tine modulates specific T cell derived cytokines as well as those involved in inflammation. The studies reported here suggested that nicotinic receptors were found on immune cells and tissues to variable extents, depending on the source of the cells. The studies then focused on functional changes following nicotine exposure, specifically apoptosis and alteration in expression of genes related to immune action. Murine lymphocytes as well as cells from human cell lines were exposed to nicotine both independently and also following stimulation with mitogens. Nicotine was found to down-regulate apoptosis induced by dexamethasone as measured by western analysis. In addition, differential gene expression techniques such as RT-PCR were used to assess the impact of nicotine on genes related to cytokine production. Preliminary data demonstrated that nicotine did in fact lead to some alterations in expression of these genes. Examination of the mechanisms of action of nicotine, whether receptor-mediated or not, establishes the molecular impact of nicotine, a drug obtained through tobacco and therapeutic means, on immunity.

585 REGULATION AND FUNCTION OF P21$^{WAF-1/CIP1/SDI1}$ INDUCTION BY OXIDANTS IN NORMAL HUMAN FIBROBLASTS.

S. Purdom, V. C. Tu and Q. M. Chen. *University of Arizona, Pharmacology, Tucson, AZ.*

p21 is a stress response gene whose transcription is regulated by p53-dependent as well as p53-independent mechanisms. In addition to its function as a cyclin-dependent kinase inhibitor, p21 also inhibits DNA replication by interacting with proliferating cell nuclear antigen. Recent studies suggest that p21 is a master gene involved in renal failure and multiple aging-associated diseases, indicating that p21 protein may exert functions beyond regulating cell proliferation. Fibroblasts are the most abundant cell type and play an important role in regulating extracellular matrix homeostasis in most organs or tissues. Normal human diploid fibroblasts (HDFs) in culture respond to oxidative damage by sustained elevation of p21 protein. Elevation of p21 mRNA was first detected at 24 hr and remained over 2 weeks after a 2-hr pulse treatment of hydrogen peroxide at a 0.75 pmol/cell dose. Inactivating p53 by expressing human papillomaviral E6 gene reduced the fold of p21 protein induction within 24-hr but not at 48 or 72 hr post hydrogen peroxide treatment. Northern blots and comparative study with cisplatin further suggest a p53-independent mechanism in regulating p21 expression by oxidants. Prolonged increase in p21 protein correlates with changes in the expression of multiple genes such as collagenase 1, α1(I)procollagen, fibronectin, osteonectin, and apolipoprotein J. Most of these genes are known to play a role in matrix remodeling and fibrotic diseases. These allow us to test the role of p21 overexpression in changes in gene expression and extracellular matrix remodeling resulting from oxidative stress.

586 TRANSCRIPTIONAL REGULATION OF TIG1, A NOVEL TCDD-INDUCIBLE GENE, BY THE ARYL HYDROCARBON RECEPTOR.

K. Baldwin and Q. Ma. *NIOSH, TMBB/HELD, Morgantown, WV.*

TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) is the prototype of a class of structurally related halogenated aromatic hydrocarbons. TCDD elicits a wide range of responses in animals and may pose a threat to human health. The aryl hydrocarbon receptor is a ligand-activated receptor/transcription factor, which, together with Arnt and other factors, mediates the biological effects of TCDD. The mechanism of action of AhR involves transcriptional regulation of target genes. However, current knowledge of the target genes regulated by AhR and their roles in TCDD toxicity is limited. By using the mRNA differential display technique, we identified and cloned a novel target gene of AhR, designated TCDD-inducible gene 1 (TIG1). In this study, we characterize the regulation of TIG1 by TCDD. Genetic analyses using AhR- and Arnt-deficient cells demonstrate AhR and Arnt are required for induction of TIG1. Moreover, the transactivation domains of both AhR and Arnt contribute to the induction of the gene. We next examined the effect of cycloheximide, a potent inhibitor of protein synthesis, on the induction of TIG1 by TCDD. Our results reveal that cycloheximide superinduces TIG1 in the presence of TCDD; the superinduction occurs in both a time- and dose-dependent manner. The mechanism of action of superinduction involves inhibition of protein synthesis. Time course studies of superinduction implicate a labile factor in the induction of TIG1. Our results demonstrate that induction of TIG1 by TCDD occurs through the AhR pathway and that a labile factor controls the transcriptional regulation of the gene by AhR. These findings support the model previously proposed by our laboratory in which a labile factor, designated AhR degradation promoting factor (ADPF), acts as a negative regulator of agonist-activated AhR by controlling the removal of AhR. Inhibition of synthesis of ADPF enhances the stability of nuclear AhR, resulting in superinduction of TIG1 and other AhR regulated genes.

587 REGULATION OF CONNEXIN 32 GENE EXPRESSION IN MH_1C_1 CELLS.

L. A. Tate, J. K. Chipman and S. D. Minchin. *University of Birmingham, School of Biosciences, Birmingham, United Kingdom.* Sponsor: T. Orton.

Connexin-mediated gap junctions are membrane channels that allow adjacent cells in multicellular organisms to communicate, and play a crucial role in the maintenance of cellular homeostasis, differentiation and growth control. Changes in the level of expression of these proteins may occur in response to toxic agents in association with altered cellular proliferation. These changes may be attributed to transcriptional or post-transcriptional regulation. We have investigated the regulation of Cx32 transcription in MH_1C_1cells using transient transfection assays with the connexin 32 promoter fused to the firefly luciferase gene. Transcriptional activation was very low in cultured rat hepatocytes, but increased to16 fold that with vector alone, in MH_1C_1 cells. Transfection of seven deletion constructs into MH_1C_1 cells has identified the region required for maximal transcription from this promoter lying between mp −257 and −152. Deletion from 257 to −152 resulted in an 80% decrease in promoter activity, with levels in construct B7 (-152 to −22) 10% above background, suggesting the presence of the basal promoter within this fragment. Transfection of a luciferase linked Cx32 wild type (ACATTT) promoter vector (a sequence previously implicated in retinoic acid receptor binding), and a mutant (CACTTT) promoter vector into the MH_1C_1 cell line resulted in a 1.5 fold increase in luciferase expression on mutation, suggesting a negative regulatory role of this site in Cx32 expression. Following retinoic acid treatment (10μM) a 3-fold increase in luciferase expression was observed, but was not dependent on this site. One potential mechanism of retinoic acid action is through antioxidant mechanisms and preliminary data suggested an oxidant-induced down-regulation in expression. However, a statistically significant decrease in expression was not found in response to non-cytotoxic concentrations of hydrogen peroxide and t-butylhydroperoxide. The regulatory role of RXR and potential heterodimer partners requires further elucidation.

588 ASSESSMENT OF A TIERED STATISTICAL APPROACH FOR THE EVALUATION OF CHEMICALS USING cDNA MICROARRAYS IN TOXICOLOGY.

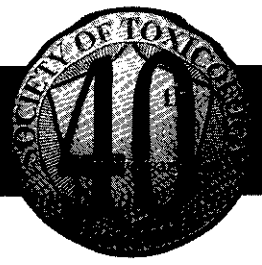
B. Sparrow[1], C. Gennings[2], B. Gollapudi[1] and W. Stott[1]. [1]*Dow Chemical Company, TERC, Midland, MI and* [2]*Virginia Commonwealth University, Biostatistics, Richmond, VA.*

While generation of cDNA microarray data is relatively straightforward, data analysis is less clear-cut. Here we demonstrate the evaluation of cDNA microarray data using a tiered statistical approach. Male B6C3F1 mice were gavaged with ciprofibrate, chloroform, alpha-naphthoflavone, beta-naphthoflavone, or vehicle. Equal masses of hepatic tissues were pooled and total RNA isolated. Subsequent hepatic mRNA was used for cDNA synthesis followed by hybridization to Clontech 120-gene Atlas Mouse Stress cDNA Expression Arrays. Each cDNA synthesis and hybridization was performed in triplicate. Resultant phosphorimages of the arrays were evaluated with AtlasImage 1.5 analysis software and a subset of 70-75 genes selected for further analysis. Genes with expression levels greater than two standard deviations from the average control value were then identified. To determine ensuing shifts in the distribution of gene expression by chemical exposure, the rank based Kruskal-Wallis test was applied. The final stage of analysis identified significant changes in gene expression following chemical exposure using principal components analysis and general linear mixed model analysis. From these analyses, 18 genes were identified as impacted by chemical exposure; 11 for ciprofibrate exposure, 6 for chloroform, 4 from beta-naphthoflavone, and 1 for alpha-naphthoflavone. Of these genes, two were identified as responsive to more than one chemical. These results build credence for using the tiered statistical analysis approach for microarray data to isolate genes that respond in similar fashion to various chemical exposures. Additionally, this approach has merit for the identification of gene expression patterns to define a response to a chemical exposure.

589 EFFECT OF SERUM ON GENE EXPRESSION PATTERNS IN HEP G2 CELLS USING MICROARRAY ANALYSES.

P. S. Rao, P. R. Harbach, J. M. Fostel, W. B. Mattes and C. S. Aaron. *Pharmacia Corporation, Kalamazoo, MI.*

Hep G2 cells are extensively used as an *in vitro* model in toxicology to predict adverse effects, screen compounds and elicit the mechanism(s) of toxicity of compounds. Due to the confounding effects of protein binding, experiments are often



Society of Toxicology

40th Annual Meeting

An Official Journal of the
Society of Toxicology
Supplement

TOXICOLOGICAL SCIENCES
Formerly Fundamental and Applied Toxicology

The Toxicologist

Abstracts of the 40th Annual Meeting

Oxford University Press

Volume 60, Number 1, March 2001

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 40th Annual Meeting of the Society of Toxicology, held at the Moscone Convention Center, San Francisco, California, March 25–29, 2001.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 451.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 479.

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

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