human AhR (hAhR) has been shown to differ biochemically from the mAhR. The ability of XAP2 to influence localization of the hAhR was examined. Results suggest that XAP2 does not influence localization or nucleocytoplasmic shuttling of the hAhR. Additionally XAP2 was unable to enhance hAhR levels, in contrast to what has been observed with the mAhR.

22 EXAMINATION OF THE AH RECEPTOR-RETINOBLASTOMA PROTEIN INTERACTION AND IMPACT ON CELL CYCLE CONTROL.

C. J. Elferink. *Pharmocology and Toxicology, University of Texas Medical Branch, Galveston, TX.* Sponsor: R. Pollenz.

The aryl hydrocarbon receptor (AhR) belongs to the basic helix-loop-helix/PAS family of transcription factors that regulate critical functions during development and tissue homeostasis. Within this family, the AhR is the only member conditionally activated in response to ligand binding, typified by 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD). The novel finding that the retinoblastoma tumor suppressor protein (pRb) interacts directly with the AhR through two distinct receptor domains spurred our interest in AhR-mediated cell cycle control. We have characterized one of the binding domains. More recent evidence revealed that TCDD-induced AhR-mediated G1 cell cycle arrest in 5L hepatoma cells depends on coactivation by the pRb. The cell cycle arrest seems to require p27Kip1 and a pRb activity apparently distinct from its function as a repressor of E2F-mediated transcription. In contrast, experiments using synchronized 5L cells linked AhR activity with a proliferative response, suggestive of a more complex role for the AhR in cell cycle control. These studies identified an unexpected connection between the persistence of AhR signaling and cell cycle control.

23 IMPACT OF AH RECEPTOR DEGRADATION ON GENE TRANSCRIPTION.

Q. Ma. *Toxicology and Molecular Biology Branch, Health Effects Laboratory Division, NIOSH/CDC, Morgantown, WV.*

Transcriptional regulation of gene expression represents a means of control for many fundamental cellular processes, such as cell growth and differentiation, and for responses to endogenous and exogenous signals, such as the adaptive/toxic responses to environmental chemicals. As such, transcription factors that mediate gene expression to specific signals are often tightly regulated to ensure physiologically adequate gene transcription and thus, the homeostasis of the cell. The ubiquitin-26S proteasome-mediated proteolysis has been implicated in the regulation of various types of cellular proteins. In this study, we analyzed the mechanism of agonist-induced degradation of the Ah receptor and its functional impact on AhR-mediated gene transcription to 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD). Biochemical and genetic data reveal that TCDD induces ubiquitination of AhR and shortening of the half-life of the protein through the 26S-proteasome mediated protein degradation. The agonist induced AhR degradation can be blocked by using inhibitors of the 26S proteasomes (MG123 and lactacystin) or cycloheximide, a potent inhibitor of protein synthesis; these data implicate a labile factor in controlling the degradation of AhR, which we designated Ah receptor degradation promoting factor (ADPF). Furthermore, inhibition of the AhR degradation by inhibitors of the 26S proteasomes or protein synthesis markedly enhances the induction of CYP1A1 by TCDD, a phenomenon termed superinduction; these findings suggest that the agonist-induced, ADPF-mediated degradation of AhR serves as a mechanism by which gene transcription by AhR is negatively controlled in cells. This view is supported by the observation that inhibition of the AhR degradation by either MG132 or cycloheximide superinduces a number of other AhR target genes, including TiPARP, a novel TCDD-inducible poly(ADP-ribose) polymerase. Our findings provide new insights into the control of the activity of agonist activated AhR through a regulated, proteasomal protein degradation pathway. Our current research is aimed at cloning of ADPF.

24 DIRECT ANALYSIS OF THE COMPLEX RELATIONSHIP BETWEEN NUCLEAR EXPORT AND AH RECEPTOR-MEDIATED GENE REGULATION.

R. S. Pollenz, Z. Song and S. Dabirshahsahebi. *Biology, University of South Florida, Tampa, FL.*

The AH receptor (AHR) is a modular protein containing distinct amino acid motifs that confer function to the receptor. Studies have revealed that the AHR contains both nuclear localization (NLS) and nuclear export (NES) signals that control in part, the subcellular location of the AHR and may influence the ability of the AHR to be activated. Thus, studies were performed to examine the relationship between the nuclear export of the AHR and AHR-mediated gene regulation. Blockage of nuclear export in human HepG2 cells with leptomycin B (LMB) resulted in a ligand-mediated increase in the level of AHR•ARNT complex in the nucleus and correlate reductions in agonist stimulated AHR degradation. However, despite the presence of high levels of the AHR•ARNT dimmer, induction of numerous AHR-responsive reporter genes and endogenous *CYP1A1* was reduced by 78-89%. To determine whether there was a direct relationship between blockage of export of the AHR and the reduced levels of gene regulation, stable cell lines were produced that expressed an AHR with a mutant NES. Immunohistochemical and biochemical analysis of these cells and direct comparison to cells expressing a wild type AHR protein showed that mutation of the NES ***did not*** affect the ability of the cells to induce endogenous *CYP1A1* following ligand exposure. To confirm these results and evaluate the temporal aspect of the signaling pathway, wild type and NES mutant AHR protein were produced *in vitro* and then microinjected directly into the nucleus of various culture cells. While export was blocked, the injected cells induced high levels of *CYP1A1* following agonist exposure. These findings demonstrate that it is possible to generate an AHR protein defective in nuclear export that functions in agonist-mediated gene induction. This implies that the negative affect of LMB on gene induction is independent of the nuclear export of the AHR and involves a novel factor that must interact with the AHR-mediated signal transduction pathway. Supported by NIEHS grants ES08980 and ES10401

25 REGULATORY AND INDUSTRY APPROACHES TO IMMUNOTOXICOLOGY ASSESSMENT OF PHARMACEUTICALS IN EUROPE, JAPAN, AND THE UNITED STATES.

J. L. Weaver and K. L. Hastings. *Div. Applied Pharmacology Reseach, USFDA, Laurel, MD.*

Assessment of pharmaceuticals for potential immunotoxicity seeks to identify adverse effects such as immunosuppression and/or activation of the immune system resulting in drug allergy or autoimmunity. A variety of methods have been used to evaluate potential drug-induced alterations in immune function. The usefulness of these assays in drug development has been extensively debated. Regulatory agencies in Europe, the United States, and Japan have published, proposed, or considered guidances for immunotoxicology assessment of pharmaceuticals over the past several years. In each case, the documents reflect the consensus of opinion within the regulatory agency as to the most reasonable and effective strategy to detect the potential of new drugs to cause adverse immune system effects. There are differences in the specific testing strategies suggested and in the scope of the issues these documents seek to address. For example, the issue of potential for drug allergy is not covered in all cases, and some of the strategies for evaluating potential immunosuppression are not completely harmonious. In this roundtable session, representatives from regulatory agencies and the pharmaceutical industry will discuss immunotoxicity testing of pharmaceuticals. Areas of agreement and disagreement among the parties will be addressed to elucidate the basis for the different approaches proposed.

26 EMEA GUIDELINES.

E. Putman. *Preclinical Assessment Group of the Medicines Evaluation Board, National Institue of Public Health, Bilthoven, Netherlands.* Sponsor: J. Weaver.

The Note for Guidance on Repeated Dose Toxicity was released in July 2000 by the CPMP (www.emea.eu.int/pdfs/human/swp/104299en.pdf). This Note for Guidance was revised to update the guidance on immunotoxicity. Immune toxicity screening is incorporated in the Note for Guidance in accordance to the tiered testing approach, i.e. an initial screening phase and extended studies. The tiered testing approach is considered sufficiently reliable to be used in a regulatory setting. Tiered testing strategies have been developed to assess direct immunotoxicity (suppression or stimulation). Hypersensitivity or testing for autoimmune potential is not in the scope of this Note for Guidance. In attempting to prevent an increase in animal use and number of studies the SWP decided not to issue a separate guideline on immunotoxicity, but to update the Note for Guidance on Repeated Dose Toxicity. As a result, repeated dose toxicity test evaluation has been extended to include immunotoxicity screening. The initial screening phase is designed to enhance the sensitivity of standard toxicity testing for immune toxic effects, preferably without the use of satellite animals. The NK-cell activity assay and lymphocyte subset phenotyping are selected because they fulfil these criteria. As an alternative, the primary antibody response to T-cell dependent antigen is suggested which allows a more holistic screen of the immune system. For the interpretation of the initial immunotoxicity screen the CPMP document advocates an integrative analysis of the changes in the immune system and other types of toxicity and the health status of the test animal. If the initial screening phase suggests direct immunotoxicity, follow-on studies in animals may be warranted on a case-by-case basis to further study

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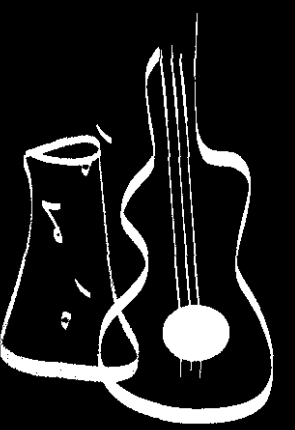

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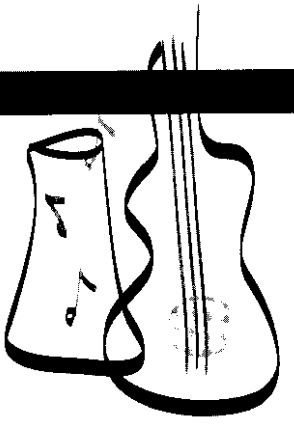

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Preface

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.

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