zene (DNCB, a known sensitizer), Vitamin E or vehicle were applied to the dorsum of both ears of mice for three consecutive days. Subsets of each treatment group were co-treated without or with the glutathione synthesis inhibitor, buthionine sulfoximine (BSO), dosed in the drinking water. Prior to sacrifice the mice were injected with bromodeoxyuridine (BrdU, i.p.) and the number of proliferating (BrdU-positive) cells of the auricular lymph nodes was determined using flow cytometry. Both DNCB and Vitamin E were found to induce lymphocyte proliferation (> 3 fold over vehicle control) and thus were positive as sensitizers in this modified LLNA. These agents were also found to increase cellular levels of reduced glutathione in the epidermal keratinocytes of mouse skin. BSO was found to dramatically inhibit agent-induced increases in cellular glutathione as determined using flow cytometry and the (fluorescent) glutathione-specific dye, monochlorobimane. Interestingly, the ability of these sensitizers to induce proliferation of lymphocytes was dramatically inhibited by treatment with BSO, and both DNCB and Vitamin E failed to function as sensitizers in BSO-treated mice. Results of these studies indicate that cellular levels of reduced glutathione may play a critical role during the induction phase of contact sensitization.

573 UTILIZATION OF SAR AND THE MURINE LOCAL LYMPH NODE ASSAY (LLNA) TO ASSESS THE SENSITIZATION POTENTIAL OF HAIR COLORANTS.

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Both oxidative (permanent) and direct (semi-permanent) dyes have an acknowledged potential to cause contact sensitization, however, this is regarded as a manageable health issue for the general population. The use of computational toxicology and the local lymph node assay (LLNA) allows us to evaluate the sensitization potential of new dye candidates "upstream" of the more traditional guinea pig assays (i.e., Buehler or GPMT methods). A number of aromatic/nitro-aromatic amines used commercially as oxidative or direct hair dyes were evaluated for allergic contact sensitization potential using the LLNA. Test materials included 10 direct and 2 oxidative dyes. The application vehicle for all materials was dimethyl sulphoxide (DMSO). Relative potency was compared to para-phenylenediamine, a more relevant although less potent allergen as compared to other OECD recommended positive controls, such as 2,4-dinitrochlorobenzene (DNCB), oxazolone, or hexyl-cinnamic aldehyde (HCA). Using a Computer Automated Structure Evaluation (CASE/MultiCASE) customized database constructed of aromatic and nitro-aromatic amines, SAR predictions and LLNA data were compared with historical data obtained with traditional guinea pig methods. In addition, the data generated from the LLNA was also used to derive a quantitative SAR model for predicting sensitization. When comparing actual LLNA and guinea pig data, an overall concordance of 77% (10/13) was achieved. Sensitivity and specificity were 92% (12/13) and 85% (11/13) respectively. These results indicate the LLNA may be successfully used to evaluate the contact sensitization potential of new and existing hair colorants.

574 A TWO-TIERED MURINE LOCAL LYMPH NODE ASSAY TO IDENTIFY CONTACT (PHOTO)ALLERGENIC POTENTIAL.

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Sponsor: A. Wolf.

To improve the predictive differentiation of contact allergy (CA) and photoallergy (CPA) from irritancy (IR) and photoirritancy (PI), induced by low molecular weight compounds, we developed a non-radioactive, two-tiered protocol of the murine local lymph node assay (LLNA). Tier I testing covering a broad concentration range of the test chemical consists of two endpoints: primary ear skin irritation and lymph node (LN) hyperplasia. The ratio of the relative changes in the ear-draining LN and in the ear skin provides information about the preferential mode of action the tested chemical. A ratio fare above 1, obtained with a certain concentration of the test chemical, indicate a (photo)allergic reaction, whereas a ratio below 1 points to (photo)irritation. Values around 1 indicate that the test chemical is likely to possess both considerable irritative and allergenic potential and, thus has to be evaluated in a tier II test schedule. For tier II testing mice were sensitised with selected tier I concentrations of chemicals or treated with vehicle (40% Dimethylacetamid, 30% Acetone, 30% Ethanol) on the shaved back on 3 consecutive days with subsequent UVA exposure in case of CPA and PI studies. Challenge was performed 12 days later on the ears on 3 consecutive days in order to reveal a possible allergy-related increase in ear swelling and ear weight as well as to investigate the expression of relevant cytokines at two stages of the evolution of an allergic response, the induction and the challenge phase. Tested chemicals were oxazolone, dinitrochlorobenzene, dinitrofluorobenzene, eugenol, isoeugenol as CA, tetrachlorosalicylanilide, chlorpromazine as CPA, croton oil as IR and 8-methoxypsoralen as well as anthracene as PI. Exclusively in case of CA and CPA, allergy-related ear swelling and ear weight increase as well as a marked increase in IL-4 and IL-10 expression by ex vivo restimulated LN CD4 T cells was observed in the challenge groups. These results were in line with the finding of high ratios between LN hyperplasia and primary skin irritation. Ratios around 1, which do not precisely indicate the mode of action of a chemical - CA/CPA or IR/PI, can occurr at high concentrations of chemicals with CA/CPA potential, but also with compounds like croton oil consisting of different ingredients. One of these ingredients of croton oil are phorbol esters affecting lymphocytes in a non-specific manner, and thus, causing LN hyperplasia. Especially in the latter case tier II testing provided the necessary information to predict or deny a CA or CPA potential.

575 ASSESSMENT OF THE SUBCUTANEOUS ROUTE OF TEST ARTICLE ADMINISTRATION USING THE MOUSE LOCAL LYMPH NODE ASSAY.

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The mouse local lymph node assay (LLNA) measures lymphocyte proliferation in draining lymph nodes of mice after topical application of a test material to the intact, non-occluded skin of both ears. There is a need to develop methods to evaluate the sensitization potential of chemicals and/or materials that are implanted or injected into the body (e.g. drugs, implanted medical devices, biologics), for which topical application in not a relevant route. In addition, it is important to distinguish between IgE mediated and cell mediated sensitizers. To evaluate the sensitization potential of implanted/injected materials, the LLNA was conducted using the subcutaneous route rather than the topical route. Two irritants (sodium lauryl sulfate (SLS), nonanoic acid), a T-cell mediated sensitizer (oxazolone), and a protein that is foreign to the mouse (human albumin) were evaluated using the subcutaneous route of administration. An IgE-mediated sensitizer (toluene diisocyanate; TDI) and a T-cell mediated sensitizer (DNFB) were used as positive controls. The irritant nonanoic acid did not induce lymphocyte proliferation compared to the vehicle control group. However, as seen with topical administration, SLS induced low level, significant lymph node cell proliferation at the two highest concentrations. Oxazolone and human albumin caused a significant concentration-dependent increase in lymphocyte proliferation versus the vehicle control. Both DNFB and TDI were associated with significant lymphocyte proliferation compared to the vehicle control. Phenotypic analysis conducted for groups of mice treated subcutaneously with vehicle (polyvinyl pyrrolidone; PVP), nonanoic acid, oxazolone, DNFB and TDI revealed a significant increases in the percent B220+ lymphocytes for the oxazolone, DNFB and TDI groups, whereas the nonanoic acid group was similar to the control. The percent IgE+B220+ lymphocytes increased from least to greatest in the oxazolone, DNFB and TDI groups, respectively whereas the nonanoic acid group was similar to the vehicle control group. TDI was associated with a 2-8 fold increase in the percent IgE+B220+ lymphocytes as compared to oxazolone or DNFB.

576 ASSESSMENT OF A MODIFIED LOCAL LYMPH NODE ASSAY TO EVALUATE THE IRRITANCY/SENSITIZATION POTENTIAL OF CHEMICALS EXPOSED TO BREACHED SKIN.

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There is a need to develop methods to evaluate the sensitization potential of chemicals and/or materials that contact breached skin (e.g. polymeric tissue adhesives, wound dressings, occupational exposure to chemicals through wounded or irritated skin). For this purpose a modified local lymph node assay (LLNA) was conducted using standardized methods for breaching the skin at the dose application site. Female BALB/C mice were exposed for three consecutive days on the ear to vehicle (.9% sterile saline) or a foreign protein (25% human albumin) via the topical route, breached skin route utilizing either a Simplate (Organon Teknika, Corp., Durham, NC) or a Multi-Test (Lincoln Diagnostics, Inc., Decatur, IL) device, or injected in the subcutaneous tissue between the ears. In order to investigate the potential for enhanced sensitivity of the assay by occlusion, for each test condition

(excluding the subcutaneous group) the exposure site was occluded using Blenderm (3M Corp., St. Paul, MN) tape in one group of animals and left unoccluded in a second group. Two days following the final exposure animals were injected intravenously with 3H thymidine, sacrificed five hours later and the draining lymph nodes excised and processed as for a standard LLNA. When unoccluded, no difference in proliferation occurred following topical application to intact skin. However, an increased proliferative response (>5 fold) was seen following both methods of breached skin with no significant differences between methods. With occlusion, exposure by all routes induced significant proliferation, however breaching with the Multi-Test device induced significantly higher (>3 fold over topical and >2 fold over Simplate) proliferation than by the other routes and induced proliferation similar to that observed following subcutaneous administration. Results indicate that the Multi-Test may provide a useful method for breaching the skin in testing compounds where exposure is anticipated to occur to compromised skin.

577 DERMAL SENSITIZATION EVALUATION OF MALEIC ANHYDRIDE AND SUBSEQUENT REACTION MATERIALS USED IN THE DEVELOPMENT OF A NOVEL SYNTHETIC FIBER.

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Maleic anhydride is a highly reactive chemical intermediate that has widespread use in the manufacture of polyester resins in the textile industry. The irritation properties of this material are well known, however the dermal sensitization potential has not been well documented. As part of a worker safety monitoring program, the dermal sensitization potential of maleic anhydride and that of several downstream synthesis materials used in the manufacture of a novel high nitrile content fiber were evaluated in a series of guinea pig dermal sensitization studies utilizing a standard closed-patch test method. Rechallenge techniques were used to selectively determine cross-reactivity between materials. The starting reaction material, maleic anhydride, was shown to be a dermal sensitizer. Reaction products of maleic anhydride and alcohols used as a stabilizer in the polymer formulation were also determined to be dermal sensitizers. The latex precursor of the novel high nitrile content polymer (before final stripping and addition of the maleate stabilizing agent) was also determined to be a weak dermal sensitizer. The polymer products (raw polymer with stabilizer prior to pellet formation, in pellet form, and in spun fiber form) were all shown to be non-sensitizers. Rechallenge applications determined that 1) animals previously sensitized to maleic anhydride were not cross-reactive to the maleate polymer stabilizer; 2) animals previously sensitized to the maleate polymer stabilizer were not cross-reactive to the final polymer; and 3) animals sensitized to the high nitrile polymer prior to addition of the maleate stabilizer were not cross-reactive to the raw polymer (with stabilizer prior to pelleting). Animals that had been previously sensitized to the stabilizer had equivocal responses when rechallenged with the raw polymer (with stabilizer prior to pelleting) indicating potential cross-reactivity. We conclude that maleic anhydride, the reaction product of maleic anhydride and alcohols and the raw nitrile polymer latex are dermal sensitizers, but no evidence of sensitization potential by the finished polymer was detected in these assays.

578 TOPICAL EXPOSURE OF MICE TO RESPIRATORY SENSITIZING ACID ANHYDRIDES STIMULATES THE EXPRESSION OF INTERLEUKIN 5.

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We have shown previously that prolonged topical exposure of BALB/c strain mice to chemical contact or respiratory allergens such as 2,4-dinitrochlorobenzene (DNCB) or trimellitic anhydride (TMA) elicits cytokine secretion patterns consistent with the selective activation of type 1 and type 2 T lymphocytes, respectively. Certain respiratory sensitizing acid anhydrides including phthalic anhydride (PA) and maleic anhydride (MA) stimulate comparable levels of the type 2 cytokine interleukin (IL)-10 to those provoked by TMA, but induce somewhat lower levels of the type 2 product IL-4. We have therefore examined the production by allergen-activated lymph node cells (LNC) of IL-5, a type 2 cytokine which plays an important role in the differentiation and recruitment of eosinophils. Exposure to TMA, PA or MA resulted in each case in the expression by LNC of relatively high levels of IL-5, with peak production ranging from 50 to 200pg/ml. In contrast, LNC derived from DNCB-treated animals failed to elaborate detectable amounts of this cytokine (<20pg/ml) under conditions where vigorous secretion of the type 1 cytokine interferon γ was observed. Furthermore, negative selection (by complement depletion) of $CD4^-$ or $CD8^+$ lymphocytes revealed that $CD4^-$ T helper (Th) 2 type cells were the exclusive source of IL-5, in addition to the other type 2 cytokines. These data demonstrate that exposure to respiratory sensitizing acid anhydrides activates predominantly Th2 type cells, resulting in the expression of IL-5, which presumably plays a central role in the lung cosinophilia which is a key feature of chemical respiratory allergy.

579 CYTOKINES FROM BRONCHOALVEOLAR LAVAGES AND THEIR *IN VITRO* PRODUCTIONS IN ASTHMATIC RATS INDUCED BY TOLUENE DIISOCYANATE.

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To investigate the status and the role of cytokines in occupational asthma induced by toluene diisocyanate (TDI), we carried out a quantitative determination of cytokine profiles from bronchalveolar lavages (BAL) and their *in vitro* productions, as well as from splenocytes and sera in an asthmatic murine model induced by TDI. Male rats were sensitized with two courses of intranasal application of 5% TDI in ethyl acetate for seven consecutive days each time separated by a one week rest. The control group of rats were similarly treated with vehicle. A week after second sensitization, both groups of rats were provoked by applying 2.5% of TDI in vehicle. BAL fluids and sera from all rats were collected, and BAL-adherent cells and splenocytes were cultured for determination the levels of IL-2, IL-4, IL-5 and IFN-r. After lavation, the lungs were intratracheally fixed and removed for histologic examination. Differential cell counts were performed on BAL fluids and peripheral bloods. The results showed that the TDI-sensitized group of rats exhibited the asthmatic symptoms. TDI exposure resulted in elevated IL-4, IL-6 in BAL fluids, IL-4, IL-6 and IFN-r in BAL-adherent cells and splenocytes, and IL-6 in sera. A marked infiltration of the peribronchial regions with eosinophils was observed in lung sections from TDI-exposed rats. The cell counts revealed that the increases of the numbers of neutrophils and cosinophils were found in BAL fluids and peripheral bloods from asthmatic rats. These findings indicate that IL-4, IL-6 and IFN-r may play an important role in the pathogenesis of TDI-induced occupational allergic asthma.

580 CYTOKINE REGULATION OF T CELL DIFFERENTIATION AND ANTIBODY ISOTYPE PRODUCTION IN A MOUSE MODEL OF OVALBUMIN SENSITIZATION.

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A mouse model featuring key components of human allergic asthma, including eosinophil recruitment into airwalls and Th2 pathway predominant antibody production has been validated. Intraperitoneal sensitization to ovalbumin followed by inhalation challenge resulted in eosinophil infiltration into peribronchial regions of the lung at 48h post-challenge. Levels of Th2 pathway antibodies (IgE, IgG1 and anti-ovalbumin antibodies AntiOVS IgE and AntiOVS IgG1) increased following sensitization, or sensitization and challenge, while Th1 pathway antibodies (IgG2a/b and anti-ovalbumin antibody AntiOVA IgG2a/b) were unchanged. This pattern of cellular and humoral change is characteristic of Th2 cell predominance leading to release of cytokines which are chemoattractant to eosinophils and stimulate B-cell switching to IgE and IgG1 isotype production.Preventive treatment of naïve mice with IFNγ, Anti IL 4 (low and high doses), Anti IL 5 (low and high doses), or IL 12 (low and high doses) was performed both before the sensitization injections and before the ovalbumin aerosol challenge, to examine their preventive effect on Th2-cell pathway commitment caused by sensitization. Anti IL 5 significantly decreased peribronchial eosinophil infiltration at 12 and 48h post aerosol challenge, however it had no effect on altering antibody production even when given before sensitization. Both IFNγ and Anti IL 4 pretreatment had little effect on preventing inflammatory eosinophil infiltration following aerosol challenge, but caused a dose-related switching of T-cell differentiation away from Th2 predominant B-cell AntiOVA IgE production to Th1 predominant B-cell AntiOVA IgG2a/b production. IL 12 treatment before sensitization caused both a consistent trend to decreased eosinophil infiltration and a dose-related B-cell isotype switching from AntiOVA IgE to AntiOVA IgG2a/b.In conclusion, it appears that IFNγ, Anti IL 4 and IL 12, when given preventively before antigen exposure, can regulate the direction of T-cell differentiation and B-cell antibody isotype production from Th2 predominance to Th1 predominance. IL 12 appears to have additional benefit in

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