hCD16A+, hCD16B+ mouse was used for preclinical studies due to appropriate receptor expression and distribution. Following repeated doses of GMA161, or its murine surrogate aglycosyl 3G8, an acute and severe hypersensitivity like reaction was observed. The first study conducted to evaluate the mechanism of action behind the hypersensitivity like reaction involved repeat dosing of aglycosyl-3G8 to double and both single Tg mice. Double Tg mice exhibited a severe reaction following repeat dosing, whereas single Tg mice displayed minor reactions, supporting that both transgenes are required for a severe hypersensitivity like reaction. Anti-GMA161 antibody titers were significantly higher in the double Tg mice compared to the single Tg mice, suggesting the severity of the reaction may be associated with the antibody response. A second study further investigated the possible mechanism of action by blocking two of the known pathways mediating murine systemic anaphylaxis: IgE – characterized by histamine release, and IgG – characterized by platelet activating factor (PAF) release. Double Tg mice were pretreated with an antihistamine [diphenhydramine (DPH)], a PAF antagonist (CV-6209), or both DPH and CV-6209 prior to repeat dosing with GMA161. Double Tg mice were tolerant of repeat dosing of GMA161 when pretreated with CV-6209 with no adverse clinical signs observed. DPH administration did not reduce the hypersensitivity like reaction, suggesting that the response to GMA161 is not histamine mediated but may be PAF mediated. Together these studies indicate that the hypersensitivity-like reaction following repeat dosing of GMA161 or aglycosyl 3G8 correlates with anti-GMA161 antibody titers, and may be alleviated by inhibiting IgG mediated hypersensitivity mechanisms in Tg mice.

PS 1505 LYMPHOCYTE GENE EXPRESSION CHARACTERISTIC OF IMMEDIATE AIRWAY RESPONSES (IAR) AND METHACHOLINE (MCH) HYPERRESPONSIVENESS IN MICE SENSITIZED AND CHALLENGED WITH ISOCYANATES.

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Exposure to isocyanates has been associated with occupational airway diseases, including asthma. Previously we reported on respiratory and immune responses following dermal sensitization and intranasal challenge of BALB/c mice with 6 different isocyanates. The purpose of this study was to determine whether differences in gene expression in the draining lymph node could be related to phenotypic differences that were observed in responses to the different isocyanates. RNA was extracted from lymph nodes of mice exposed to 2% dicyclohexylmethane-4,4'diisocyanate (HMDI), 1% toluene diisocyanate (TDI), and 1% meta-tetramethylene xylene isocyanate (TMXDI) following the intranasal challenge. IAR were observed in mice treated with TDI and HMDI, but not TMXDI. Increased MCH hyperresponsiveness was observed in mice treated with HMDI and TMXDI, but not TDI. Affymetrix chips were used to analyze treatment-associated changes in gene expression RNA. A statistical filter was used to assess difference from vehicle control ($p<0.05$) corrected for multiple observations. A Venn diagram was generated showing changes in expression of 2475 genes in common with HMDI and TDI, hence possibly associated with the IAR, and in expression of 88 genes in common with HMDI and TMXDI, hence possibly associated with the MCH response. Amongst the 2475 genes pathway analysis revealed a number of genes associated with the T-cell receptor and T-cell activation. In contrast, amongst the 88 genes, pathway analysis identified genes associated with cell cycle and tight junctions, but T-cell activation genes were notably absent. These genes (and/or pathways) could potentially be used to predict airway responses to other isocyanates and possibly other low molecular weight chemicals. This abstract does not reflect EPA policy.

PS 1506 A MODIFIED LOCAL LYMPH NODE ASSAY (LLNA) DOSING THE EAR CANAL FOR THE CHARACTERIZATION OF INDUSTRIAL ENZYMES.

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Industrial enzymes are proteinacous water soluble test materials, known to be potential respiratory allergens in case of occupational exposure to dust or aerosols. There is a high need for a test model to be able to discriminate such sensitizers from contact sensitizers. We have assessed the potential of a modified LLNA for this purpose. The effects of dosing selected test materials using an aqueous vehicle into the ear canal have been investigated by analyzing the proliferative response of the draining lymph nodes. The OECD protocol 429 was used; however, the difficulties associated with the skin application of aqueous solutions in the traditional model were overcome by using 1% Pluronic in PBS as vehicle and by the dosing into the ear canal instead of on the ear dorsum. The following test materials were tested: The skin sensitizer hexyl cinnamic aldehyde, normally used as positive control substance, the chemical respiratory sensitizer ammonium hexa chloro-platinate, a standard protein ovalbumin, tuberculin purified protein derivative and two industrial enzymes, a protease and an amylase. Proliferative responses in terms of stimulation indexes were established both *in vivo* and *in vitro*, 5 hours after pulse with H3-thymidin, followed by a cytokine profiling with and without re-stimulation.
The aqueous vehicle worked very well with all test substances and the various allergens showed a clear increase in the total amount of proliferating cells in comparison to the solvent control-treated mice. The *in vitro* and *in vivo* stimulation indexes showed a strong correlation, indicating that refining the model by using the *in vitro* pulsing alone is feasible. In addition, the cytokine phenotyping of the proliferating cell populations provided information with regard to discrimination between a Th1- and a Th2-type response. The present LLNA approach offers an easy to perform, significantly refined method for the assessment of sensitizers and the influence on the proliferation and production of cytokines *in vitro.*

PS 1507 TOLUENE DIISOCYANATE (TDI)-SPECIFIC MONOCLONAL ANTIBODIES: PRODUCTION AND EPITOPE MAPPING.

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Toluene diisocyanate (TDI) is a commonly used diisocyanate (dNCO). TDI is a reactive low molecular weight chemical that is widely used in industry, especially in the manufacture of polyurethane foams and adhesives. Diisocyanate exposure is one of the most commonly reported causes of occupational asthma. The production of well characterized TDI-specific monoclonal antibodies (mAbs) will allow for the development of standardized immunoassays for exposure and biomarker assessment. Such mAbs may also be useful to isolate and study dNCO protein targets. BALB/c mice were immunized with 2,4- or 2,6-TDI conjugated keyhole limpet hemocyanin (KLH), spleen cells isolated and hybridomas made. Resultant mAbs produced by these hybridomas were screened for their ability to bind TDI conjugated human serum albumin (HSA) by ELISA. MAbs were further characterized by ELISA and western blot against various monoisocyanate (mNCO) and dNCO and diisothiocyanate conjugated proteins to identify reactivity toward urea, amide or thiourea linkages. A total of 35 mAbs were produced (25 IgG1, 8 IgG2a, 2 IgG2b) were obtained against 2,4-TDI-KLH sensitized mice. Seven mAbs were found to recognize 2,4-TDI-HSA and 28 mAbs reacted with both 2,4- and 2,6-TDI-HSA. mAbs specific for only 2,4-TDI-conjugated protein did not recognize 2,6-TDI-HSA, mNCO-HSA or other dNCO–HSAs. Other cross-reactive mAbs reacted with 2,4-TDI-HSA, 2,6-TDI-HSA, and other dNCO protein conjugates like methylene diphenyldiisocyanate- and hexamethelene diisocyanate-HSA, but not mNCO-HSA. All mAb reactivities were carrier protein independent. The mAbs produced can differentiate between specific dNCO in a carrier independent fashion and may be useful as hapten-specific reagents for immunoassay development and research into dNCO related diseases. Funded in part by NIEHS-NIOSH IAG#Y1-ES-0001

PS 1508 IDENTIFYING THE LOW DOSE CHEMICAL-INDUCED RESPIRATORY ALLERGIC RESPONSE IN MICE.

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The inhalation of many types of chemicals, including pesticides, perfumes, and other low-molecular-weight chemicals, is a leading cause of allergic respiratory diseases. We attempted to develop a new test protocol to detect environmental chemical–related respiratory hypersensitivity at low and weakly immunogenic doses. We used long-term dermal sensitization followed by a low-dose intratracheal challenge to evaluate sensitization by the well-known respiratory sensitizers trimellitic anhydride (TMA) and toluene diisocyanate (TDI) and the contact sensitizer 2,4-dinitrochlorobenzene (DNCB). Female Balb/c mice aged 8 week were divided into five subgroups for each chemical: Subgroup A-C (sensitized and challenged with solvent or test solution); Subgroup D1-D2 (sensitized with test solution and challenged with low or high dose of test solution). On days 1 to 3, 8 to 10, and 15 to 17, a 25-μL aliquot of test solution was applied to the dorsum of each ear for dermal sensitization. Two weeks after the last sensitization, mice were intratracheally challenged with a 50-μL aliquot of test solution. After topically sensitizing and challenging them intratracheally, we assayed differential cell counts and chemokine levels in bronchoalveolar lavage fluid (BALF); lymphocyte counts, surface antigen expression of B cells, and local cytokine production in lung-associated lymph nodes (LNs); and antigen-specific IgE levels in serum and BALF. TMA induced marked increases in antigen-specific IgE levels in both serum and BALF, proliferation of eosinophils and chemokines (MCP-1, eotaxin, and MIP-1β) in BALF, and prolifer-

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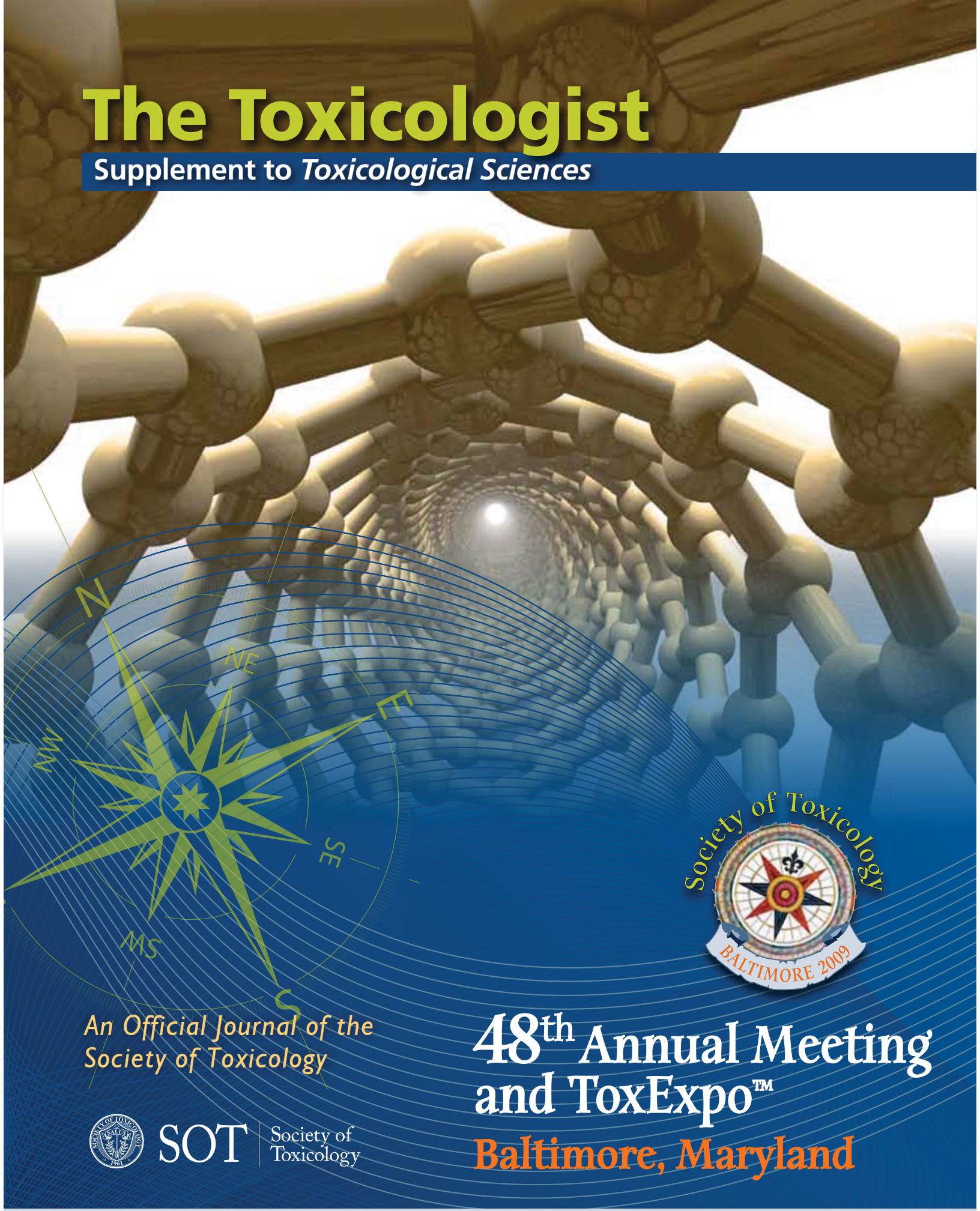

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