Neurotoxicants in our environment: Induced Eosinophilia in a Mouse Model of Occupational Asthma

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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 μg) or OA (30 μg). Nonsensitized mice received corn oil vehicle intra-dermally and MSA (30 μg) intratracheally. The allergic response was elicited 3 weeks later by intratracheal instillation of 30 or 400 μg 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; Lun; Pulmonary Allergy

Introduction

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). 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,

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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 non-sensitized group was challenged intratracheally with 30 μg 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 (~2 × 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.

**Statistical methods**

All data were log transformed to equalize variances. Figures show the geometric mean ± 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, 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, 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, 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.
**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 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.

**References**


creatic β-Cells: Voltage-Independent Calcium Channels Mediate Slow Oscillations of Cytosolic Calcium