

Advances in acid anhydride induced occupational asthma

C. Raymond Zeiss

The acid anhydrides have been signal occupational agents for study because of their inherent and complex biological activity and the wide range of associated occupational airway disease that is manifest in exposed workers in a variety of occupational settings. This year in review has shed light on many of the complexities of anhydride exposure, biologic reactivity and airway disease. *Curr Opin Allergy Clin Immunol* 2:89–92. © 2002 Lippincott

Williams & Wilkins.

Northwestern University Medical School, Chicago, Illinois, USA

Correspondence to C. Raymond Zeiss, MD, Northwestern University Medical School, 303 E. Chicago Ave., Allergy Division, Chicago, IL 60611, USA
Tel: +1 312 649 2101; e-mail: crz528@nwu.edu

Current Opinion in Allergy and Clinical Immunology 2002, 2:89–92

Abbreviations

HHPA	hexahydrophthalic anhydride
MHHPA	methylhexahydrophthalic anhydride
MTHPA	methyltetrahydrophthalic
TMA	trimellitic anhydride

© 2002 Lippincott Williams & Wilkins
1528-4050

Introduction

The year has seen a range of publications that encompass three areas of acid anhydride induced occupational respiratory disease: the impact of anhydride exposure and removal from exposure; the biologic consequences of adduction (binding) of anhydrides to chemical moieties, proteins and airway epithelium; and animal models of immunoglobulin E-mediated lung inflammation, the role of anhydride induced airway complement activation, and anhydride mediated respiratory irritant effects.

Exposure

In a 10-year prospective study in three plants of workers exposed to the phthalic anhydride related acid anhydrides, hexahydrophthalic anhydride (HHPA), methylhexahydrophthalic anhydride (MHHPA), and methyltetrahydrophthalic anhydride (MTHPA). Welinder and co-authors [1••] reported the central role of exposure in the induction of immunoglobulin E and G antibodies to these anhydrides. There was an exposure driven response relationship from $2.5 \mu\text{g}/\text{m}^3$ to greater than $15 \mu\text{g}/\text{m}^3$ (as determined by personal and area sampling at frequent intervals).

The effect of smoking on antibody induction was not statistically significant, but the effect of atopy as defined by a positive skin prick test response was significant with an odds ratio of 5.9 (2.0–17.3). Of interest, the presence of smoking and atopy only modestly added to the effect of exposure in their multivariate analysis, however the study did not have enough statistical power to determine if atopics were sensitized at lower exposure levels. This study is of significant interest because of the prospectively gathered exposure data demonstrating exposure as the key driver in immunologic sensitization, modified only marginally by smoking and atopy.

In an excellent study, Rosqvist and co-authors [2••] have determined the value of total plasma protein adducts as a biomarker in workers exposed to HHPA and MHHPA. The urinary metabolites of these anhydrides served as the index of work shift exposure. There was a strong positive correlation comparing plasma total plasma protein adduct levels and urinary metabolite levels (r , 0.920–0.997). In addition, specific anhydride adducted hemoglobin levels in plasma were highly correlated with urinary anhydride metabolite levels. The half-lives of the total plasma protein adducts were 22–24 days making them excellent potential biomarkers of long-term exposure. The availability of quantitative plasmas and

urinary metabolite markers of anhydride exposure may revolutionize our assessment of exposure and therefore the risk of sensitization in acid anhydride exposed workers, where traditional air sampling methods may be confounded by undetected peak exposures and the use or non-use of respiratory protective devices.

In a further study of the binding/adduction of the acid anhydride HHPA [3•], Johannesson and co-authors determined that serum albumin was the major plasma protein adducted by the inhalation of HHPA in exposed workers and guinea pigs. Using rigorous biochemical techniques they located the predominant HHPA adduct/hapten to serum albumin. In guinea pigs exposed to tritium-labeled HHPA, by nose only inhalation exposure, autoradiography of plasma proteins demonstrated a single band with a molecular weight of 66 kDa, equivalent to that of serum albumin. In addition, they showed that immunoglobulin E and G antibodies from sensitized workers bond preferentially to albumin, when the target was human plasma adducted with HHPA. These studies elegantly demonstrate that small but detectable amounts of reactive inhaled anhydride, HHPA bind with (haptinize/adduct) proteins, predominantly albumin, in the circulation of anhydride-exposed humans and guinea pigs.

Lemiere and co-authors [4•] investigated the changes in airway inflammation induced by exposure to occupational agents in subjects with and without occupational asthma. They set out to determine which changes in sputum eosinophil numbers and bronchial responsiveness to methacholine should be regarded as clinically significant for predicting occupational asthma. They performed specific inhalation challenges in three groups of subjects: subjects reporting a history consistent with occupational asthma with a positive specific inhalation challenge response ($n=17$); subjects reporting a history consistent with occupational asthma with a negative specific inhalation challenge response ($n=14$); and asthmatic subjects without any history of occupational asthma ($n=10$). Induced sputum and methacholine challenges were performed at the end of the control day and again at the end of the last day of exposure.

There was an increase in median sputum eosinophil and neutrophil numbers in those subjects with occupational asthma. Cell counts remained unchanged after exposure in asthmatic subjects without occupational asthma. Sputum eosinophilia and a significant increase in methacholine sensitivity identified those with occupational asthma.

They concluded that exposure to occupational agents per se did not induce airway inflammation and that

changes in both sputum eosinophil counts and methacholine responsiveness are satisfactory predictors of a significant bronchial responsiveness to occupational agents.

Grammer and co-workers [5•] describe 42 workers with trimellitic anhydride (TMA)-induced lung disease (four late asthma, 13 late respiratory systemic syndrome, four both late asthma and late respiratory systemic syndrome, and 21 with asthma) moved from the direct manufacture of TMA to low exposure jobs in this large industrial complex. They were evaluated 1 year after the move by physician-administered questionnaire (symptoms), spirometry, immunoglobulin E levels to trimellitate-human serum albumin, and chest radiography. Thirty-five of 42 workers had improved symptoms, spirometry and lower levels of immunoglobulin E to trimellitate-human serum albumin. Seven workers with immunoglobulin E mediated asthma did not improve and were transferred to no exposure jobs. There were no chest radiographic abnormalities related to TMA in any group.

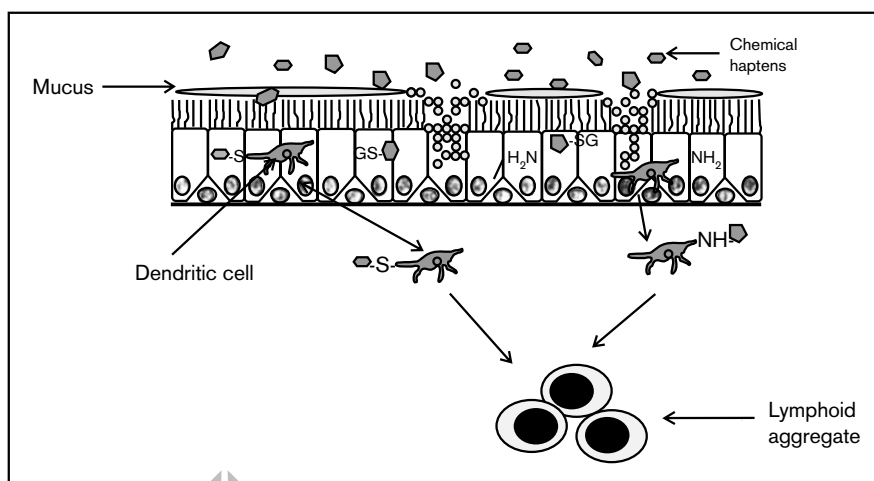
Binding and adduction

Griffen and co-authors [6•] report a preliminary study of the localization of TMA to cultured lung epithelial cell proteins. They developed a monoclonal antibody with primary specificity for the TMA hapten and used this as a probe to identify TMA haptenized proteins extracted from cultured lung epithelial cells, which had been exposed previously to TMA in culture. Western blot analysis demonstrated that the monoclonal antibody probe reacted with TMA haptenized proteins in a restricted low molecular weight range (20–35 Kd). They suggest that the low molecular weight cellular proteins may be as important as albumin in TMA-induced lung disease. It is clear from the studies just reviewed and recent animal studies (below) that albumin, when adducted by reactive acid anhydrides, is a major antigen eliciting a pathogenic antibody response.

The study by Griffin [6•] was accompanied by an excellent and wide-ranging editorial comment by M. Karol [7•]. This editorial reviewed newer information on reactive chemical adduction of epithelial cell proteins and other intracellular/extracellular moieties. Of particular interest was the recent observation that glutathione is readily adducted by di-isocyanates and that isocyanate adducted glutathione could transfer the isocyanate hapten to reactive groups on proteins under physiologic conditions. This work suggests that glutathione, a ubiquitous intracellular and extracellular transfer agent, might play a role in the transport and adduction of reactive chemicals to a variety of biologically important macromolecules. The complexity of the interplay

Figure 1. Exposure of the large airways to reactive chemical allergens

The inhaled chemical hapten reacts with nucleophiles such as R-NH₂ and R-SH in the airway epithelium to form a hapten conjugate. The hapten may transfer from the original conjugate to a preferred nucleophile. The dendritic cell embodying the hapten-conjugate migrates to lymphoid tissue and presents the conjugate to T lymphocytes thereby initiating the sensitization process. Reproduced with permission [7••].



between reactive chemicals such as the acid anhydrides/isocyanates and the airway epithelium is illustrated in this editorial (Fig. 1).

Animal models

Larsen and co-authors [8••] report the role of complement activation in the airway of guinea pigs sensitized to TMA and challenged with intratracheal TMA-guinea pig serum albumin. In challenged, sensitized animals there was airway infiltration with eosinophils, neutrophils, macrophages with red blood cells and protein in the air spaces.

The complement activation product, C3a, was detected in bronchoalveolar lavage fluid and was correlated with all measures of cell airway infiltration.

Regal and co-authors [9••] reported a model of immunoglobulin E-mediated hypersensitivity to TMA in BALB/c mice accompanied by lung eosinophilia and the product, eosinophil peroxidase, after intratracheal instillation of TMA mouse serum albumin in mice previously sensitized to TMA. There was a parallel increase in total immunoglobulin E in plasma and bronchoalveolar lavage fluid. The magnitude of the response to inhaled TMA mouse serum albumin was equivalent to that seen in their standard model, BALB/c mice sensitized to and exposed to ovalbumin.

Arts and co-authors [10•] reported a modest lower airway irritant response to inhaled TMA in rats exposed to increasing concentrations of TMA (10–300 mg/m³) for 30 min. Irritant responses were assessed by breathing pattern and frequency changes and necropsy lung weight. No irritant effects were detected at or below

14 mg/m³. There were no changes in lung weight at any level of exposure.

Conclusion

This year in review has seen significant scientific advances in the study of occupational asthma induced by acid anhydrides. The strong linkage of exposure to sensitization, the newer methods of exposure assessment, the exciting and ongoing exploration of anhydride binding/adduction of key cellular and extracellular biologic targets and the use of animal models to explore the complex pathophysiology of anhydride asthma speak for the certainty of future insights and knowledge to come.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

- 1 Welinder H, Nielsen J, Rylander L, Stahlbom B. A prospective study of the relationship between exposure and specific antibodies in workers exposed to organic acid anhydrides. *Allergy* 2001; 56:506–511.

This study is of significant interest, because of the prospectively gathered exposure data demonstrating exposure as the key driver in immunologic sensitization modified only marginally by smoking and atopy. This report clarifies the acid anhydride literature to date that has presented conflicting evidence from studies that examined the effect of smoking and atopy on immunologic sensitization to the acid anhydrides, in that the effect of these modifiers may be manifest only under conditions of low exposure.

- 2 Rosqvist S, Johannesson G, Lindh CH, Jonsson BA. Total plasma protein adducts of allergenic hexahydrophthalic and methylhexahydrophthalic anhydrides as biomarkers of long-term exposure. *Scand J Work Environ Health* 2001; 27:133–139.

The availability of quantitative plasmas and urinary metabolite markers of anhydride exposure may revolutionize our assessment of exposure and therefore the risk of sensitization in acid anhydride exposed workers, where traditional air sampling methods may be confounded by undetected peak exposures and the use or non-use of respiratory protective devices.

- 3 Johannesson G, Rosqvist S, Lindh CH, *et al*. Serum albumins are the major site
 •• for in vivo formation of hapten-carrier protein adducts in plasma from humans and guinea-pigs exposed to type 1 allergy inducing hexahydrophthalic anhydride. *Clin Exp Allergy* 2001; 31:1021–1030.

These studies elegantly demonstrate that small but detectable amounts of the reactive inhaled anhydride HHPA bind with proteins, predominantly albumin, in the circulation of anhydride-exposed humans and guinea pigs. Immunoglobulin E and G antibodies recognize haptenized albumin as the major antigen when HHPA adducted plasma proteins serve as the substrate for antibody binding. These observations do not preclude potentially important respiratory tract cellular proteins as haptenized antigens that may induce a pathogenic immune response.

- 4 Lemièrre C, Chabouille S, Malo JL, Cartier A. Changes in sputum cell counts
 •• after exposure to occupational agents: what do they mean? *J Allergy Clin Immunol* 2001; 107: 1063–1068.

This is an important study that demonstrates not only the predictive value of sputum eosinophilia induced by occupational allergen exposure, but also the finding that inhalation exposure of asthmatics without occupational asthma to a variety of occupational agents does not induce airway inflammation or increases in airway reactivity.

- 5 Grammer LC, Shaughnessy MA, Kenamore BD. Clinical and immunologic
 •• outcome of 42 individuals with trimellitic anhydride-induced immunologic lung disease after transfer to low exposure. *Allergy Asthma Proc* 2000; 21:355–359.

This group of investigators has continuously assessed workers in this large industrial chemical manufacturing complex (one unit of which manufactures TMA) for over 25 years. This report indicates that most workers with TMA-induced lung disease can be safely moved to minimal exposure jobs and have resolution of symptoms and other clinically relevant measures. However, there are workers with immunoglobulin E mediated asthma that cannot have even minimal TMA exposure and must be moved to no-exposure jobs.

- 6 Griffin P, Allan L, Beckett P, *et al*. The development of an antibody to trimellitic
 •• anhydride. *Clin Exp Allergy* 2001; 31:453–457.

Although these results are intriguing, the authors did not show that immunoglobulin E or G antibodies from exposed workers with TMA lung disease would bind to these low molecular weight TMA-haptenized proteins, which would have demonstrated their pathogenic potential in TMA-induced lung disease.

- 7 Karol MH. Bonding and transfer: do epithelial conjugates have a role in chemical
 •• asthma? *Clin Exp Allergy* 2001; 31:357–360.

The study by Griffin (above) was accompanied by this excellent and wide-ranging editorial comment. Of particular interest was the recent observation that glutathione is readily adducted by di-isocyanates and that isocyanate adducted glutathione could transfer the isocyanate hapten to reactive groups on proteins under physiologic conditions.

- 8 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.

These studies add important and potent inflammatory agents, the complement cascade cleavage products, to the pathogenesis of anhydride-induced asthma. The analysis of bronchoalveolar lavage fluid for mediators of inflammation continues to be a fruitful area of investigation.

- 9 Regal JF, Mohrman ME, Sailstad DM. Trimellitic anhydride-induced eosinophilia
 •• in a mouse model of occupational asthma. *Toxicol Appl Pharmacol* 2001; 175:234–242.

This is an important development, the establishment of an anhydride (TMA) induced model of occupational asthma in the BALB/c mouse. This will allow the dissection of this immunoglobulin E mediated response by the newer gene manipulation techniques developed in the mouse, such as the study of 'knockout' mice missing key cytokine and other genes central to immunoglobulin E mediated inflammation.

- 10 Arts JH, de Koning MW, Bloksma N, Kuper CF. Respiratory irritation by
 •• trimellitic anhydride in Brown Norway and Wistar rats. *Inhal Toxicol* 2001; 13:719–728.

These studies confirm clinical observations that TMA is not a potent respiratory irritant under circumstances of usual occupational exposure. However, accidental exposure to high concentrations of TMA as a fume can lead to the acute chemical pneumonitis. The acid anhydrides have a spectrum of irritant properties with, for example, maleic anhydride, having marked irritant properties at low concentrations. Anhydrides with lower irritant potential but high sensitizing potential are a concern in that inhalation at levels that may be sensitizing are not accompanied by evident irritant symptoms.

- 11 Malo JL, Chan-Yeung M. Occupational asthma. *J Allergy Clin Immunol* 2001;
 •• 108:317–328.

This is an important review of the state of the art in occupational asthma from pathogenesis to guidance on evaluation for impairment and worker compensation that places the acid anhydrides in the context of the broader picture of the range of work that has been accomplished in the study of occupational asthma.

WILLIAMS & WILKINS

Unauthorized Use
Prohibited