

1209 TOPICAL EXPOSURE OF MICE TO PHTHALATE IS WITHOUT ADJUVANT EFFECT.

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It has been suggested that one possible contributor to the increasing prevalence of IgE-mediated allergic diseases (including respiratory allergy and asthma) in Western Europe and the US is the presence in the environment of chemicals that may act as adjuvants. It has been reported previously that certain commonly used phthalate plasticizers, including that used most widely worldwide, di-(2-ethylhexyl) phthalate (DEHP), are able under some circumstances to modify immune responses induced in mice by the common allergen ovalbumin (OVA). Thus, it was reported that subcutaneous administration of DEHP (at 2mg/ml) in the presence of OVA resulted in an increase in anti-OVA IgG1 antibody production compared with mice exposed to OVA alone. Murine IgG1 antibody can be considered a surrogate of IgE antibody as cytokine regulation of this subclass of antibody parallels that of IgE. However, the significance of these observations for human health, and for the development of allergic disease is unclear; not least because the studies have been conducted exclusively using subcutaneous administration of phthalates. We have therefore investigated the ability of DEHP when applied topically to affect anti-OVA IgG1 antibody responses induced by subcutaneous exposure to OVA in BALB/c strain mice. Concentrations of DEHP (500mg/ml) were used that resulted in a marked (approximately 30%) increase in liver weight. Under conditions where anti-OVA IgG1 antibody responses were sub-optimal (immunization with 1mg OVA) topical administration of DEHP was without impact on anti-OVA antibody responses, regardless of whether DEHP was applied local or distant to the site of OVA immunization. These data suggest that the reported adjuvant effect of DEHP is a feature of the subcutaneous route of exposure as topical application of concentrations of DEHP that provoked marked systemic effects were without effect on the induction of immune responses.

1210 AUGMENTATION OF OVALBUMIN-INDUCED IGE AND AIRWAY HYPERREACTIVITY RESPONSE BY PERFLUOROOCCTANOIC ACID (PFOA).

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Studies were conducted to investigate the role of dermal exposure to Perfluorooctanoic acid (PFOA), an immunosuppressant with widespread use as a carpet and fabric protectant, on the hypersensitivity response to ovalbumin in a murine model. BALB/c mice were exposed dermally to concentrations of PFOA ranging from 0.01-2.0% (0.25-50mg/kg) for 4 days. In hypersensitivity studies, mice were also intraperitoneally injected with 7.5µg ovalbumin and 2mg alum on days 1 and 10 and in some studies, intratracheally challenged with 250µg ovalbumin on days 17 and 26. Endpoints for studies included body and organ weights and cellularities, IgE, airway hyperreactivity, and lung histopathology. Following exposure to PFOA, an increase in liver weights and a decrease in thymus and spleen weights and cellularities were observed. Similar immunomodulatory trends were demonstrated in mice co-administered PFOA and ovalbumin. Greater than a 2-fold increase in total IgE was demonstrated when mice were co-exposed with concentrations of PFOA ranging from 0.75-1.5%, while the ovalbumin-specific IgE response peaked after a 3-fold increase (p<0.01) in 0.75% PFOA co-exposed animals as compared to the ovalbumin alone exposed animals. Antigen-specific airway hyperreactivity was increased (p<0.05) in the 1.0% PFOA co-exposed group, with a dose-responsive pleiotropic cell response characterized by eosinophilia and mucin production, in animals co-exposed to concentrations of PFOA up to 1.0%, as compared to the ovalbumin alone exposed animals. PFOA was demonstrated to be immunotoxic in a murine model following dermal exposure, with an enhancement of the hypersensitivity response to ovalbumin, suggesting that PFOA exposure may augment the IgE response to environmental allergens.

1211 EVALUATION OF DERMAL SENSITIZATION TO WESTERN RED CEDAR EXTRACT AND ABIETIC ACID USING THE LOCAL LYMPH NODE ASSAY.

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Western Red Cedar (WRC, *Thuja plicata*) induces sensitization and asthma after occupational exposure. Plicatic acid, a low molecular weight (LMW) compound present at high concentrations in WRC wood has been implicated as the active agent. Another wood product, abietic acid (ABA), a terpenoid present in the resin of conifer species, has also been identified as a LMW agent causing occupational sensitization and asthma. We used the mouse local lymph node assay to address the question of whether these agents could induce sensitization via the dermal route.

WRC was extracted in water and the extract was lyophilized (WRC Extract). Mass spectrometry was consistent with plicatic acid being a major, but not the only, component of WRC extract. WRC extract and ABA were applied in concentrations of 12.5, 25, and 50% (w/v) to the ears of Balb/C mice. Vehicle treatment was used as a negative control and 30% -hexylcinnamaldehyde was used as a positive control. Neither WRC nor ABA caused increases in ear thickness, suggesting that neither was a dermal irritant. *In vivo* cell proliferation in draining lymph nodes was measured as 3H-thymidine incorporation following 3 days of exposure. Lymph node cell populations were evaluated for the presence of B220+ IgE+ B cells by flow cytometry on day 10, following 4 days of exposure. 3H-thymidine incorporation in draining lymph nodes was significantly increased by exposure to all three concentrations of WRC extract and the highest concentration of ABA (50%). Stimulation indices were: 3.4, 6.9, and 11.0 for 12.5%, 25%, and 50% WRC extract; and 3.0 for 50% ABA. Only the 50% WRC extract caused significant increases in B220+ IgE+ B cell percentage in draining nodes. Serum total IgE was increased 2 weeks after dermal exposure every other day to 25% and 50% WRC extract. Our results show that both WRC extract and ABA can induce sensitization by the dermal route and that WRC extract and ABA are weak dermal sensitizers (EC3 10% and 50%, respectively).

1212 INDUCTION OF CONTACT SENSITIZATION BY ANNATTO EXTRACT BIXIN BUT NOT BY NORBIXIN IN FEMALE BALB/C MICE.

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Annatto seeds, also known as 'achiote seeds', are commonly used in South American cooking. Annatto dye is an orange-yellow food coloring extracted from the seeds of the tree *Bixa orellana*. It has been used to color butter, margarine, cheese, smoked fish, snack foods, beverages, and cereals. Reported adverse reactions associated with annatto dye ingestion include urticaria and angioedema. The present studies were carried out to examine a possible contact allergic effect of two purified carotenoid pigments from annatto extract: bixin (BIX: C25H30O4) and norbixin (NOR: C24H28O4). The experiments were conducted in female BALB/c mice by applying BIX (1-25% w/v) and NOR (1-20% w/v) in acetone topically, and assays performed including the local lymph node assay (LLNA), the irritation assay, the mouse ear swelling test (MEST) and flow cytometric analysis. There was no treatment-related adverse effect on the body weights. However, a three-fold increase in the proliferation of auricular lymph node cells was observed at BIX concentrations of 5%-25% (p≤0.01). A significant increase in percent ear swelling was also produced in mice treated with BIX (5-10%) at 24 hr after challenge as measured in MEST assay. Further study indicated that BIX was not an irritant at the concentrations tested. Additional study demonstrated that exposure to BIX induced a significant increase in the percentage of B cells (Ig+) in the draining lymph node. In contrast, the results from studies on NOR showed that exposure to NOR at concentration levels of 1-20% did not alter the lymph node cell proliferation in the LLNA assay. Taken together, these results suggested that in annatto extract bixin but not norbixin is a contact sensitizer in female BALB/c mice (Supported by the NIEHS Contract ES 05454).

1213 DIFFERENTIAL ALLERGIC AND NEUROTROPHIN RESPONSES TO FUNGAL COMPONENT EXTRACTS IN MICE.

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Metarhizium anisopliae mycelium (MYC), conidia (CON) and inducible protease (IND) extracts were combined to produce the antigen MACA to screen for allergic potential. Involuntary aspiration (IA) exposure to MACA in BALB/c mice has caused immune, inflammatory and physiological responses characteristic of allergic lung disease. Our objective here was to determine if the component extracts have similar allergic potential. BALB/c mice received 4 IA of 10 µg of MACA, CON, MYC, IND, or BSA (negative control) in 50 µl HBSS, or HBSS alone (vehicle control) over a 4-week period. Additional mice received 1 exposure to agents as non-allergic controls. Serum and bronchoalveolar lavage fluid (BALF) were collected 3 days (D3) after the final exposure. Mice were assessed for immediate responses following IA exposure and airway hyperresponsiveness to methacholine (MCH; D1 & D3) by whole-body plethysmography, an index of pulmonary resistance and bronchoconstriction (PenH). All fungal extract exposures resulted in elevated BALF total protein, LDH, total cell counts, total IgE and serum IgE compared to HBSS and BSA controls. These responses were highest in MYC exposed mice, but their PenH was significantly lower compared to the other fungal treatments. To investigate this dichotomy, the level of BALF neurotrophins (NGF, NT-3, NT-4) that have been associated with allergic lung disease were evaluated. All extract exposures resulted in increased levels of the neurotrophins compared to controls. The IND treated mice had the lowest levels of the neurotrophins and significantly higher PenH compared to the other fungal treatments. The rank order of



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