific airway challenge was performed by inhalation of 40 mg/m^3 TMA aerosol for 10 min. Immediately following challenge the rats were moved to the whole-body plethysmography chambers (Buxco Electronics Inc., Wilmington, DE, USA) to record Penh every 30 s for 16–24 h. Nose-only exposure was used to avoid deposition of the TMA powder on the animal's fur and in the chamber. Animals in the whole-body plethysmography chambers were conscious, unrestrained with food and water provided *ad libitum*.

Criteria for evaluation of the Penh data have been previously published [16]. An increase in Penh after airway challenge with TMA aerosol was defined as 3.29 standard deviations (SD, 99.9% confidence interval upper limit) greater than the running average of sham-sensitized, TMA-aerosol-challenged rats. EAR develops during the first hour after airway challenge with TMA aerosol, while LAR develops after 1 h following airway challenge with TMA aerosol. LAR may last for several hours and the running average curve was used to determine the area under the curve (AUC) between the start point and end-point of the LAR.

Lung pathology

Rats were euthanized (100 mg/kg pentobarbital, i.p.) 24 h following the final TMA aerosol challenge. Each rat's trachea was cannulated and the lungs lavaged five times with 7 mL physiological saline. Cells from each lavage were recovered by centrifugation and resuspended in 1 mL saline for eosinophil, neutrophil, macrophage and lymphocyte enumeration using the Coulter® Multisizer II and microscopic differential counts. Lungs were then perfused and fixed with 10% phosphate-buffered formalin. The lungs were embedded in paraffin, sectioned (5 μm), and stained with haematoxylin and eosin for histopathological assessment. Semi-quantitative pathology scores were assigned by a Board Certified Veterinarian Pathologist according to the Best Practices Guidelines: Toxicological Histopathology [17].

Data analyses

Student's *t*-test was used for comparisons of two groups. Paired *t*-test was used to compare the Penh response from the same animals following the initial day 35 TMA challenge and subsequent 18–24 months TMA challenge. Mann–Whitney *U*-test was used for data quantified as percentage. A Wilcoxon Rank Sum test with exact *P*-values calculated using SAS Proc NPAR1WAY was conducted to assess significant changes in pathological assessment scores between groups. *P*-values < 0.05 were considered to be significant.

Results

Dose-dependent dermal sensitization and airway challenge responses

TMA-specific IgE was not detectable in rats from Group 1a.

Groups 1b and 1c (sensitized to 4 and 40 mg of TMA) had detectable TMA-specific IgE by day 21 following the first application of TMA. TMA-specific IgE levels of the rats exposed to the 40 mg TMA were significantly higher

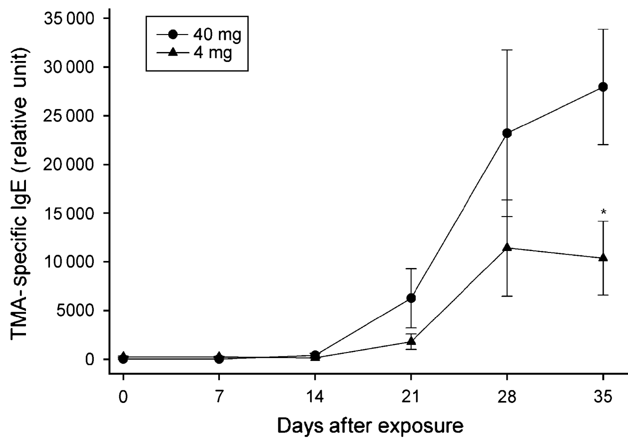

Fig. 1. Development of specific IgE. Dry trimellitic anhydride powder, 40 or 4 mg, was applied on the dorsum of two groups of Brown Norway rats ($n = 8$/group) on days 0, 7, 14 and 21. Dose-dependent specific IgE of rats exposed to TMA was observed by day 21, and peaked by day 35. *Significantly different between the two groups ($P < 0.05$).

Table 1. Serum TMA-specific IgE* and AUC of Penh from EAR and LAR after challenge with TMA

Group	TMA-specific IgE	AUC of EAR	AUC of LAR
0 mg	Not detectable	–	–
4 mg	10 362±3782	78±10	2046±336
40 mg	27 940±5918**	100±12	3451±552**

*Relative unit extrapolated from titration of pooled TMA-specific IgE containing rat sera.
**Significantly different between 4 and 40 mg TMA-sensitized rats (student's t-test, $P < 0.05$).
Rats sensitized by dermal exposure to TMA at the dose of 0 ($n = 6$), 4 and 40 mg ($n = 8$/group).
AUC, area under the curve; EAR, early-phase airway response; LAR, late-phase airway response; TMA, trimellitic anhydride.

by day 35 compared with the 4 mg group ($P < 0.05$, Fig. 1, Table 1).

Group 1a (controls): The mean Penh of the six sham-sensitized, TMA-aerosol-challenged rats was 0.5± 0.09 SD. The mean Penh plus 3.29 SD was 0.8 (Fig. 2a).

Group 1b and 1c (sensitized to 4 and 40 mg of TMA powder applied for 4 h/application without occlusion): The rats in both exposure groups developed EAR and LAR following specific airway challenge (Fig. 2b). The difference between the two groups in the Penh AUC of EARs was not statistically significant. The AUC values of the Penh from LARs in the group sensitized with 40 mg of TMA were significantly higher than that in the 4 mg TMA dermally sensitized group (Table 1).

Persistence studies of sensitization and airway challenge responses

TMA-specific IgE was not detected in the aged control rats. Group 2b TMA-sensitized, aerosol-challenged rats

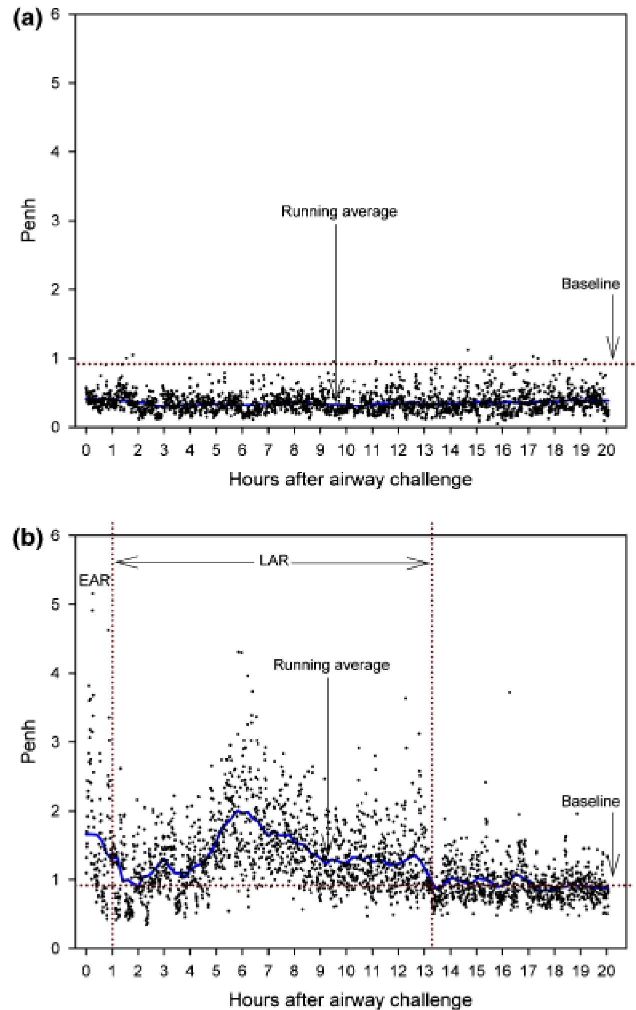

Fig. 2. Brown Norway rats received 4 h of dermal exposure to TMA on the clipped dorsum under anaesthesia on days 0, 7, 14 and 21 ($n = 8$/group). Airway challenge to 40 mg of TMA aerosol for 10 min was performed on day 35 and Penh was recorded every 30 s for 20 h (dot = individual Penh reading). Early-phase (EAR) or late-phase airway responses (LAR) from representative rats: (a) Non-sensitized rats given sham airway exposure (0 mg of TMA). No measurable EAR or LAR observed. (b) Rats sensitized with 4 mg TMA and challenged on day 35 to 40 mg/m^3 TMA. EAR and LAR are prominent. A Penh response is considered to be positive when it exceeds 3.29 standard deviations (SD, 99.9% confidence interval upper limit) above the running average Penh of non-sensitized, TMA-challenged rats. The 99.9% confidence interval upper limit is noted as base line in the figure.

(long-term follow-up study) TMA-specific IgE serum levels on day 35 were significantly higher than that measured 18–24 months after removal from TMA exposures (Group 2b, $P < 0.05$, Table 2).

Group 2a (aged controls): The mean Penh of the 8 aged, non-sensitized rats following TMA challenge was 0.5±0.064 SD. The mean Penh plus 3.29 SD was 0.7. There was no observable difference in base-line Penh between young and old rats.

Group 2b (long-term study): All rats developed both EAR and LAR following TMA airway challenge on day 35. Following the second TMA aerosol challenge given between 18 and 24 months after sensitization, all rats developed EAR and LAR again, but the duration of EAR was shorter compared with the day 35 challenge (21±2.6 vs. 7±2.9 min, $P<0.05$). Attenuation of the EAR after 18–24 months was also noted by assessing Penh AUC (Table 2, $P<0.05$). There was no significant difference in LAR duration (11.7±1 vs. 14.2±1.9 h, $P>0.05$) or Penh AUC between challenge to TMA on day 35 vs.18–24 months later. The 18–24-month post-sensitization rats were combined into one group as no age-related trend was found by regression analyses (EAR: $r=0.36$, $P=0.3$; LAR: $r=0.49$, $P=0.16$) among rats challenged to TMA between 18 and 24 months.

Cell differentiation of bronchoalveolar lavage fluid (BALF) from both sensitized and control TMA-challenged rats was performed. Concurrent non-sensitized control BALF assessment on young rats was not performed. BALF cell differential of the aged control rats is not significantly different than that observed in younger Brown Norway rats in our facility with macrophages making up >95% of the BAL cell population, although the older rat BALF macrophage numbers are much higher than previously observed [16]. Control Brown Norway rats BALF alveolar macrophage count ($n=9$) from a separate study performed in our laboratory during the same time period as this study was 28.55 (±1.9)×10^4. This is approximately 2.5-fold fewer alveolar macrophages than that recovered from the 20-month-old aged controls. In addition, an age-related effect on alveolar macrophage count was suggested from a regression analyses of the TMA sensitized, 18–24 months TMA-challenged aged rats ($r=0.74$, $P=0.058$), although the correlation did not reach a statistical significance of <0.05. The eosinophils in the sensitized and challenged rats were significantly higher than that in non-sensitized but TMA-aerosol-challenged rats. There was no observed age-related difference in BALF eosinophils in sensitized rats challenged from 18 to 24 months ($r=0.23$, $P=0.615$). The total number of macrophages in the sensitized and challenged rats were significantly lower than the controls. There were no significant differences in neutrophils and lymphocytes between the sensitized and non-sensitized groups (Table 3).

Histopathology findings in control rats were either absent or consisted of normal background findings in aged rats, such as mild hyperplasia of bronchus-associated lymphoid tissue or mild alveolar histiocytosis. However, one of the control rats had a mild, multifocal eosinophilic granulomatous pneumonia. This is consistent with the well-described background eosinophilic granulomatous response in control Brown Norway rats [18, 19]. The principal histopathologic response to TMA challenge in sensitized Brown Norway rats was granulomatous to eosinophilic granulomatous pneumonia but the severity of the response was dramatically increased relative to controls. Eosinophilic granulomatous inflammation was most prominent in younger rats that were sensitized and challenged 35 days later. In the younger rats the response was characterized by marked to severe, multifocal and coalescent eosinophilic granulomatous pneumonia with a fibrohistiocytic component and extension of inflammation to the pleura in some foci. Eosinophilic and lymphoplasmacytic perivascular cuffing was also observed and

Table 2. Serum TMA-specific IgE* and AUC of Penh from EAR and LAR following two challenges/rat to TMA aerosol, the first on day 35 and the second between months 18 and 24 after dermal exposure to 40 mg/m^3 of TMA

Challenge on	TMA-specific IgE	AUC of EAR	AUC of LAR
Day 35	35 502±6120	81±19	2985±513
Months 18–24	13 848±2880**	26±14**	3699±713
Month 20 (controls)	Not detectable	–	–

*Relative unit extrapolated from titration of pooled TMA-specific IgE containing rat sera.
**Significantly different (student's t-test, $P<0.05$) between sensitized rats' response to challenge to 40 mg/m^3 TMA on day 35 from that on months 18–24.
TMA-specific IgE was not detectable in sham-treated controls.
AUC, area under the curve; EAR, early-phase airway response; LAR, late-phase airway response; TMA, trimellitic anhydride.

Table 3. Differential of BALF cells and percentage (mean±SEM) following airway challenge with TMA

	Total cell numbers in BALF and percentage			
Challenge on	Eosinophils	Neutrophils	Macrophages	Lymphocytes
Day 35	(13.27±4.66)×10^4	(7.43±3.22)×10^4	(6.89±0.97)×10^4	(1.25±1.10)×10^4
($n=8$)	(42±3.9)%†	(23±4.0)%	(31±4.4)%	(4±0.8)%
Months 18–24	(6.62±2.25)×10^4	(20.28±9.85)×10^4	(40.72±3.86)×10^4†	(3.21±1.17)×10^4
($n=10$)	(8±1.9)%	(22±7.8)%	(66±10)% †	(4±1)%
20-month-old controls	(0.08±0.08)×10^4↓	(9.58±7.10)×10^4↓	(73.75±8.71)×10^4↑	(1.04±0.66)×10^4↓
($n=8$)	(0.1±0.1)%↓	(7±3.7)%↓	(91.9±3.9)%↑	(1±0.4)%↓

†Significantly different ($P<0.05$) between the groups challenged on day 35 and months 18–24.
↓ or ↑ stands for significantly lower or higher ($P<0.05$) than the other groups.
BALF, bronchoalveolar lavage fluid; TMA, trimellitic anhydride.

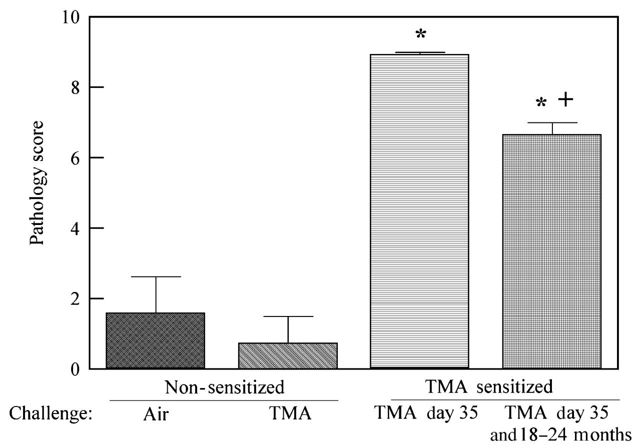

Fig. 3. Pathology assessment of Brown Norway rat lungs following sensitization and TMA challenge. Challenge on day 35 produced significant allergic asthma-like pathological changes in the lungs of dermally sensitized rats ($^{*}P = 0.00067$ vs. TMA-challenged non-sensitized rats). Removal from TMA exposure and rechallenge at 18–24 months produced a less sever pathology score than at the day 35 challenge ($^{+}P = 0.00033$).

may be a component of the eosinophilic granulomatous pneumonia. The response of rats sensitized when young and challenged as aged rats was distinctly muted in comparison with the rats that were challenged when younger. The pulmonary inflammation varied from granulomatous to eosinophilic granulomatous. Rats that were not sensitized had a response to TMA exposure that was similar to the control group. The differences between semi-quantitative pathology scoring of the severity of the pulmonary response to TMA challenge on day 35 vs. extended removal from TMA exposure and rechallenge at 18–24 months was statistically significant ($P = 0.00033$, Fig. 3).

Discussion

Occupational exposures leading to TMA sensitization and asthma occurs primarily through the airway (mucosa) and/or skin. A unique feature in the present Brown Norway rat model is that sensitization developed following a non-invasive skin short duration exposure to dry TMA powder, without occlusion or solubilization in a solvent. This method of dermal exposure better represents skin exposure that may occur in the workplace when compared with the previous exposure methods. Workers can potentially be exposed to TMA powder or dust during manufacturing, loading, packing and unpacking procedures or to TMA fume that is released when resins containing TMA are applied to hot metal.

While there is currently a debate if Penh is an index of airway resistance, our studies and that of others [20, 21] find that allergen challenge induced increased Penh and alteration of the waveform showing a more prominent early expiration peak are good indices to define EAR and LAR.

Both 4 and 40 mg TMA dermally sensitized rats developed EARs and LARs after airway challenge with TMA aerosol 2 weeks after the final dermal TMA powder exposure (day 35). Several authors have investigated the mechanisms underlying EAR and LAR development in murine models and found that specific IgE-mediated mast-cell activation was responsible for EAR. While IgE allergen interaction may enhance the magnitude of LAR, it is not essential for LAR. Th2-type cytokines produced by activated T cells were essential for LAR as characterized by airway hyperresponsiveness and influx of inflammatory cells [22–25]. In the present long-term follow-up study, rats were challenged with TMA aerosol on day 35 and again 18–24 months later. TMA aerosol-induced EAR significantly declined over the long rest period, while LAR responses remained robust. The decline in TMA-specific IgE and pulmonary inflammation paralleled that of EAR, but not LAR. The results confirm the role of IgE in the EAR and the strong potential of IgE independent mechanisms of chemical allergen-induced LAR.

The long-term follow-up observation of asthma-like responses following airway challenge has not been previously reported in a model of low-molecular-weight chemical sensitization. Rodent models are limited for this type of study, due to their short life span. Initially 14 rats were sensitized, but four age-related deaths occurred before the second challenge could be administered. It is difficult to compare the persistence of allergic sensitization in a man's life span to that in our rat model. The results of the present study are consistent with that of clinical toluene diisocyanate (TDI) rechallenge studies of TDI asthmatics following extended periods of removal from exposure [26–28].

Grammer et al. [29], reported that TMA-sensitized workers would continuously have symptoms for years until they were moved to the jobs with no TMA exposure/challenge. Lemière [30] also summarized studies on occupational asthma from 1985 to 2001 and concluded that patients who have been ill with asthma are at risk of asthma attacks when exposed to the allergen again even many years after they have left the workplace and no longer have hyper-reactive airways. Non-occupational allergic asthma in the general population is most commonly manifested in the second decade of life. Some patients enjoy a transient or even a permanent remission, while others have persistent symptoms [31]. The findings of persistence of TMA-induced pulmonary responses long after sensitization and initial asthma-like airway manifestations is consistent with reported clinical observations of the persistence of occupational asthma.

It must be noted that the present design cannot separate out whether the muted IgE and EAR responses were the result of the age of the rats when challenged or the result

of the increased time duration between the sensitization and challenge. It is doubtful, however, that the effects observed can be attributed to age. Busse et al. [32] examined the effect of age in an ovalbumin-sensitized mouse model. Eighteen-month-old mice mounted specific IgE responses and eosinophilic inflammatory responses greater than that observed in 6-month-old mice. Non-specific airway reactivity was decreased in the older mice, but specific EAR or LAR was not measured. Asthma prevalence in the elderly (>65 years of age) was reported to be 6.8%, with 5.9% reporting current asthma [33]. Features of asthma in the elderly have been recently reviewed by Stupka and deShazo including those that contribute to its underdiagnosis and undertreatment [34]. Also discussed in this review is the evidence of increased severity at the time of asthma diagnosis and higher asthma mortality among seniors.

An additional limitation of this study is that histological sampling pulmonary sites were not identical for all groups; however, the differences observed cannot be explained by the sample site variation. Importantly, these findings were supported by BAL findings, which did not have these limitations. It must be noted that there is a well-recognized variable idopathic eosinophilic granulomatous pneumonia in Brown Norway rats that cannot be controlled in animal facilities. This background condition was minimal in our rats and the dramatic decrease in the TMA-specific challenge pulmonary inflammatory response over time, between day 35 and 18–24 months, is fully attributable to loss of TMA reactivity over time. This would suggest that if TMA exposure is avoided for an extended period, there may be a decreased risk of severe responses to TMA exposure, provided response in workers is similar to that in the rat model.

In summary, dermal contact to dry TMA powder may induce sensitization and specific airway responses to TMA aerosol. TMA-induced allergic asthma-like responses that persisted through the life span of the Brown Norway rat, although pulmonary inflammation, TMA-specific IgE and EAR, but not LAR, decreased with time away from exposure.

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