Published in final edited form as:

Toxicol Appl Pharmacol. 2001 September 15; 175(3): 234–242. doi:10.1006/taap.2001.9251.

Trimellitic Anhydride-Induced Eosinophilia in a Mouse Model of Occupational Asthma

Jean P. Regal^{*}, Margaret E. Mohrman^{*}, and Denise M. Sailstad[†]

^{*} Department of Pharmacology, Toxicology Graduate Program, University of Minnesota, Duluth, Minnesota 55812

[†] Office of Research and Development, National Health and Environmental Effects Research Lab, U.S. EPA, Research Triangle Park, North Carolina 27711

Abstract

Trimellitic anhydride (TMA) is a low-molecular-weight chemical known to cause occupational asthma. The present study was designed to determine if TMA elicited eosinophil infiltration into lungs of sensitized mice similar to previous studies with the protein allergen ovalbumin (OA). BALB/c mice were sensitized intra-dermally with 0.1 ml of 3% TMA or 0.3% OA in corn oil followed by intratracheal instillation with TMA conjugated to mouse serum albumin (TMA-MSA; 30 or 400 μ g) or OA (30 μ g). Nonsensitized mice received corn oil vehicle intra-dermally and MSA (30 μ g) intratracheally. The allergic response was elicited 3 weeks later by intratracheal instillation of 30 or 400 μ g TMA-MSA, OA, or control MSA. Cellular infiltration into bronchoalveolar lavage fluid (BAL) was determined 12 h later. Eosinophil peroxidase (EPO) and myeloperoxidase (MPO) activity in lung homogenates was used as an estimate of numbers of eosinophils and neutrophils, respectively, in lung tissue. In TMA-sensitized mice, TMA-MSA challenge significantly increased numbers of eosinophils in BAL and EPO in lung, indicating an increase in number of eosinophils in the airway and tissue. In nonsensitized mice, TMA-MSA challenge also caused a small but significant increase in eosinophils in BAL compared to MSA control. Total IgE in both plasma and BAL was significantly higher in TMA-sensitized compared to nonsensitized mice. The eosinophil infiltration in TMA-sensitized mice was similar in magnitude to the response in OA-sensitized mice. These studies are the first to demonstrate TMA-induced eosinophilia in mouse lung and to provide a model for comparing mechanisms and mediators responsible for the substantial eosinophilia induced by TMA and OA.

Keywords

trimellitic anhydride; mouse; occupational asthma; eosinophils; lung; pulmonary allergy

Asthma is a chronic inflammatory lung disease characterized by reversible airway obstruction, airway eosinophilia, and increased airway responsiveness to a variety of stimuli (Busse and Parry, 1998; Mapp *et al.*, 1999; Banks and Wang, 2000). Exposure to both high- and low-molecular-weight substances can result in the development of asthma. Estimates indicate that anywhere from 2 to 15% of the workforce is affected by occupational asthma (Sarlo and Karol, 1999). Trimellitic anhydride (TMA),¹ a low-molecular-weight chemical that is used in the paint and plastics industry, is a known cause of occupational asthma (Zeiss *et al.*, 1977).

¹Abbreviations used: BAL, bronchoalveolar lavage fluid; EPO, eosinophil peroxidase; MPO, myeloperoxidase; MSA, mouse serum albumin; OA, ovalbumin; PBS, phosphate-buffered saline; RBC, red blood cells; TMA, trimellitic anhydride; TMA-MSA, trimellitic anhydride conjugated to mouse serum albumin.

Approximately 20,000 or more workers annually are exposed to acid anhydrides such as TMA, either by inhalation or dermally (Zeiss *et al.*, 1990; NIOSH, 1978). In addition, exposure to numerous other low-molecular-weight compounds results in respiratory allergy (van Kampen *et al.*, 2000).

Various animal model systems have been used to examine mechanisms of occupational asthma (Sarlo and Karol, 1999; Hayes and Newman Taylor, 1995). Studies in the guinea pig by ourselves and by Hayes *et al.* (1992a,b) have demonstrated differences in the mechanism of the allergic response elicited by TMA in comparison to the large-molecular-weight protein ovalbumin (OA). The bronchoconstrictor response to TMA relies heavily on cyclooxygenase products of arachidonate metabolism (Arakawa *et al.*, 1993), whereas OA-induced bronchoconstriction is mediated by lipoxygenase products with cyclooxygenase inhibitors enhancing the OA-induced bronchoconstriction (Regal, 1989; Andersson, 1982). During the allergic response to TMA in the rat, deposition of the third component of complement occurs in the lung (Leach *et al.*, 1987). Our previous studies in the guinea pig have demonstrated that complement depletion inhibits cellular infiltration after TMA challenge (Fraser *et al.*, 1995; Regal, 1997a,b) but not after OA challenge (Regal and Fraser, 1996), suggesting that the complement system plays a role in cellular infiltration into the lung with one antigen but not the other. Recently, using C3a receptor-deficient mice, Humbles *et al.* (2000) found that the OA-induced eosinophil infiltration in the lung was not affected by the absence of the C3a receptor. However, the C3a receptor was essential in the elicitation phase of OA-induced airway hyperreactivity.

The guinea pig has long been used as a model for pulmonary hypersensitivity. A major advantage of the guinea pig is that, similarly to humans, the lung is the target organ for the allergic response to antigens (Sarlo and Karol, 1999). The guinea pig airway reacts to histamine with bronchoconstriction, whereas the mouse airway responds to serotonin. In the mouse models of respiratory hypersensitivity to OA, repetitive challenge with OA has been necessary to elicit eosinophil infiltration in the mouse lung (Brewer *et al.*, 1999; Herz *et al.*, 1996; Zhang *et al.*, 1997), whereas eosinophil infiltration is more readily elicited in the guinea pig lung. However, the guinea pig does not readily produce IgE antibody after antigen exposure, whereas various strains of mice, similarly to humans, readily produce IgE (Zhang *et al.*, 1997). Additionally, only a few inbred strains of guinea pigs are available, in contrast to the mouse for which numerous inbred strains are available as well as gene knockout technology and extensive information on the mouse genome. Thus, the potential for examining mechanisms in the murine system holds great promise.

The purpose of our present study was to determine if TMA was able to elicit an allergic pulmonary response in the mouse as measured by the infiltration of inflammatory cells, particularly eosinophils, into the lung. Numerous publications have examined the mechanism of the OA-induced allergic pulmonary response, i.e., allergen-induced asthma, in the mouse. However, no studies to date have established such a model of TMA-induced occupational asthma in the mouse. In the OA model, studies have examined the influence of allergen administration and genetic background using many different mouse strains (Zhang *et al.*, 1997). In view of the differences noted, it was clearly important to directly compare OA-induced cellular infiltration to TMA-induced cellular infiltration using similar sensitization and challenge modes in the same strain of mouse. Our present study demonstrates that TMA can induce significant eosinophilia in the Balb/c mouse. These studies provide the basis for examination of mechanisms and/or mediators responsible for the substantial TMA-induced eosinophilia. In addition, this model provides a baseline response to compare alternate methods of sensitization and elicitation that may be useful as screening methods for low-molecular-weight respiratory allergens.

METHODS

Sensitization and elicitation: experimental groups

Female BALB/c mice (BALB/cAnNHsd) were purchased from Harlan (Portage, MI) and were 8 to 9 weeks of age (18.3 ± 0.2 g) at time of sensitization. Mice were fed Purina Rodent Chow and water *ad libitum* and maintained on a 12-h light–dark cycle. 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 U.S. National Institutes of Health. Intratracheal instillation was performed by aspiration as described by Ward *et al.*, (1998) using mice anesthetized with 1 mg ketamine and 0.2 mg xylazine im. TMA was conjugated to MSA as described previously in our studies using guinea pig serum albumin (Fraser *et al.*, 1995). Mouse serum albumin was obtained from Sigma Chemical, Co. (St. Louis, MO). The degree of substitution was 18–21 mol TMA per mol MSA.

The experimental groups of animals are outlined in Table 1. Animals were sensitized on days 1 and 3 intradermally with 0.1 ml of OA (0.3%) suspended in corn oil, TMA (3%) suspended in corn oil, or the corn oil vehicle as a control. Animals sensitized with the corn oil vehicle are referred to as non-sensitized. On day 12 animals were additionally sensitized intratracheally with 0.04 ml OA. TMA conjugated to mouse serum albumin (TMA–MSA), or MSA dissolved in water. As seen in Table 1, animals are grouped into either TMA treatment or OA treatment. Within each of these treatments, a nonsensitized group was challenged intratracheally with 30 μ g MSA. The MSA used in the TMA treatment groups was carried through the procedure for conjugating TMA to MSA, without adding any TMA to the reaction mixture. The MSA used in the OA treatment groups was dissolved in water without any other manipulation. Thus, the two nonsensitized MSA-challenged groups are analyzed as two separate groups using the appropriate group of sensitized animals.

For elicitation of the allergic response, mice were challenged intratracheally beginning on day 19 with 0.04 ml of aqueous solutions of OA, TMA–MSA, or MSA under ketamine/xylazine anesthesia. At the designated time after the last intratracheal instillation, the mice were anesthetized with pentobarbital, EDTA plasma was collected by cardiac puncture, the trachea was cannulated, and the lungs were lavaged with two 0.9-ml aliquots of phosphate-buffered saline (PBS) to obtain bronchoalveolar lavage fluid (BAL). BAL volume recovered ranged from 1.15 to 1.59 ml. Finally, the lungs were removed for homogenization and analysis of eosinophil peroxidase (EPO) and myeloperoxidase (MPO), as an estimate of the number of eosinophils and neutrophils, respectively, in the lung.

Evaluation of cell infiltration

BAL was centrifuged, the BAL supernatant was removed for analysis, and the cell pellet was resuspended in PBS to 0.125 ml. Total white blood cells in the pellet were counted by standard methods in a hemacytometer. Cytospin preparations of BAL cells ($\sim 2 \times 10^4$ cells) were made using a Shandon Cytospin 3 centrifuge (Shandon Lipshaw Inc., Pittsburgh, PA). Cells were stained with a modified Wrights' stain (Diff Quik, American Scientific Products, McGraw Park, IL) and at least 400 cells were counted and categorized as neutrophils, eosinophils, or macrophages as determined by their morphology. Lung lobes were processed as previously described for guinea pigs (Fraser *et al.*, 1995) for the measurement of EPO and MPO activity as estimates of the number of eosinophils and neutrophils, respectively. These methods have been used successfully in the mouse by other investigators as an indicator of cell infiltration into the lung and other tissues (Dimayuga *et al.*, 1991; Hamelmann *et al.*, 1997; Hessel *et al.*, 1997; Strath *et al.*, 1985). Measurement of EPO and MPO activity provides a quantitative and

efficient measure of cellular infiltration into the tissue while sampling the whole lung rather than representative histological sections.

Total protein and red blood cells (RBC) in the BAL

Total protein in the BAL supernatant was measured using the method of Lowry *et al.*, (1951). Bovine serum albumin was used as the standard. RBC in the BAL were used as an indicator of lung hemorrhage. The BAL cell pellet was freeze-thawed to lyse the RBC and centrifuged to remove the cell debris. The absorbance at 412 nm (hemoglobin) of this supernatant was assessed as an estimate of the number of RBC in the original BAL cell pellet (Fraser *et al.*, 1995).

Measurement of total IgE

The ELISA for total IgE was a modification of the previously described method by Ward *et al.*, (1998). Briefly, 96-well plates (Costar Corp., Cambridge, MA) were coated with 100 μ l mAb rat anti-mouse IgE (Southern Biotechnology, Birmingham, AL) at 2.5–5 μ g/ml in a carbonate-bicarbonate pH 9.4 buffer (BupH buffer pack, Pierce, Rockford, IL) overnight. All incubations were at room temperature in a volume of 100 μ l/well with the exception of the blocking step, which used 200 μ l. Plates were washed between each incubation step using a Tris-buffered saline solution (TBS, BupH buffer pack, Pierce) containing 0.5% Tween 20 (Sigma Chemical Co., Grand Island, NY). Plates were sequentially incubated for 1 h each with blocking buffer (TBS plus 1% BSA), diluted samples or standards, 2.5 μ g/ml biotinylated sheep anti-mouse IgE (The Binding Site, Birmingham, England), and 0.25 μ g/ml of streptavidin alkaline phosphatase (Jackson Labs, West-grove, PA). Finally, *p*-nitrophenyl phosphate disodium (Sigma Chemical Co.) was added to the plate and optical density at 405 nm was read on a Thermomax plate reader (Molecular Devices Corp., Menlo Park, CA) after incubation. The IgE standards, mouse plasma diluted 1:20 and 1:40, and BAL supernatant diluted 1:2 were prepared in TBS buffer containing 0.5% Tween 20 and 3% BSA. The standard curve was used to calculate the concentration of IgE in the unknown samples. For mouse plasma, values are reported as the μ g/ml total IgE in the plasma sample. For BAL supernatant, the total IgE (ng) in the BAL was calculated using the concentration of IgE in the BAL supernatant and the total volume of BAL recovered from the mouse.

Statistical methods

All data were log transformed to equalize variances. Figures show the geometric mean $\pm$ 1 SE, with significant comparisons indicated by an asterisk. Statistical significance was defined as $p < 0.05$. Statistical analyses were done using JMP and SAS software (SAS Institute Inc., Cary, NC).

Three different analyses were conducted on data from the BAL and lung in Figs. 1–4, 6, and 7. First, MSA control and TMA–MSA challenge within each sensitization group were compared by ANOVA with one-tailed single degree of freedom contrasts (short brackets in Figs. 1–4, 6, and 7). Second, to determine if the MSA control values for the variables changed between the nonsensitized and sensitized groups, a one-way ANOVA was used (long brackets in Figs. 1–4, 6, and 7). Third, ANOVA with one-tailed single degree of freedom contrasts was used to test for effects of different sensitization and challenge protocols on the magnitude of the MSA/TMA–MSA effect or MSA/OA effects (Tables 2 and 3). In Tables 2 and 3, a p value of less than 0.05 indicates that the magnitude of the MSA vs TMA–MSA effect varies between the two sensitization groups being compared.

For plasma IgE results shown in Fig. 5, ANOVA with one-tailed single degree of freedom contrasts using log transformed values was used. In measuring total IgE in the BAL supernatant, many of the values were below the limit of detection of the assay. Since these data

were known to be in a range below the limit of detection of the assay, they are termed left censored data. Thus, data for IgE in the BAL supernatant were analyzed employing censored data techniques that accounted for these values below the detection limit of the assay. Estimated population means and standard errors were computed treating the below detectable values as left censored. Computations were done with SAS procedure LIFEREG using a log normal distribution. Sample means and standard errors were left undefined when all values in a group were below detection. For comparisons involving a group with all below detectable values, the nonparametric Wilcoxon rank sum test was used to test for significance. For comparing groups with some above detectable data, the Wilcoxon results were similar to the LIFEREG parametric results. For consistency, the non-parametric Wilcoxon results are reported for all group comparisons of IgE in BAL supernatant.

RESULTS

TMA-Induced Cellular Infiltration into the Lung

The protocol used for sensitization and elicitation of the cellular infiltration into the mouse lung in the different TMA treatment groups is shown in Table 1. Lungs were lavaged on day 26, 72 h after the last intratracheal challenge, and cell infiltration was assessed. As shown in Fig. 1, TMA-MSA challenge significantly increased the numbers of eosinophils in the BAL compared to the MSA challenge in both nonsensitized animals and TMA-sensitized animals challenged with either 30 or 400 μ g TMA-MSA. In contrast, the numbers of neutrophils and macrophages did not significantly increase after TMA-MSA challenge in any of the groups. As shown by the long brackets in Fig. 1, the baseline number of eosinophils and neutrophils in the airspace was not significantly different in MSA-challenged animals, whether animals were sensitized or not. However, the number of macrophages did differ significantly in MSA-challenged animals between sensitization groups, suggesting an effect of sensitization itself on the cells residing in the lung.

The numbers of eosinophils and neutrophils infiltrating the lung were assessed by determining, respectively, the EPO and MPO in lung homogenates. As shown in Fig. 2, TMA-MSA challenge significantly increased the lung EPO in sensitized animals (short brackets). In nonsensitized animals, TMA-MSA challenge tended to increase the lung EPO, but the increase was not statistically significant ($p = 0.18$). Lung MPO was not significantly affected by TMA-MSA challenge compared to MSA challenge. To determine if sensitization altered the cell types residing in the lung, animals challenged with MSA were compared between groups. As indicated by the long brackets in Fig. 2, lung EPO but not lung MPO in MSA-challenged animals differed significantly between groups.

The mean amount of protein in the BAL supernatant of the groups ranged from 286 to 342 μ g total protein. TMA-MSA challenge did not significantly increase the amount of total protein compared to MSA challenge in any of the groups (data not shown). Similarly, the number of red blood cells in the BAL also did not differ between any of the groups (data not shown), suggesting that appreciable lung injury was not occurring with sensitization or with TMA-MSA challenge.

The magnitude of the MSA/TMA-MSA effect was compared between the different sensitization groups. As shown in Table 2, the difference in eosinophils in the BAL and the lung EPO between MSA control and TMA-MSA-challenged animals varied significantly comparing nonsensitized 30- μ g animals with TMA-sensitized 30- μ g animals ($p < 0.05$). When the analysis compared the TMA-sensitized 30- μ g and TMA-sensitized 400- μ g groups, the lung EPO was significantly different. These data suggest that the extent of eosinophilia in the nonsensitized animals differed from the TMA-sensitized animals. In addition, the extent of

eosinophilia also differed in the TMA-sensitized animals depending on the dose of TMA-MSA used for challenge.

In an additional set of experiments, animals were sensitized and challenged as described for the 400- μ g TMA group, but were lavaged at 48 h rather than 72 h after the last intratracheal challenge. A comparison of the results at 48 vs 72 h is shown in Figs. 3 and 4. At 48 h after lavage, TMA-MSA challenge did not significantly affect the number of neutrophils or macrophages in the BAL nor the lung MPO activity compared to MSA challenge. However, TMA-MSA caused a significant increase in the number of eosinophils in the BAL as well as in the lung EPO 48 h after challenge, similarly to 72 h after challenge. The neutrophil infiltration did not differ between MSA and TMA-MSA challenge at either 48 or 72 h. Thus, TMA-MSA-induced eosinophil infiltration was apparent by 48 h compared to the MSA infiltration and was maintained at 72 h. In MSA-challenged animals, the number of neutrophils and eosinophils in the 48-h lavage was greater than in the 72-h lavage, suggesting nonspecific cellular infiltration due to instillation of protein.

Effect of TMA Sensitization and Challenge on Total IgE in the Plasma and BAL

In the TMA treatment group, total IgE was determined in the BAL supernatant obtained 72 h after the last intratracheal challenge, as well as the concentration of IgE in the plasma of these same animals at the time of lavage. An additional group of four naive animals was included in this portion of the study to ensure that the oil sensitization itself was not affecting the amount of IgE in the BAL supernatant or plasma. Naive animals received no injections or intratracheal instillations and were subjected only to the lavage procedure. As shown in Fig. 5, TMA-MSA challenge significantly increased plasma IgE in the TMA-sensitized animals challenged with 400 μ g TMA-MSA compared to MSA challenge. In addition, sensitization itself, as expected, resulted in an increase in plasma IgE as evidenced by the significant difference found when comparing MSA challenged animals in the nonsensitized vs TMA-sensitized 30- μ g or TMA-sensitized 400- μ g group. Comparing the naive animals to the nonsensitized MSA challenged animals, no significant difference was detected.

Results evaluating IgE in the BAL supernatant were complicated by levels of IgE below detection in all naive animals and all nonsensitized animals. In addition, values of IgE in the BAL supernatant were below detection in the following TMA-sensitized groups: two of eight animals, 30 μ g MSA challenged; one of eight animals, 30 μ g TMA challenged; two of four animals, 400 μ g MSA challenged. However, evaluation of this censored data by the appropriate statistical techniques demonstrated that TMA-MSA challenge significantly increased the IgE in the BAL supernatant in both the 30- and 400- μ g groups of TMA-sensitized animals compared to the respective MSA control. However, in nonsensitized mice, TMA-MSA challenge did not significantly increase IgE in the BAL compared to the MSA control. Within the MSA-challenged animals, TMA sensitization caused a significant increase in IgE in the 30- μ g TMA-sensitized group. However, when the 400- μ g TMA-sensitized, MSA-challenged animals were compared to MSA-challenged nonsensitized animals, the IgE in the BAL supernatant was not significantly different. This is likely due to the high variability and the fact that two of four animals in the MSA-challenged, 400- μ g TMA-sensitized group were below the limits of detection of the assay. No significant difference was detected between naive animals and the nonsensitized, MSA-challenged group.

OA-Induced Cellular Infiltration into the Lung

Previous studies by others using various sensitization and challenge protocols have indicated that OA sensitization and challenge can elicit cellular infiltration, in particular, eosinophilia in the mouse lung. However, none of these protocols in mice have utilized sensitization of antigen in corn oil. Thus, experiments were also conducted using OA suspended in oil as an antigen

with the same sensitization protocol as used for TMA, lavaging animals 72 h after the last intratracheal challenge. OA treatment groups are shown in Table 1. In the OA treatment groups, MSA and OA used for challenge were dissolved in water.

As seen in Fig. 6, OA challenge caused a significant increase in the number of eosinophils in the BAL in OA-sensitized but not nonsensitized animals (short brackets). Unexpectedly, the number of neutrophils significantly increased after OA challenge in nonsensitized but not in sensitized animals. OA challenge caused a slight but significant increase in the number of macrophages in the OA-sensitized animals but not nonsensitized animals. As seen in Fig. 7 (short brackets), the lung EPO and lung MPO significantly increased after OA challenge in OA-sensitized animals but not in nonsensitized animals. Also, as seen in Figs. 6 and 7, no significant difference was detected when comparing the baseline number of BAL cells or the lung EPO and lung MPO in MSA-challenged animals (long brackets).

ANOVA with single degree of freedom contrasts was used to determine if this MSA/OA effect differed comparing non-sensitized vs OA-sensitized animals. Clearly, as seen in Table 3, the MSA/OA effect on eosinophils in the BAL and EPO in the lung differed between nonsensitized and sensitized animals, as did the MPO in lung.

DISCUSSION

The purpose of the present study was to determine if eosinophilia, a hallmark of asthma, occurred in mice in response to exposure to the occupational allergen TMA. Numerous studies have been done in mice examining the mechanisms and mediators of cellular infiltration into the lungs using OA as the antigen. However, the ability of the low-molecular-weight occupational allergen TMA to induce eosinophil infiltration in the mouse lung had not yet been demonstrated. In the present study, we utilized intradermal TMA sensitization and intratracheal challenge with TMA conjugated to the carrier MSA to determine if eosinophilia could be induced. Clearly, an increased number of eosinophils in both the BAL and lung tissue were seen in TMA-sensitized and -challenged animals. The time course of this response was consistent with that seen in OA models of asthma in the mouse. Forty-eight hours after TMA challenge, eosinophils as well as neutrophils were evident in the lung. Seventy-two hours after TMA challenge, the neutrophil numbers had declined but the eosinophilia was maintained. Thus, the mouse clearly responds to TMA sensitization and challenge with eosinophil infiltration into the lung.

In addition to an increase in eosinophils in TMA-sensitized and -challenged animals, we also documented a statistically significant increase in eosinophils in the BAL of nonsensitized animals challenged with TMA-MSA. In our previous studies in the guinea pig (Fraser *et al.*, 1995; Larsen *et al.*, 2001), challenge of nonsensitized guinea pigs with TMA conjugated to guinea pig serum albumin caused complement activation in the absence of specific antibody. Both sensitization and the complement system were shown to be required for TMA-induced eosinophilia. Our current studies in the mouse indicate that TMA causes sensitization-independent effects on eosinophilia.

Since TMA decomposes in water, it is given as a suspension in oil for sensitization. To parallel the method of TMA sensitization, OA was also administered in oil. The TMA-MSA conjugate was used to elicit the response by intratracheal instillation to avoid the technical difficulties associated with generating and containing TMA dust. Previous studies by others in the guinea pig used a similar design to compare the bronchoconstrictor response induced by OA and TMA following intradermal sensitization in oil (Arakawa *et al.*, 1995). In sensitized animals, Arakawa *et al.* (1995) found that intratracheal instillation of OA caused an abrupt increase in

airway resistance whereas instillation of TMA protein conjugate caused a slowly progressive increase in airway resistance.

In the present study, the cell infiltration response induced by TMA was compared to that of OA. A parallel sensitization and challenge procedure was used for OA as the antigen, since studies of others had indicated that mechanisms of the allergic response in mice could differ depending on the route of sensitization and challenge with OA (Zhang *et al.*, 1997). The eosinophilia in TMA-sensitized and -challenged mice was similar to the response seen in OA-sensitized and -challenged mice using a parallel procedure. These studies provide the basis for future studies to compare mechanisms and mediators responsible for the substantial TMA-induced eosinophilia compared to the OA response. Our previous studies in the guinea pig have suggested that the mechanism of eosinophilia differs depending on the allergen, i.e., TMA vs OA (Fraser *et al.*, 1995; Regal and Fraser, 1996; Regal, 1997a,b). The mouse has the advantage that knockout strains and information on the mouse genome are available. Thus, testing hypotheses to compare mechanisms and mediators of the TMA- and OA-induced allergic response is more feasible in the mouse than in the guinea pig.

Whether low-molecular-weight allergens cause effects independent of IgE has been debated and remains controversial (Banks and Wang, 2000; Grammer *et al.*, 1998). Previous studies have clearly shown that exposure to TMA via inhalation or dermal routes causes an increase in TMA-specific IgE in the plasma of mice (Dearman *et al.*, 1991; Potter and Wederbrand, 1995). In our present mouse model, the eosinophilia was accompanied by increases in total IgE in both plasma and BAL of the mice. However, some eosinophilia also occurred in the absence of any sensitization. Future studies can more directly focus on the potential IgE-dependent vs -independent components of the response.

Clearly, screening methods are needed for hazard identification of respiratory allergens and the mouse is considered an acceptable rodent for the development of such screening methods. This TMA model provides a baseline response of eosinophilia to compare alternate methods of sensitization and elicitation in developing screening methods for low-molecular-weight respiratory allergens. Realistically, both sensitization and challenge with TMA would most likely occur by inhalation in an occupational exposure, with the possibility of dermal exposure also being significant. Preliminary studies of Boykin *et al.* (2001) and Sailstad *et al.* (2001) have demonstrated cellular infiltration into the lung of mice sensitized and challenged intratracheally with TMA in water. However, significant eosinophilia was not documented at the doses employed. The significant eosinophilia described in the present study can serve as a positive control for the evaluation of sensitization and elicitation regimens more suitable for screening low-molecular-weight compounds.

In conclusion, we have demonstrated that TMA sensitization and challenge can cause significant eosinophilia in the lung of the mouse, similar to the characteristic eosinophilia seen in human asthma. In addition, sensitization-independent eosinophilia was also observed in response to challenge with TMA-MSA. Future studies can now be directed toward examining the mechanisms and mediators of the response.

Acknowledgments

The authors acknowledge Liz Boykin for her excellent technical assistance and Susan Kurki for her excellent secretarial assistance. The authors also thank Dr. Ronald Regal, Department of Mathematics and Statistics, University of Minnesota Duluth, for assistance in the statistical analysis of the data. This study was supported by NIH ES 07406. This work does not reflect EPA policy.

References

- Andersson P. Effects of inhibitors of anaphylactic mediators in two models of bronchial anaphylaxis in anaesthetized guinea-pigs. *Br J Pharmacol* 1982;77:301–307. [PubMed: 6814558]
- Arakawa H, Lotvall J, Kawikova I, Morikawa A, Lofdahl CG, Skoogh BE. Airway responses following intradermal sensitization to different types of allergens: Ovalbumin, trimellitic anhydride and *Dermatophagoides farinae*. *Int Arch Allergy Immunol* 1995;108:274–280. [PubMed: 7580293]
- Arakawa H, Kawikova I, Skoogh BE, Hayes J, Morikawa A, Lofdahl CG, Lotvall J. Role of arachidonic acid metabolites in airway responses induced by trimellitic anhydride in actively sensitized guinea pigs. *Am Rev Respir Dis* 1993;147:1116–1121. [PubMed: 8484619]
- Banks DE, Wang M. Occupational asthma: “The Big Picture”. *Occup Med State of the Art Rev* 2000;15:335–357.
- Boykin E, Ward M, Selgrade MJ, Sailstad D. Trimellitic anhydride (TMA) hypersensitivity in mice after dermal and intratracheal (IT) exposures. *Toxicologist* 2001;60:9.
- Brewer JP, Kisselgof AB, Martin TR. Genetic variability in pulmonary physiological, cellular, and antibody responses to antigen in mice. *Am J Respir Crit Care Med* 1999;160:1150–1156. [PubMed: 10508801]
- Busse, WW.; Parry, DE. The biology of asthma. In: Fishman, AP., editor. *Fishman’s Pulmonary Diseases and Disorders*. 3. McGraw Hill; New York: 1998. p. 721-733.
- Dearman RJ, Hegarty JM, Kimber I. Inhalation exposure of mice to trimellitic anhydride induces both IgG and IgE anti-hapten antibody. *Int Arch Allergy Appl Immunol* 1991;95:70–76. [PubMed: 1917113]
- Dimayuga E, Stober M, Kayes SG. Eosinophil peroxidase levels in hearts and lungs of mice infected with *Toxocara canis*. *J Parasitol* 1991;77:461–466. [PubMed: 1953850]
- Fraser DG, Regal JF, Arndt ML. Trimellitic anhydride-induced allergic response in the lung: Role of the complement system in cellular changes. *J Pharmacol Exp Ther* 1995;273:793–801. [PubMed: 7752082]
- Grammer L, Shaughnessy M, Kenamore B. Utility of antibody in identifying individuals who have or will develop anhydride-induced respiratory disease. *Chest* 1998;114:1199–1202. [PubMed: 9792595]
- Hamelmann E, Oshiba A, Loader J, Larsen GL, Gleich G, Lee J, Gelfand EW. Antiinterleukin-5 antibody prevents airway hyper-responsiveness in a murine model of airway sensitization. *Am J Respir Crit Care Med* 1997;155:819–825. [PubMed: 9117011]
- Hayes JP, Daniels R, Tee RD, Barnes PJ, Chung KF, Newman Taylor AJ. Specific immunological and bronchopulmonary responses following intradermal sensitization to free trimellitic anhydride in guinea pigs. *Clin Exp Allergy* 1992a;22:694–700. [PubMed: 1504892]
- Hayes JP, Lotvall JO, Barnes PJ, Newman Taylor AJ, Chung KF. Involvement of inflammatory mediators in the airway responses to trimellitic anhydride in sensitized guinea pigs. *Br J Pharmacol* 1992b; 106:828–832. [PubMed: 1382788]
- Hayes JP, Newman Taylor AJ. In vivo models of occupational asthma due to low molecular weight chemicals. *Occup Environ Med* 1995;52:539–543. [PubMed: 7663640]
- Herz U, Lumpp U, Da Palma JC, Enssle K, Takatsu K, Schnoy N, Daser A, Kottgen E, Wahn U, Renz H. The relevance of murine animal models to study the development of allergic bronchial asthma. *Immunol Cell Biol* 1996;74:209–217. [PubMed: 8724012]
- Hessel EM, Van Oosterhout AJM, Van Ark I, Van Esch B, Hofman G, Van Loveren H, Savelkoul HFJ, Nijkamp FP. Development of airway hyperresponsiveness is dependent on interferon gamma and independent of eosinophil infiltration. *Am J Respir Cell Mol Biol* 1997;16:325–334. [PubMed: 9070618]
- Humbles AA, Lu B, Nilsson CA, Lilly C, Israel E, Fujiwara Y, Gerard NP, Gerard C. A role for the C3a anaphylatoxin receptor in the effector phase of asthma. *Nature* 2000;406:998–1001. [PubMed: 10984054]
- Larsen CP, Regal RR, Regal JF. Trimellitic anhydride-induced allergic response in the guinea pig lung involves antibody-dependent and -independent complement system activation. *J Pharmacol Exp Ther* 2001;296:284–292. [PubMed: 11160609]

- Leach CL, Hatoum NS, Ratajczak HV, Zeiss CR, Roger JC, Garvin PJ. The pathologic and immunologic response to inhaled trimellitic anhydride in rats. *Toxicol Appl Pharmacol* 1987;87:67–80. [PubMed: 3798453]
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951;193:265–275. [PubMed: 14907713]
- Mapp C, Boschetto P, Miotto D, De Rosa E, Fabbri LM. Mechanisms of occupational asthma. *Ann Allergy Asthma Immunol* 1999;83:645–664. [PubMed: 10619337]
- NIOSH. Current Intelligence Bulletin 21. Publication 78–121. U.S. Dept. of Health Education and Welfare (NIOSH); Cincinnati, OH: 1978. Trimellitic Anhydride (TMA).
- Potter DW, Wederbrand KS. Total IgE antibody production in BALB/c mice after dermal exposure to chemicals. *Fundam Appl Toxicol* 1995;26:127–135. [PubMed: 7657056]
- Regal, JF. Mechanism of IgG- and IgE-mediated contraction of airway smooth muscle. In: Hollinger, MA., editor. *Focus on Pulmonary Pharmacology and Toxicology*. Vol. I. CRC Press; Boca Raton, FL: 1989. p. 73-90.
- Regal, JF. Hypersensitivity reactions in the lung. In: Lawrence, DA., editor. *Toxicology of the Immune System*. Vol. 5. Elsevier Science; New York: 1997a. p. 339-351. *Comprehensive Toxicology*
- Regal JF. Role of the complement system in pulmonary disorders. *Immunopharmacology* 1997b;38:17–25. [PubMed: 9476111]
- Regal JF, Fraser DG. Systemic complement system depletion does not inhibit cellular accumulation in antihistamine pretreated allergic guinea pig lung. *Int Arch Allergy Immunol* 1996;109:150–160. [PubMed: 8563490]
- Sailstad D, Boykin E, Ward M, Selgrade MJ. Trimellitic anhydride (TMA) hypersensitivity in mice after multiple intratracheal (IT) exposures. *Toxicologist* 2001;60:173.
- Sarlo, K.; Karol, M. Animal models of occupational asthma. In: Bernstein, IL.; Chan-Yeung, M.; Malo, JL.; Bernstein, DL., editors. *Asthma in the Workplace*. 2. Dekker; New York: 1999. p. 111-127.
- Strath M, Warren DJ, Sanderson CJ. Detection of eosinophils using an eosinophil peroxidase assay. Its use as an assay for eosinophil differentiation factors. *J Immunol Methods* 1985;83:209–215. [PubMed: 3840509]
- Van Kampen V, Merget R, Baur X. Occupational airway sensitizers: An overview on the respective literature. *Am J Ind Med* 2000;38:164–218. [PubMed: 10893510]
- Ward MDW, Sailstad DM, Selgrade MJK. Allergic responses to the biopesticide *Matarhizium anipoliae* in Balb/c mice. *Toxicol Sci* 1998;45:195–203. [PubMed: 9848126]
- Zeiss CR, Patterson R, Pruzansky JJ, Miller MM, Rosenberg M, Levitz D. Trimellitic anhydride-induced airway syndromes: Clinical and immunologic studies. *J Allergy Clin Immunol* 1977;60:96–103. [PubMed: 68964]
- Zeiss CR, Mitchell JH, Peenen V, Harris J, Levitz D. A twelve year clinical and immunologic evaluation of workers involved in the manufacture of trimellitic anhydride (TMA). *Allergy Proc* 1990;11:71–77. [PubMed: 2338240]
- Zhang Y, Lamm WJE, Albert RK, Chi EY, Henderson WR Jr, Lewis DB. Influence of the route of allergen administration and genetic background on the murine allergic pulmonary response. *Am J Respir Crit Care Med* 1997;155:661–669. [PubMed: 9032210]

FIG. 1.

Total numbers of eosinophils, neutrophils, and macrophages in BAL of nonsensitized and TMA-sensitized animals 72 h after intratracheal instillation of 30 or 400 µg MSA or TMA-MSA. Values represent the geometric mean $\pm$ SE of the response in 4–10 animals. Short brackets, comparison of MSA and TMA-MSA within a sensitization group. Long brackets, comparison of MSA control between all three groups. * $p < 0.05$; ns, not significant.

FIG. 2.

Eosinophil peroxidase (EPO) and myeloperoxidase (MPO) activity in lung tissue of nonsensitized and TMA-sensitized animals 72 h after intratracheal instillation of 30 or 400 µg MSA or TMA-MSA. EPO and MPO are indicators of the numbers of eosinophils and neutrophils, respectively. Values represent the geometric mean $\pm$ SE of the response in 4–10 animals. Short brackets, comparison of MSA and TMA-MSA within a sensitization group. Long brackets, comparison of MSA control between all three groups. * $p < 0.05$; ns, not significant.

FIG. 3.

Total numbers of eosinophils, neutrophils, and macrophages in BAL of TMA-sensitized animals 48 or 72 h after intratracheal instillation of either 400 µg MSA or TMA-MSA. Values represent the geometric mean $\pm$ SE of the response in four or five animals. Short brackets, comparison of MSA and TMA-MSA within a time of lavage group. Long brackets, comparison of MSA control between 48- and 72-h groups. * $p < 0.05$; ns, not significant.

FIG. 4.

Eosinophil peroxidase (EPO) and myeloperoxidase (MPO) activity in lung tissue of TMA-sensitized animals 48 or 72 h after intratracheal instillation of either 400 μ g MSA or TMA-MSA. EPO and MPO are indicators of the numbers of eosinophils and neutrophils, respectively. Values represent the geometric mean $\pm$ SE of the response in four or five animals. Short brackets, comparison of MSA and TMA-MSA within a time of lavage group. Long brackets, comparison of MSA control between 48- and 72-h groups. * $p < 0.05$; ns, not significant.

FIG. 5.

The concentration of total IgE in plasma (µg/ml) or the total IgE in BAL supernatant (ng) in either naive, nonsensitized or TMA-sensitized animals 72 h after challenge with either 30 or 400 µg MSA or TMA-MSA. Naive mice ($n = 4$) were neither sensitized nor challenged. Values represent the mean $\pm$ SE of the response in 4–8 animals. Brackets indicate the statistical comparisons made as described under Methods. * $p < 0.05$; ns, not significant; B.D., below detection.

FIG. 6.

Total numbers of eosinophils, neutrophils, and macrophages in BAL of nonsensitized and OA-sensitized animals 72 h after intratracheal instillation of 30 µg MSA or OA. Values represent the geometric mean $\pm$ SE of the response in 4–8 animals. Short brackets, comparison of MSA and OA within a sensitization group. Long brackets, comparison of MSA control in both groups. * $p < 0.05$; ns, not significant.

FIG. 7. Eosinophil peroxidase (EPO) and myeloperoxidase (MPO) activity in lung tissue of nonsensitized and OA-sensitized animals 72 h after intratracheal instillation of 30 μ g MSA or OA. EPO and MPO are indicators of the numbers of eosinophils and neutrophils, respectively. Values represent the geometric mean $\pm$ SE of the response in 4–8 animals. Short brackets, comparison of MSA and OA within a sensitization group. Long brackets, comparison of MSA control in both groups. * $p < 0.05$; ns, not significant.

TABLE 1

Sensitization and Elicitation Protocols for Experimental Groups of Mice

	Sensitization		Elicitation	
Group	Intradermal Days 1 and 3	Intratracheal Day 12	Intratracheal Days 19, 22, 23	<i>n</i>
TMA treatments ^a				
Nonsensitized, 30 µg	Corn oil	30 µg MSA	30 µg MSA	4
			30 µg TMA–MSA	4
Sensitized, 30 µg	3% TMA in corn oil	30 µg TMA–MSA	30 µg MSA	10
			30 µg TMA–MSA	10
Sensitized, 400 µg	3% TMA in corn oil	400 µg TMA–MSA	400 µg MSA	4
			400 µg TMA–MSA	4
OA treatments				
Nonsensitized, 30 µg	Corn oil	30 µg MSA	30 µg MSA	4
			30 µg OA	4
Sensitized, 30 µg	0.3% OA in corn oil	30 µg OA	30 µg MSA	8
			30 µg OA	8

^a MSA for these groups of animals was carried through the conjugation procedure without the addition of TMA.

TABLE 2

Comparison of the MSA/TMA–MSA Effect between Nonsensitized 30-μg, TMA-Sensitized 30-μg, and TMA-Sensitized 400-μg Groups

	<i>p</i> values ^a	
	Nonsensitized 30 μg vs TMA-sensitized 30 μg	TMA-sensitized 30 μg vs TMA-sensitized 400 μg
Cell infiltration into airspace		
Eosinophils	0.02 *	0.24
Neutrophils	0.25	0.16
Macrophages	0.41	0.38
Cell infiltration into lung		
Lung EPO	0.02 *	0.01 *
Lung MPO	0.42	0.22

^aData were log transformed before analysis. ANOVA with one-tailed single degree of freedom contrasts.

* Significantly different, $p < 0.05$.

TABLE 3

Comparison of the MSA/OA Effect between Nonsensitized and OA-Sensitized Animals

Nonsensitized vs OA-Sensitized <i>p</i> value ^a	
Cell infiltration into airspace	
Eosinophils	<0.01 *
Neutrophils	0.11
Macrophages	0.26
Cell infiltration into lung	
Lung EPO	<0.01 *
Lung MPO	0.02 *

^aData were log transformed before analysis. ANOVA with one-tailed single degree of freedom contrasts.

* Significantly different, $p < 0.05$.