949 POPLITEAL LYMPH NODE ASSAY AND RECENT MECHANISTIC STUDIES.

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Generally applicable animal models to predict drug hypersensitivity based on systemic or clinical effects do not exist, although some chemicals induce adverse immune-mediated effects in some animal strains. This unpredictibility is thought to be due to a complex interplay of predisposing idiosyncratic and environmental factors. Because drug hypersensitivity reactions often mimick graft-versus-host (GVH) reactions the popliteal lymph node assay (PLNA), originally used to measure GVH activity, was introduced to study chemical induced hypersensitivity. To date, around 140 different chemicals (drugs and homologues thereof as well as other compounds) have been tested in various isolated PLNA studies. When the lack of metabolism in the PLNA is taken into account the outcomes of this simple screening test correlated well with documented adverse effects in man. Modifications of the simple primary PLNA by including immunologically more relevant parameters than cell proliferation have allowed mechanism-based and therefore better assessment of sensitizing potential of chemicals. Recent studies, using KO-mice and specific blocking monoclonals have shown that costimulatory molecules (CD40L and CD86) are crucial for induction of PLNA responses by Th-2 sensitizing model-drugs like D-penicillamine and DPH studies. The response to the Th-1 stimulating model compound streptozotocin however, particularly involves CD80, and is more or less independent of CD40L. This indicates that immunosensitization by drugs follows general immunological patterns, but also that expression of costimulation may be used as an alternative read-out parameter. In all, the PLNA appears not only suitable to screen for the sensitizing potential of series of drugs (including structural homologues) but also to study fundamental aspects of drug-induced hypersensitivity.

950 MODIFICATION OF THE LOCAL LYMPH NODE ASSAY TO EVALUATE THE POTENTIAL FOR ADVERSE IMMUNOLOGICALLY-MEDIATED DRUG REACTIONS.

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There is no generally accepted preclinical method to predict the potential for drug allergy to systemically administered drugs. A collaborative research project, involving members of industry, government and academia, was initiated to evaluate an assay which uses the basic protocol of the Local Lymph Node Assay (LLNA, an assay accepted by regulatory agencies for hazard identification of skin sensitizers) modified by administration of test article by subcutaneous injections between the ears, rather than the prescribed topical exposure. Test compounds were selected from marketed drugs known to cause human drug allergy. Selection of active drugs was based on reports of adverse events found in at least two independent data sources including FDA postmarketing data, drug labeling, clinical trial data, and publications. The following drugs associated with adverse reactions were tested by at least three laboratories: streptozotocin, hydralazine, sulfamethoxazole, diphenhydramine, procainamide, clonidine, ofloxacin, chlorpromazine, nevirapine, abacavir, lamotrigine, and zomepirac. Clinically negative drugs tested were phenobarbital and metformin. The pure compound was used if available; otherwise the marketed drug formulation was used. Range finding studies in mice were performed to allow selection of the highest dose that did not produce systemic toxicity. Both clinically negative drugs tested negative in the assay. Six of the 12 clinically positive drugs were positive in the modified LLNA (mLLNA). For a limited set of drugs, a concurrent Popliteal Lymph Node Assay was performed and the results were comparable to those obtained in the mLLNA. Evaluation of the lymph node cell phenotypes by flow cytometry showed population changes in general agreement with the mLLNA data. These results suggest that the mLLNA holds promise as a screen for systemic hypersensitivity inducing drugs and the use of concurrent flow cytometry may provide insight into the underlying mechanisms.

951 THE USE OF THE PLNA IN RELATION TO ORAL ROUTE OF EXPOSURE TO LMWCS.

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This work examined diclofenac (an NSAID) and oxazolone (a contact sensitizer) in the Direct- and RA-PLNAs in naive mice and mice that had been orally pretreated with the respective LMWC. Both caused T cell-mediated PLN reactions in naive mice. Oxazolone increased the percent of CD4+ PLN T cells and decreased the percent of CD8+ PLN T cells, with a significant increase in CD4+ T cells having an activated phenotype (highCD44, lowCD62L). With diclofenac the PLN reaction was significantly attenuated in T cell-deficient mice, while wild-type mice showed an accumulation of CD4+ and CD8+ T cells with an activated phenotype. When co-injected with TNP-OVA, diclofenac also selectively induced IgG1 AFCs, IgE AFCs, and CD4+IL-4+INFg- T cells. Oral pretreatment with either compound appeared to affect the respective PLN reactions. Oral pretreatment with oxazolone (3 doses) rendered mice hypo-responsive to a footpad injection with oxazolone and the suppression could be adoptively transferred using CD8+ splenocytes. Oral pretreatment with diclofenac yielded a mixed effect on a subsequent diclofenac-induced PLN reaction. For example, mice gavaged a single time had augmented and accelerated diclofenac-induced PLN reactions both in the direct PLNA and when co-injected with TNP-Ficoll (increases of IgM, IgG1 and IgG3 AFCs). In contrast, 3 oral doses with diclofenac rendered mice hypo-responsive following footpad injection by an as of yet unidentified mechanism. These observations suggest the PLNA may be well suited to study the systemic immunomodulating effects of chemicals following oral priming and support further examination of the assays.

952 SCIENCE IN THE LEGISLATIVE PROCESS: A CONGRESSIONAL AND SCIENTIFIC VIEW.

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A primary goal for the Society of Toxicology is to promote the use of sound toxicological science in regulatory and legislative practices and policies. To achieve this goal, the SOT focuses significant activity to support science-related functions in regulatory agencies and legislative bodies with the objective of achieving better working relationships with policy makers. In this session, representatives from Congress and experienced SOT members will explore those practices that have worked best in developing and promoting good science policy. Topics to be included in this session will be a Congressional view on the "rights" and the "wrongs" of working with Congressional committees or individual representatives. It is anticipated that a speaker from both the Senate and House of Representatives will provide special insights for the SOT membership on developing relationships with Congressional members and their staff. Also, SOT members with significant experience with presentations before Congress or have worked closely with Congressional members will present on the practices that work best for the scientific community. A general discussion between speakers will address the importance of strategy and effective communication between policy makers and scientists during those times when scientific topics are being addressed by Congress. A key outcome from this roundtable discussion will be to provide SOT members with greater perspective of how the process of legislation works and how scientists can be a part of that process.

953 THE ROLE OF ESTROGEN IN THE MAINTENANCE OF AH RECEPTOR EXPRESSION IN MCF-7 BREAST CANCER CELLS.

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Numerous studies indicate cross-talk between estrogen receptor-alpha- and Ah receptor(AhR)-mediated signaling in breast and endometrial cells. In this study we examined the effects of long-term estrogen deprivation and estrogen re-exposure on Ah responsiveness in MCF-7 human breast cancer cells. Estrogen-deprived MCF-7 cells were obtained by long-term culture in medium prepared with 5% bovine calf serum, which contains minimal estrogen. These estrogen-deprived cells showed depressed levels of AhR mRNA and protein and reduced responsiveness for induction of CYP1A1 and CYP1B1 by dioxin. The reduced Ah-responsiveness was reversed by culture for four passages in medium supplemented with 1 nM estradiol (E2). Expression from a transiently transfected CYP1B1-promoter-luciferase reporter construct was reduced in the long-term estrogen-deprived cells, but was restored by co-transfection with an AhR expression construct, indicating that AhR expression was limiting in the estrogen-deprived cells. To determine whether the reduced AhR expression in estrogen-deprived cells involved alterations in transcription of the AhR, we investigated AhR promoter activity using a vector that contained 5.8 kb of the human AhR gene promoter cloned into the luciferase reporter vector, pGL3-Basic. A deletion construct containing 1.9 kb of the AhR proximal promoter was also used. Use of either promoter-reporter construct showed significantly lower AhR promoter activity in long-term estrogen-deprived MCF-7 cells compared with cells maintained in estrogen-containing medium. When estrogen- deprived cells were cultured for four passages in medium containing 1 nM E2, AhR promoter activity was restored, implicating E2 in the regulation of AhR expression. These studies indicate that the continued presence of estrogen is required to maintain high levels of AhR expression and inducibility of CYP1A1 and CYP1B1 in MCF-7 cells and that the reduced expression of AhR in long-term estrogen-deprived MCF-7 cells involves altered transcriptional regulation of the AhR. (Supported by NIH grant CA81243.)

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, workshop, roundtable, platform and poster sessions of the 43rd Annual Meeting of the Society of Toxicology, held at the Baltimore Convention Center, Baltimore, Maryland, March 21-25, 2004.

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