1178 RESTRAINT STRESS MODULATION OF EAR SWELLING IN ACD IS INDEPENDENT OF SENSITIZING DOSE OF CHEMICAL.

M. S. Flint, R. R. Salmen, K. Brumbaugh and S. S. Tinkle. *TMBB, NIOSH, Morgantown, WV.*

Stress plays an important role in numerous chronic health problems including modulation of immunologic diseases, however, the interaction between the stress response and the cutaneous immune response are incompletely understood. We have previously shown that acute stress suppresses the immune response in allergic contact dermatitis (ACD), and additional studies have shown a significant interaction between the concentration of chemical required for sensitization and for challenge in the development of ACD. We hypothesized that 2 hour restraint stress would change the sensitizing concentration of chemical required to elicit a full ear swelling response to chemical challenge. Male BALB/c mice were exposed on the back with increasing doses (0.05%-0.5%) of 2, 4 dinitrofluorobenzene (DNFB) on days 1 & 2 and challenged onto the ear with 0.1% DNFB on day 6. Mice were restrained for 2 hours prior to chemical application on day 1. We assessed ear swelling 24, 48 and 72 h post-challenge. Quantitative analysis of the data indicated that the ear swelling response to chemical challenge is dependent upon the sensitization concentration of DNFB and is dose-responsive. The curve shows a quadratic trend with 0.1% as a possible maximum dose for sensitization. Application of restraint stress did not alter the shape of this curve but significantly suppressed ear swelling at all concentrations of DNFB. These data suggest that restraint modulation of ACD is independent of the sensitizing dose of chemical.

1179 EXPRESSION OF TH2 CYTOKINES IS A GENERAL FEATURE OF MURINE CONTACT ALLERGY.

J. Streich, N. Runser, W. Suter and P. Ulrich. *Genetic and Experimental Toxicology, Novartis Pharma AG, Basel, Switzerland.* Sponsor: A. Wolf.

The cytokine response pattern following sensitisation (induction) and elicitation (challenge) of contact allergy in BALB/c mice with different chemicals (dinitrochlorobenzene, dinitrofluorobenzene, oxazolone, glutaraldehyde, formaldehyde, trimellitic anhydride, croton oil) was investigated. The results of our investigations showed that contact allergens induced both T helper (Th) 1 cytokines interleukin (IL) 2, interferon gamma (IFNg) and Th2 cytokines (IL-4, IL-10) at different stages during murine contact allergy. We also confirmed our previous findings that IL-4 and IL-10 release were up-regulated during the challenge phase regardless the contact allergen used, whereas the release of IFNg did not show a clear preference for being up- or down-regulated. In our hands, the increased expression of Th2 cytokines after challenge exposure to contact allergens appeared as a stable marker of secondary contact allergenic responses. Quantitative differences in the expression of IL-4 were observed between different contact allergens. The present results clearly indicate that skin sensitisers were able to elicit cytokine response patterns, which could not be related to a clear-cut Th1 or Th2 type of cytokine response. Furthermore, topical application of contact allergens produced different kinetics of cytokine secretion upon induction and challenge. In our hands, the co-expression of Th1 and Th2 type cytokines appeared as a universal consequence of dermal application of contact allergens to responsive mice. Our results also indicate that chemicals differ in their potency to induce the expression of these cytokines. Furthermore, the results do not support the view that different chemicals induce Th1 or Th2 cytokines in a mutually exclusive manner depending on their preference to induce either contact or respiratory allergy. The results are expected to renew the discussion about the usefulness of the Th1/Th2 paradigm in certain areas of immunotoxicology.

1180 THE USE OF CD54 AND CD86 EXPRESSION ON THE HUMAN THP-1 AND KG-1 CELL LINES TO PREDICT CONTACT SENSITIZERS.

Y. Yoshida, Y. Ito, H. Sakaguchi, M. Okuda and H. Suzuki. *Kao Corporation, Tochigi, Japan.* Sponsor: J. Avalos.

In regard to *in vitro* skin sensitization tests, dendritic cells (DCs) derived from human peripheral blood have been considered in the development of new tests. However, there are some problems with the use of human DCs in skin sensitization tests. These include availability of human blood, donor to donor variability and other problems. In the present study, we evaluated the use of two human cell lines, THP-1 (monocytic leukemia cell line) and KG-1 (acute myelogenous leukemia cell line) in developing a method to identify contact sensitizers. Naive cells were used in our studies after comparing the usefulness of cytokine-derived cells and naive cells. The expression of CD54 and CD86 on THP-1 and KG-1 cells was measured using flow cytometry after a 24-hour exposure to known allergens (e.g., DNCB, 2-MBT, $NiSO_4$) and non-allergens (e.g., SLS, methyl salicylate, DMSO). We found that only the allergens enhanced the expression of CD54 and/or CD86 significantly in a dose dependent manner. In particular, metal salts, such as $NiSO_4$ and $CoSO_4$, increased only the CD54 expression. Additionally, we examined the signaling pathways of DNCB in order to study the intracellular mechanisms involved in the activation of THP-1 by DNCB. Results obtained using Western blotting showed that the phosphorylation of p38 MAPK was increased after 30-minute exposure to DNCB. Furthermore, SB203580, a specific inhibitor of the p38 MAPK, inhibited the up-regulation of CD54 and CD86 upon stimulation with DNCB. These results suggested that the up-regulation of CD54 and CD86 was coupled to the phosphorylation of p38 MAPK. This method was further evaluated using more than 20 of our materials. The expression of CD54 and CD86 on THP-1 and KG-1 cells used for predicting the sensitization potential of these materials was compared to guinea pig test results. A higher accuracy was observed with THP-1 (>70%) than with KG-1 cells. These results suggest that *in vitro* sensitization tests using these cell lines, especially THP-1 cells are useful to predict various contact sensitizers.

1181 INTEGRATION OF THE PASSIVE CUTANEOUS ANAPHYLAXIS (PCA) ASSAY INTO STUDIES TO IDENTIFY POTENTIAL CHEMICAL ALLERGENS.

J. A. Hotchkiss, M. R. Woolhiser and M. P. Holsapple. *Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI.*

Establishing a predictive test to identify chemicals that may induce respiratory sensitization is a pressing need in safety assessment. Concerns over the introduction of novel protein allergens has resurrected an interest in the PCA assay to measure antigen-specific IgE. This study compared the ability of the PCA assay to identify known protein (ovalbumin; OVA) and chemical (trimellitic anhydride; TMA) respiratory sensitizers. Balb/C mice were systemically sensitized to OVA *via* two i.p. injections seven days apart with 5% OVA in PBS (or with PBS alone; control). Test serum was collected 21 and 28 days following initial exposure. Serial dilutions of test sera were injected i.d. into the ears of Balb/c mice or the shaved backs of Sprague-Dawley (SD) rats. OVA and Evan's blue dye were injected i.v. 48 h later. The lowest dilution producing a positive reaction (≥ 2 x 2 mm spot) was recorded. Both heterologous and homologous PCA assays indicated that Day 28 OVA serum had the highest titer. For the chemical allergens, A/J mice were treated topically with 25% TMA, 1% dinitrochlorobenzene (DNCB; dermal sensitizer) or acetone:olive oil (AOO; vehicle) on their shaved backs. Seven days later, 12.5% TMA, 0.5% DNCB, or AOO was topically applied to the ears. Serial dilutions of test sera, collected 7 days later, were injected into the ears of A/J mice or the shaved backs of SD rats. Four hours later, 12.5% TMA or 0.5% DNCB was topically applied at the injection sites and 0.5% Evan's Blue dye was injected i.v. Only mice injected with TMA sera and challenged with TMA displayed a positive PCA reaction. ELISA confirmed the presence of a modest increase of total IgE in the DNCB serum, a marked increase of total IgE in TMA serum and no elevation of IgE in AOO serum. The timing of serum injection, test chemical application, and assay endpoint must be optimized. These data suggest that the PCA assay may be used to identify elevations in specific IgE induced by chemicals which may induce respiratory sensitization.

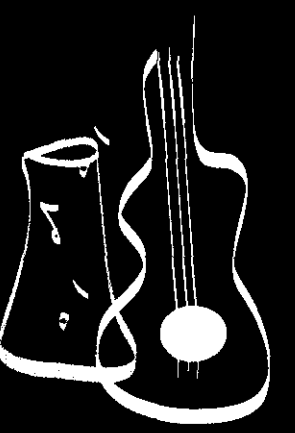
1182 CYTOKINE PROFILING FOR CHEMICAL SENSITIZERS: EFFECT OF DOSE.

R. J. Smialowicz[1], L. M. Plitnick[2], S. E. Loveless[3], G. S. Ladics[3], M. P. Holsapple[4], D. M. Sailstad[1] and M. J. Selgrade[1]. [1]*NHEERL, USEPA, Research Triangle Park, NC,* [2]*Curr. in Toxicology, UNC, Chapel Hill, NC,* [3]*Haskell Laboratory, DuPont Co., Newark, DE and* [4]*Dow Chemical, Midland, MI.*

Chemicals encountered in both domestic and occupational settings may result in airway hypersensitivity (AHS). Because cytokine profiles generated during a T cell response are indicative of AHS (Th2) or contact hypersensitivity (CHS) (Th1), cytokine expression in draining lymph nodes (LN) has been used to identify chemicals with AHS potential. In this study, female BALB/c mice were sensitized twice on the flanks and challenged 3 times on the ears using the airway sensitizer trimellitic anhydride (TMA), or the contact sensitizer dinitrochlorobenzene (DNCB). Doses were varied during either: sensitization, challenge or both simultaneously. At various times following challenge, total mRNA was isolated from draining LN and analyzed by ribonuclease protection assay (RPA). When dose varied during sensitization only, TMA induced higher levels of the Th2 cytokines IL4, IL10 and IL13 than DNCB. However, no dose-dependent responses were observed with either chemical. DNCB did not induce Th1 cytokines at any dose tested. Variation of TMA dose during both sensitization and challenge also induced IL4, IL10 and IL13, and again showed no dose dependency. Dose only appeared to be a factor when TMA concentration was varied during challenge alone, suggesting the magnitude of the response depends upon the challenge phase. Thus, dose appears not to affect quantitative differences in Th2 responses between TMA and DNCB. These results suggest conventional cytokine profiling studies in which the same dose is

Society of Toxicology

41st Annual Meeting & ToxExpo™

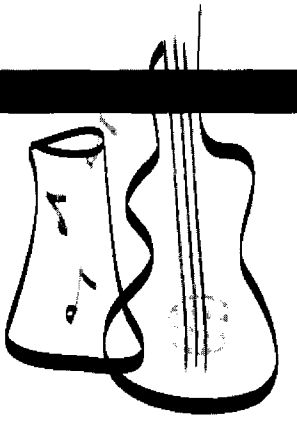


An Official Journal of the Society of Toxicology
Supplement

TOXICOLOGICAL SCIENCES

Society of Toxicology

41st Annual Meeting & ToxExpo™



An Official Journal of the Society of Toxicology
Supplement

TOXICOLOGICAL SCIENCES
Formerly Fundamental and Applied Toxicology

Abstracts of the 41st Annual Meeting

The Toxicologist

Oxford University Press

Volume 66, Number 1-S, March 2002

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 41st Annual Meeting of the Society of Toxicology, held at the Opryland Hotel and Convention Center, Nashville, Tennessee, March 17–21, 2002.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 385.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 411.

The abstracts are reproduced as accepted by the Program Committee of the Society of Toxicology and appear in numerical sequence.

Additional Late-Breaking Abstracts are issued in a supplement to this publication and are available at the 41st Annual Meeting and through the Society of Toxicology Headquarters office.

Copies of *The Toxicologist* are available at $45 each plus $5 postage and handling (U.S. funds) from:

Society of Toxicology
1767 Business Center Drive, Suite 302
Reston, VA 20190-5332

http://www.toxicology.org