807 KINETIC CHANGES IN CYTOKINE GENE EXPRESSION PATTERNS PROVOKED IN MURINE LYMPH NODE CELLS BY CHEMICAL ALLERGENS.

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Prolonged (13 day) topical exposure of BALB/c strain mice to the chemical respiratory allergen trimellitic anhydride (TMA) induces a selective T helper (Th) 2 profile of cytokine secretion in cells isolated from the draining lymph node. Thus, TMA-stimulated lymph node cells (LNC) produce little of the Th1 cytokines interferon γ(IFN-γ) and interleukin (IL) 12, but secrete relatively high levels of the Th2 products IL-5, IL-10 and IL-13 and mitogen-inducible IL-4. We have now compared changes in cytokine gene expression following acute (3 day) and prolonged (13 day) exposure to TMA using a multi-probe ribonuclease protection assay. Acute exposure to TMA resulted in the expression of mRNA for both Th1- and Th2-type cytokines, including IL-4, IL-10, IL-15 and IFN-γ. In each of 4 independent experiments performed, however, following more prolonged exposure a polarized phenotype was observed, with down-regulation of message for IL-15 and IFN-γ, and concomitant upregulation of mRNA for IL-4 and IL-10 and, to a lesser extent, IL-13. Interestingly, there was also a marked increase with time of mRNA for the Th2 cytokine IL-9, identified recently to be associated with asthma, bronchial hyperresponsiveness and elevated total serum IgE. These data show that as the immune response to TMA develops, the cytokine gene expression profile of allergen-activated LNC evolves from a Th0-type phenotype to a Th2 profile.

808 IMPACT OF DURATION OF EXPOSURE TO LOW MOLECULAR WEIGHT COMPOUNDS ON mRNA EXPRESSION AND PRODUCTION OF INTERFERON-γ AND INTERLEUKIN-4 USING THE LOCAL LYMPH NODE ASSAY.

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The local lymph node assay (LLNA) is used to identify chemical allergens by means of dermal exposure of mice. In addition, the test is used to classify the potency of the allergens. It comprises a sensitizing phase only. This assay may also be used to discriminate contact and respiratory allergens. We have previously shown that IL-4 production by stimulated LN cells was able to identify respiratory allergens. We have also found that next to IL-4 production, also IL-4 mRNA expression could be used to distinguish contact from respiratory allergens. We failed to discriminate contact from respiratory allergens on the basis of IFN-γ. We have studied whether prolonged exposure to the allergen would enhance the discriminatory capacity of the test. Using the prolonged protocol, dose response relationships were often less clear. In addition, mRNA expression and production of IFN-γ and IL-4 showed a similar result compared to the shorter exposure protocol. This suggests that using IFN-γ and IL-4 as parameters the prolonged protocol has no advantages over the shorter protocol. The LLNA, that includes the induction phase only, is thus preferred since it is shorter, and combines both potency assessment and identification of respiratory allergens.

809 EVALUATION OF THE UNDERLYING GENETIC MECHANISMS OF DERMAL SENSITIZATION USING THE LOCAL LYMPH NODE ASSAY AS A MODEL.

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The objective of this study was to use the local lymph node assay in conjunction with the high-density DNA array to identify the underlying genetic mechanisms of dermal sensitization. Initial studies examined the kinetics of gene expression changes following the administration of a known dermal sensitizer, 1-chloro-2,4-dinitrobenzene (DNCB). Female CBA/J mice were dosed with either 4:1 acetone:olive oil (AOO) vehicle-control or 0.25% DNCB for 3 consecutive days. Groups of vehicle control (n=15) and DNCB treated (n=15) mice were sacrificed and auricular lymph nodes were removed and pooled within each group 5 hours following the first (test day 1) and third dose (test day 3) of DNCB. An additional group of vehicle-control (n=15) and DNCB treated mice (n=10) were sacrificed on test day 6. Total RNA was purified, reverse transcribed, and radioactively labeled using [a-32P]dATP and gene-specific primers for probing a murine cDNA expression array (Atlas™ Mouse 1.2 Array; Clontech Labs, Inc.). Treatment-induced changes in gene expression levels were detected in approximately 0.7%, 2.5%, and 0.8% of the arrayed genes on test days 1, 3, and 6, respectively. Three genes were up regulated (>1.8-fold relative to control) at all time points examined. These included prothymosin alpha, thymosin beta-4, and hematopoetic-specific retinoic acid-inducible E3 protein. Seven genes were up regulated at 2 of the 3 time points. Of interest was an elevation in PAX5, GLYCAM1, vimentin, YB1 DNA binding protein, and GPI. Apolipoprotein E precursor was up regulated on test day 1 and down regulated on test day 6. Seventeen genes were up regulated exclusively on test day 3 and included GM-CSF-R, IL3-R, TGFB1, and CISH2, a suppressor of cytokines signaling protein 2. One gene, programmed cell death 1 protein precursor, was up regulated exclusively on test day 6. These data begin to demonstrate the differential and temporally dependent gene regulation that occurs following the topical application of a known dermal sensitizer.

810 ACTIVATION OF THE MITOGEN-ACTIVATED PROTEIN KINASE PATHWAY IN THE HUMAN KG-1 DENDRITIC CELL LINE BY XENOBIOTICS.

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Despite progress in elucidating the mechanisms of xenobiotics-induced hypersensitivity, the first steps of the sensitization phase including activation of dendritic cells (DC) by haptens is still poorly understood. To evaluate the mechanisms of DC stimulation by haptens, we worked on the activation of Mitogen-activated protein kinases (MAPKs) which are stimulated by several factors, including stress and cytokines. MAPKs are known to play a key role in the regulation of expression of target genes. We tested the effect of a sensitizing molecule, dinitrochlorobenzene (DNCB), on MAPKs activation using the human cell line KG1. This CD34+ cell line was recently described as an immature « DC-like » cell. We evaluate the activation state of the MAPKs by the phosphorylation of specific substrates (c-Jun, ATF-2) bound to Glutathion S-Transferase (GST) [c-Jun to evaluate the activity of the c-Jun N-terminal kinase (JNK), ATF-2 for the activity of the p38 MAPK and Elk-1 for the Extracellular signal-Regulated Kinase (ERK) activity]. Kinase activities of JNK and p38 MAPK were detected upon 30 min of stimulation of KG1 cells with DNCB at 25 µM and lasted for 3 hours. In contrast ERK which play a role in cell proliferation was not activated by DNCB in the same conditions. In addition, at equivalent concentrations, Sodium Lauryl Sulfate (SLS), a well-known irritant, activated also p38 MAPK into KG1 cells. These results showed the capacity of xenobiotics to activate the MAPK pathway, this activation could take part in the mechanisms of sensitization by chemicals.

811 LOCAL LYMPH NODE ASSAY AS A QUANTITATIVE RISK ASSESSMENT TOOL TO MODEL BIOLOGIC SKIN RESPONSE OF ALLERGIC CONTACT DERMATITIS IN THE MOUSE.

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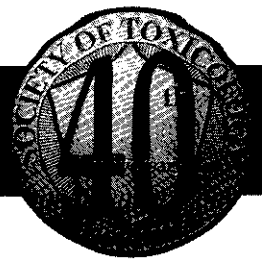
The murine local lymph node assay (LLNA) is a method used to screen allergic contact sensitizing chemicals and as such, has been suggested for use as a risk assessment tool to predict the sensitizing and irritant potential of chemicals. Its reproducibility (precision and accuracy) has been validated through an international inter-laboratory collaborative trial. Because the mathematically derived EC3 parameter predicts the chemical concentration at which skin sensitization occurs, and the LLNA appears to be dose responsive, we asked if the LLNA could be adapted for use as an occupational risk assessment tool. We explored in a mouse model, the relationship between sensitizing and challenge concentrations and the quantitative biological parameters of tritiated thymidine incorporation in auricular lymph node cells and of skin thickness after challenge. Our results indicate that: 1) LLNA data fit highly significant dose response models for the chemicals dinitrochlorobenzene (DNCB) and squaric acid dibutylester (SADBE) ($R2=0.884$ $p<0.0001$ and $R2=0.855$, $p<0.0001$ respectively). 2) We report a novel EC3 for SADBE equal to 0.1% in acetone olive oil. 3) Using two different skin sensitization-challenge models, we confirm that DNCB chemical sensitization is dose responsive. In contrast, our data suggest that the quantitative increase in skin thickness following chemical challenge is dependent on a threshold concentration and that the interaction of sensitization and challenge concentration is highly significant ($p<0.0001$). 4) Finally, we show an empirical association of sensitization dose response in the LLNA with the post-challenge skin thickness. On the basis of these studies, we propose that the LLNA may be adapted in future studies for use in quantitative assessment of occupational exposure to sensitizing chemicals.



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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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