reduction, a measure of mitochondrial function, were 100, 30 and 300 nM, respectively. Neutral red (NR) uptake, an index of cell membrane function, was decreased 20% at >300, 75 and 300 nM, respectively. EC_{20}values for decreased protein content, a crude measure of cell viability, were 200, 100 and 200 nM, respectively. EC_{50} values of TBT for inhibition of the various endpoints were about 200 nM (EROD, NR, protein) and 100 nM (aromatase, MTT). None of the organotins inhibited aromatase or EROD activity by direct exposure of the cells only for the duration of the catalytic assays. We conclude that the inhibitory effects of the organotin compounds on aromatase and EROD activity occur at cytotoxic concentrations, indicating that the decreased CYP activities in H295R cells are not due to selective catalytic inhibition, but likely to the high affinity of organotins for sulfhydryl-containing proteins and/or secondary to decreased cell function.

39 ANALYSIS OF CYTOKINE PRODUCTION FOLLOWING EXPOSURE OF RATS TO CHEMICAL ALLERGENS.

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The cellular and molecular mechanisms that result in the induction of respiratory sensitization and asthma to low molecular weight chemicals are unclear, although there is evidence for the development of T helper (Th) 2 type responses and, in some cases, the production of IgE. We have shown previously that prolonged topical exposure of BALB/c strain mice to different classes of chemical allergen induces cytokine secretion patterns consistent with the selective activation of discrete functional subsets of T cells. Thus, topical exposure of mice to the respiratory sensitizer trimellitic anhydride (TMA) stimulates a Th2 cytokine secretion profile, whereas the contact allergen 2,4-dinitrochlorobenzene (DNCB), which apparently lacks respiratory sensitizing potential, elicits a selective Th1-type pattern. We have now examined whether these divergent responses are a feature only of murine immune responses, by analyzing cytokine secretion profiles induced following topical exposure of Brown Norway rats to TMA and DNCB. Culture of lymph node cells isolated 13 days following TMA treatment resulted in the expression of high levels of mitogen-inducible interleukin (IL)-4 and spontaneous IL-10 production, but low levels of the type 1 cytokine, interferon gamma. The converse cytokine secretion pattern was induced following identical treatment of rats with DNCB. The innate ability of DNCB and TMA to promote preferential type 1 and type 2 responses, respectively, would appear to be species independent and provide additional evidence that chemicals which cause allergic sensitization of the respiratory tract are associated with polarized Th2 type immune responses.

40 LACK OF ASSOCIATION OF ANTIBODIES WITH DIISOCYANATE - INDUCED AIRWAY INFLAMMATION, HYPERREACTIVITY AND ASTHMA.

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Diisocyanates are the most frequent cause of chemically-induced occupational asthma. Chemically-induced asthma differs from occupational asthma caused by high molecular weight allergens, in that specific antibodies are rarely found in the sera of patients with chemically-induced asthma. To investigate the role of antibodies in the pathogenesis of the disease we used both animal and human data. Mice deficient in tumor necrosis factor (TNF) activity, *i.e.*, TNF receptor knockout animals and animals pretreated with TNF neutralizing antisera were exposed to toluene diisocyanate (TDI). We have shown that these animals fail to develop sensitization to TDI as evidenced by the absence of both airway inflammation and hyperreactivity following TDI exposure. C57BL/6J mice were sensitized by sc injection and challenged on 3 occasions by inhalation of the chemical. Blood was drawn and sera were evaluated for TDI-specific IgE and IgG as well as for IgG subclasses. Using ELISA, TDI-specific IgE was not detected. TDI-specific IgG antibodies, with titers ranging from 800 - 1200, were found in all TDI-exposed groups. Antibodies were present in animals pretreated with anti-TNF antiserum, and in TNFR knockout animals, although these animals had either greatly diminished or no evidence of TDI asthma. In human studies, sera from 200 automobile painters were evaluated for hexamethylene diisocyanate (HDI)-specific antibodies. Thirty % of the painters had specific IgG antibodies, 4% had specific IgE antibodies, and 4% had clinically diagnosed asthma but none of the latter asthmatics had specific IgE. These results indicate the lack of association of antibodies with symptoms of asthma in an animal model and in humans. The results imply that diisocyanate asthma occurs though an antibody-independent mechanism. Supported by NIEHS 05651 and OH03457.

41 ELEVATION OF IL-9, IL-10, IL-13 AND IL-15 AS BIOMARKERS OF DERMAL EXPOSURE TO RESPIRATORY SENSITIZERS.

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Differential modulation of mRNA and protein levels for interleukin-4 (IL-4), IL-10 and interferon gamma by respiratory and T cell mediated sensitizers has been demonstrated. The following investigation examined the mRNA expression patterns of multiple cytokines associated with respiratory sensitization for modulation using a Multiprobe Ribonuclease Protection Assay. Female BALB/c mice were dermally exposed on the shaven dorsal lumbar area with either vehicle or test article on Day 0 and Day 5 followed 5 days later by exposure on the ear pinna for 3 consecutive days to chemicals known to primarily induce irritation (Sodium Lauryl Sulfate), respiratory sensitization (Toluene Diisocyanate), or T cell-mediated hypersensitivity (Dinitrofluorobenzene) responses. Draining lymph nodes were excised and mRNA isolated immediately or following 24 or 48 hrs in culture with Concanavalin A (Con A). Differential expression of cytokine mRNA was most notable in animals receiving test article only on the ears (no previous induction) following 24 hr incubation with Con A. The response for IL-4, IL-10, and IFN-γ was consistent with previous studies by others. In addition, IL-9, IL-13 and IL-15 were significantly elevated only following TDI exposure. The most robust modulation (> 200 fold increase over control) was observed for IL-13 which shares many biological activities with IL-4 and has been shown to be required for the induction of airway hyper-responsiveness in mice. Further investigations of these cytokines may provide additional insight into the mechanisms of chemically induced respiratory sensitization and provide endpoints for screening methods aimed at identifying chemicals with the capacity to elicit pulmonary sensitization.These studies were supported in part by the NIEHS. (Contract NO1-ES-55387 and interagency agreement #Y1-ES-0049-03.)

42 USE OF THE RIBONUCLEASE PROTECTION ASSAY (RPA) FOR IDENTIFYING CHEMICALS THAT ELICIT HYPERSENSITIVITY RESPONSES.

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The incidence of allergy and asthma has increased dramatically over the last 20 years and there is concern that exposure to chemicals such as diisocyanates and acid anhydrides in both domestic and occupational settings may contribute to this rise. Methods are needed to efficiently screen chemicals for the potential to cause airway hypersensitivity (AHS) reactions. AHS is driven by Th2 cells, whereas the contact response is driven by Th1 cells and the distinct cytokine profiles produced by these cells provide a means of distinguishing respiratory from contact sensitizers. ELISA assays have previously been conducted for this purpose but we have used the more efficient RPA to examine cytokine profiles. In these studies, mice were topically exposed to the known airway sensitizer trimellitic anhydride (TMA), and the contact sensitizers dinitrofluorobenzene (DNFB) and dinitrochlorobenzene (DNCB) on days 0 and 5 and challenged on days 10-12. At various times following challenge, total mRNA was isolated from draining lymph nodes and analyzed by RPA. Cytokines produced by Th2 cells (IL4, IL10 and IL13) were significantly increased in response to TMA as compared to DNCB and DNFB. Levels of the Th1 cytokine IFNγ were significantly elevated over TMA in DNCB and DNFB treated mice however, this occurred at only one point during the timecourse. A dose response experiment confirmed these results and showed that TMA induces higher levels of IL4, IL10 and IL13 than DNCB and DNFB over a range of doses. The RPA appears to be an effective method for the detection of cytokines characteristic of AHS responses following dermal sensitization and elicitation with a respiratory sensitizer which is more efficient than traditional ELISA methods in that multiple cytokines can be assessed at one time and an additional *in vitro* stimulation step is not required. (This abstract does not reflect EPA policy. This work was supported in part by The Dow Chemical Co. & DuPont Co.)

43 TRIMELLITIC ANHYDRIDE-INDUCED EOSINOPHILIA IN A MURINE MODEL OF OCCUPATIONAL ASTHMA.

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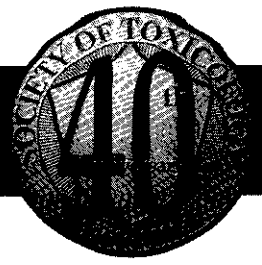
Trimellitic anhydride (TMA) is a small molecular weight chemical known to cause occupational asthma. To determine if TMA elicits cell infiltration into the lung similar to eosinophilia described for the protein allergen ovalbumin (OA), BALB/c



Society of Toxicology

40th Annual Meeting

An Official Journal of the
Society of Toxicology
Supplement

TOXICOLOGICAL SCIENCES
Formerly Fundamental and Applied Toxicology

The Toxicologist

Abstracts of the 40th Annual Meeting

Oxford University Press

Volume 60, Number 1, March 2001

Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster discussion, workshop, roundtable, and poster sessions of the 40th Annual Meeting of the Society of Toxicology, held at the Moscone Convention Center, San Francisco, California, March 25–29, 2001.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 451.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 479.

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