1329 INHIBITION OF NF-κB/Rel NUCLEAR TRANSLOCATION BY ACETYLAMINOFLUORENE (AAF): MECHANISM FOR THE INHIBITION OF iNOS GENE EXPRESSION.

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In the present study, the mechanism by which AAF inhibited nitric oxide (NO) formation, in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells was investigated. The decrease in NO, as demonstrated by measurements of nitrite was found to correlate well with a decrease in inducible nitric oxide synthase (iNOS) mRNA. Since it has been reported that nuclear factor-κB (NF-κB/Rel) is involved in the regulation of the iNOS gene expression, the effects of AAF on the activation of NF-κB/Rel was determined. Treatment of RAW 264.7 cells with AAF inhibited protein/DNA binding of NF-κB/Rel to its cognate site as measured by electrophoretic mobility shift assay. In addition, AAF treatment caused a significant reduction of nuclear c-rel, p65, and p50 protein levels, and these decrease was paralled by the accumulation of cytoplasmic c-rel, p65, and p50. Pyrrolidine dithiocarbamate, a relatively specific inhibitor of NF-κB/Rel, also inhibited the production of iNOS mRNA. These data suggest that AAF inhibited iNOS gene expression by a mechanism involving a blockade of LPS-induced nuclear translocation of NF-κB/Rel (Supported by the KOSEF Grant 971-0503-017-2).

1330 HYDROQUINONE INHIBITS NF-κB IN PRIMARY HUMAN CD4$^+$ T LYMPHOCYTES.

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Hydroquinone (HQ), a reactive metabolite of benzene, is present in cigarette smoke and is known to inhibit mitogen stimulated activation of both T and B lymphocytes. Despite extensive study, the underlying mechanism for HQ's immunotoxicity is not clear. NF-κB is a transcription factor known to regulate the expression of a number of genes critical for normal T cell activation. We therefore hypothesized that NF-κB might be involved in HQ induced immuno-suppression. In this study, we demonstrate that 1 uM HQ inhibits TNF-α induced activation of NF-κB in primary Human CD4$^+$ T cells. This inhibition is not accompanied by a loss in viability, and HQ treated T cells maintain other active signaling pathways throughout the exposure duration. Additionally, the inhibition of NF-κB is reversible as HQ treated T cells regain normal functioning after 72 hours in culture. HQ does not appear to alter NF-κB directly as pre-incubation of nuclear extracts with HQ does not diminish activity of this protein. We further demonstrate that 1 uM HQ inhibits intracellular IL-2 production in T cells stimulated with phorbol ester but does not alter surface expression of CD25 (the α subunit of the IL-2 receptor). These data suggest that NF-κB may be an important molecular mediator of HQ's (and benzene's) immunotoxicity.

1331 POST-TRANSCRIPTIONAL INHIBITION OF INTERFERONγ PRODUCTION BY LEAD IS DIFFERENTIALLY MODULATED BY IL-12 BUT NOT BY TGFβ, ZINC OR IRON.

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Lead (Pb), an environmental toxicant, preferentially suppresses type-1 helper T cell (Th1) activities, demonstrated by decreased IFNγ production and lowered serum IFNγ levels in Pb-exposed mice. IL-12, an important cytokine for Th1 development, was reported to overcome the inhibition of Pb on mouse resistance to *Listeria monocytogenes* and on IFNγ secretion by Th1 cells stimulated with antigen *in vitro*. TGFβ is an important modulator of the activation and development of T cells, but the regulatory influence of TGFβ on activities of the helper T cell subsets is debated. Intracellular mechanisms leading to Pb-induced modulation of IFNγ production by Th1 cells have been investigated. Expression of IFNγ mRNA by a Th1 clone stimulated with antigen and antigen-presenting cells for 4, 8, 12, and 21h was examined with a ribonuclease protection assay. No effect of Pb on mRNA expression was observed at each time point after stimulation, whereas the IFNγ levels in the culture supernatants were significantly downregulated by Pb. To assess Pb effects on IFNγ secretion, IFNγ levels in Th1 cell lysates obtained 18 and 36h after stimulation were examined by western blot and ELISA. Cellular IFNγ levels were at the lowest level of detection, but the levels in Pb-exposed cells were always lower than in controls, suggesting a Pb effect on biosynthesis and not secretion. The influence of Pb on IFNγ biosynthesis was investigated using ^{35}S-incorporation and immunoprecipitation. The synthesis of IFNγ was reduced during a 0–6h and 6–12h but not a 12–18h pulse with antigen. Addition of TGFβ, zinc, or iron could not overcome the suppression of IFNγ production caused by Pb, whereas IL-12 completely overcame this suppression for Th1 cells stimulated with antigen or PMA plus ionomycin, suggesting that IL-12 prevents the Pb inhibition at the translational level. Supported by NIH grant ES03179.

1332 EFFECT OF ESTROGENS ON IL-1β PROMOTER ACTIVITY.

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Steroid hormones modulate cytokine gene expression, however, estrogens have been shown to both suppress and enhance cytokine production. This study investigated the regulatory mechanisms which underlie the modulation of the interleukin-1β (IL-1β) gene at the transcription level. A macrophage cell line was stably transfected with the human estrogen receptor (ERα) and an IL-1β promoter-chloramphenicol acetyltransferase (CAT) reporter construct. 17β-Estradiol (E_2) markedly enhanced lipopolysaccharide (LPS)-induced IL-β promoter-driven CAT activity in a dose dependent manner. This response was estrogen specific since no synergism was observed between LPS and testosterone or progesterone and 17α-estradiol only stimulated at 10–100 times the amount required for E_2. Triphenylethylene antiestrogens and ICI 182, 780 inhibited the estrogen stimulated enhancement. The estrogenic effect appeared to be indirect and time dependent since the addition of E_2 was required hours prior to LPS stimulation. Surprisingly, the estrogen metabolites 17-epiestriol and 16-keto-17β-E_2 displayed an estrogenic response indistinguishable from E_2 while other metabolites were either less effective or ineffective. The mycotoxins, zearalenone and zearalenol, were also effective estrogens while the phytoestrogens, naringenin and luteolin, and other environmental estrogens, such as methoxychlor, 4-octylphenyl, and bis-phenol A demonstrated no agonist effect in the dose range 50–2000 nM, although they did display some antiestrogenic properties in the higher dose ranges. These results demonstrate that various classes of estrogens synergize with LPS to markedly enhance IL-1β promoter activity through ER mediated processes. Support: NIH ES05968.

1333 ESTROGEN WITHDRAWAL INDUCES ENHANCED PRODUCTION OF PROINFLAMMATORY CYTOKINES FROM KIDNEY AND LIVER.

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Tumor necrosis factor-α (TNF-α), interleukin (IL)-1, and IL-6 are thought to play important roles in pathophysiology in various organs including kidney, liver and bones. In this study, we investigate the effect of estrogens withdrawl in proinflammatory cytokine production in kidney, liver and bone marrow. To examine local production of the cytokine without the contribution of other organs, we used semiquantitative polymerase chain reaction (RT-PCR) analysis and kidney and liver slice culture model from tissues of mice ovariectomized (Ovx) with or without 17beta-estradiole (E2) replacement. In addition, IL-6 secretion from kidney was also monitored by urinary IL-6 exretion. The mRNA expression of IL-6 in renal glomeruli was elevated by Ovx while IL-6 expression was reduced by E2 replacement. TNF-α, IL-1β, and IL-6 secretions from kidney tissues are elevated by Ovx and the increments were prevented by E2 replacement. On the other hand, IL-1α alone from liver slice tissue was increased significantly by Ovx. IL-6 concentrations in urine were also elevated by Ovx and the changes were prevented by E2 replacement. Splenocyte and bone marrow cells produced more IL-6 after Ovx. Taken together, these studies indicate that, like the other immune competent cells, inflammatory cytokine production is influenced by estrogen status in kidney, liver and bone marrow cells. These altarations of cytokine production may be associated partly to acute phase response and to neuroendocrineimmunological axis.

1334 ROLE OF TNF-RECEPTORS (TNFR) IN CARBON TETRACHLORIDE-INDUCED LIVER TOXICITY.

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Carbon tetrachloride (CCl_4), a classical hepatotoxicant, causes acute, reversible liver injury characterized by centrilobular necrosis, fat accumulation, influx of neutrophils followed by hepatic regeneration and tissue repair. Although, TNF-α appears to be an important mediator in the pathogenesis of these processes, the role and the mechanisms responsible are still not well

understood. TNF-α, a pleiotropic and multifunctional cytokine, transduces regulatory abilities by two distinct cell surface receptors of 55 kd (TNFRp55) and 75 kd (TNFRp75) with most of the known cellular TNF responses attributed to TNFRp55 activation. We used TNFR—deficient mice to characterized the contributions of these receptors in chemical-induced liver toxicity. Single injections of CCl_4 in double knockout mice dose dependently induced larger centrilobular necrosis, prominent destruction of the surrounding hepatocytes and minimal inflammatory response comparing to the same exposure in the background control mice. Liver regeneration, measured as PCNA staining, was impaired in TNFR deficient mice particularly at exposures to high doses of CCl_4. TNFRp55 is sufficient for mediating TNF effects in this model since mice deficient for this receptor demonstrated liver toxicity with similar characteristics as the double TNFR deficient mice. The protective role of TNF-a in CCl_4 -induced liver toxicity may be associated with activation of several transcription factors including NF-kB and AP-1 and generation of secondary inflammatory mediators such as C-C chemokines.

1335 TUMOR NECROSIS FACTOR α MODULATES TRANSFORMING GROWTH FACTOR α EXPRESSION FOLLOWING CHEMICALLY-INDUCED HEPATOTOXICITY.

R M Gallucci, W Toriumi, P P Simeonova, and M I Luster. *NIOSH, Morgantown, WV.*

Previous studies have shown that Tumor Necrosis Factor-α (TNF-α) expression is increased in the livers of experimental animals following exposure to the hepatotoxin carbon tetrachloride (CCl_4). In addition, administering neutralizing antibodies to TNF-α (anti-TNF-α) prior to exposure to CCl_4 delays the regenerative process of the liver.

We hypothesized that TNF-α may influence liver regenerative processes through modulation of liver derived growth factors. Mice were administered anti-TNF-α prior to CCl_4 treatment. Antibody treatment did not affect the mRNA expression, as assessed by RT-PCR, of hepatocyte growth factor, its receptor c-*met*, epidermal growth factor, epidermal growth factor receptor (EGFR), or acidic fibroblast growth factor. Anti-TNF-α also did not affect liver EGFR number or binding. However, anti-TNF-α decreased the expression of transforming growth factor alpha (TGF-α) approximately 2.5 fold 12 and 24 hours post CCl_4 administration. To confirm this finding ribonuclease protection assays were performed and similar results to that shown with RT-PCR were found. Interleukin-6 (IL6) has been shown to be necessary for liver regeneration following partial hepatectomy, and TNF-α can induce the expression of IL6 in liver. To assess whether TNF-α directly modulates TGF-α expression in the murine liver, recombinant murine TNF-α or recombinant murine IL6 was administered. TNF-α upregulated TGF-α expression approximately four fold above control levels, whereas IL6 did not affect TGF-α mRNA expression 90 minutes post injection. Taken together, these data indicate that TNF-α modulates the expression of TGF-α in the liver following hepatotoxic injury with CCl_4, and that this modulation is independent of IL6 induction.

1336 ENDOGENOUS METALLOTHIONEIN CAN ALTER AN ANTIGEN SPECIFIC HUMORAL RESPONSE.

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Metallothionein (MT) acts as a reservoir of essential heavy metals, it can regulate Zn-dependent transcription factor activity, scavenge free radicals, and participate in the cellular defense against toxic heavy metals. These various roles are clearly essential to cell homeostasis, thus it is somewhat unexpected that mice deficient in MT-1 and MT-2 synthesis are without a profound effect on phenotype. This may be due to the fact that MT's role in biological systems is most important during periods of cellular stress, and that other mechanisms substitute during periods of normal cellular activity. In previous work, we have shown that exogenous MT can reduce a specific immune response to a T-dependent antigen when simultaneously presented with antigen to an animal. We extend our findings here to address the effect of the pool of MT that is synthesized as a consequence of immune cell responses to antigen. The absence of MT-1 and MT-2 in MT-null animals correlates with a twofold increase in antibody titer to the T-dependent antigen (ovalbumin, OVA). The onset of this response remains unaltered. Moreover, this effect appears to depend, at least partially, on endogenous extracellular MT, since injection of a monoclonal anti-MT antibody (clone UC1MT) enhances the humoral response to OVA in animals with a normal complement of MT genes when compared to syngeneic animals immunized in the presence of an isotype-matched antibody control. Our experiments suggest that MT can be a potent modifier of humoral responses, and that manipulation of MT levels during autoimmune or inflammatory disease may have beneficial consequences.

1337 ROLE OF GLUTATHIONE IN LYMPHOCYTE REDOX BALANCE AND FUNCTIONAL DEVELOPMENT.

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Lymphocytes encounter oxidative stress at sites of inflammation, in association with HIV infection, and with exposure to certain immunotoxicants. We investigated the role of the cellular antioxidant glutathione (GSH) in the maintenance of redox homeostasis and cellular function of T lymphocytes. Human peripheral T cells treated with a short pulse of H_2O_2 prior to stimulation by cell surface receptor engagement demonstrated a greatly reduced proliferative response, dose-dependent decrease in cell activation markers, and impaired cytokine production. H_2O_2 treatment rapidly activated GSH biosynthesis by glutamate cysteine ligase (GLCL), which led to rapid replenishment of intracellular GSH. We found that normal resting T cells were much more sensitive to H_2O_2 treatment than T cells which had been previously activated with phytohemaglutinin and undergone a round of proliferation (PHA blasts). The PHA blasts were capable of proliferation upon restimulation following pulses of H_2O_2 at concentrations 10 to 20-fold greater than those which completely inhibited proliferation of normal T cells. GLCL was induced by T cell activation, and expression of both the catalytic and regulatory subunits was increased by day 1 following stimulation. However by day 5, expression of both GLCL subunits had returned to levels similar to those found in unstimulated T cells. The GLCL enzyme activation following H_2O_2 treatment was also the same for T cells and day 5 PHA blasts. Therefore while GLCL is induced by lymphocyte activation, this induction is transient and does not account for the increased resistance to oxidative stress seen in PHA blasts. Increased GLCL activity may protect lymphocytes early after activation, whereas other inducible enzymes may be involved in the sustained protection seen in previously activated T cells. This work was supported by Bristol-Myers Squibb and National Institutes of Health grants ES04696 and ES07032.

1338 IMMUNODEFICIENCY INDUCED BY METHYLMERCURY IN C57BL/6 MICE IS RELATED TO INCREASE OF FAS-MEDIATED APOPTOSIS IN SPLENIC AND THYMIC CD4+ T CELLS.

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Methylmercury chloride (MeHgCl) is known to reduce both humoral and cell-mediated immune responses, rendering animals more sensitive to infectious diseases. The mechanism(s) by which mercury elicits immunodeficiency remains unclear. We have postulated that MeHgCl increase, at subtoxic doses, the apoptosis process in T cells from sensitive C57BL/6 mice. We have studied, *in vitro*, the number of apoptotic cells in splenic and thymic T subpopulations exposed to MeHgCl by double immunostaining with anti-CD4, anti-CD8, anti-Thy1 or propidium iodide (IP). Cytofluorometric analysis revealed that low doses (0.001 to 0.01 mM) of MeHgCl increased apoptosis, as defined by FSC/SCC, IP staining and the TUNEL method, in total and in T splenic cells while toxic effects occurred with higher doses (0.1 to 1 mM). ConA-activated splenic cells were more sensitive to MeHgCl-induced apoptosis, particularly the CD4+ subpopulation. Apoptosis was also increased in thymic mature CD4+ cells treated with lower concentrations of MeHgCl. Cell cytotoxicity analysis showed that MeHgCl acts by increasing the apoptosis induced by anti-Fas antibodies. In addition, the splenic CD4+Vb8 subset was preferentially depleted whereas CD4+Vβ6 cells increased. These results suggest that immunodeficiency induced by MeHgCl may be caused by induction of an apoptotic process in thymic and splenic T cells via the Fas antigen.

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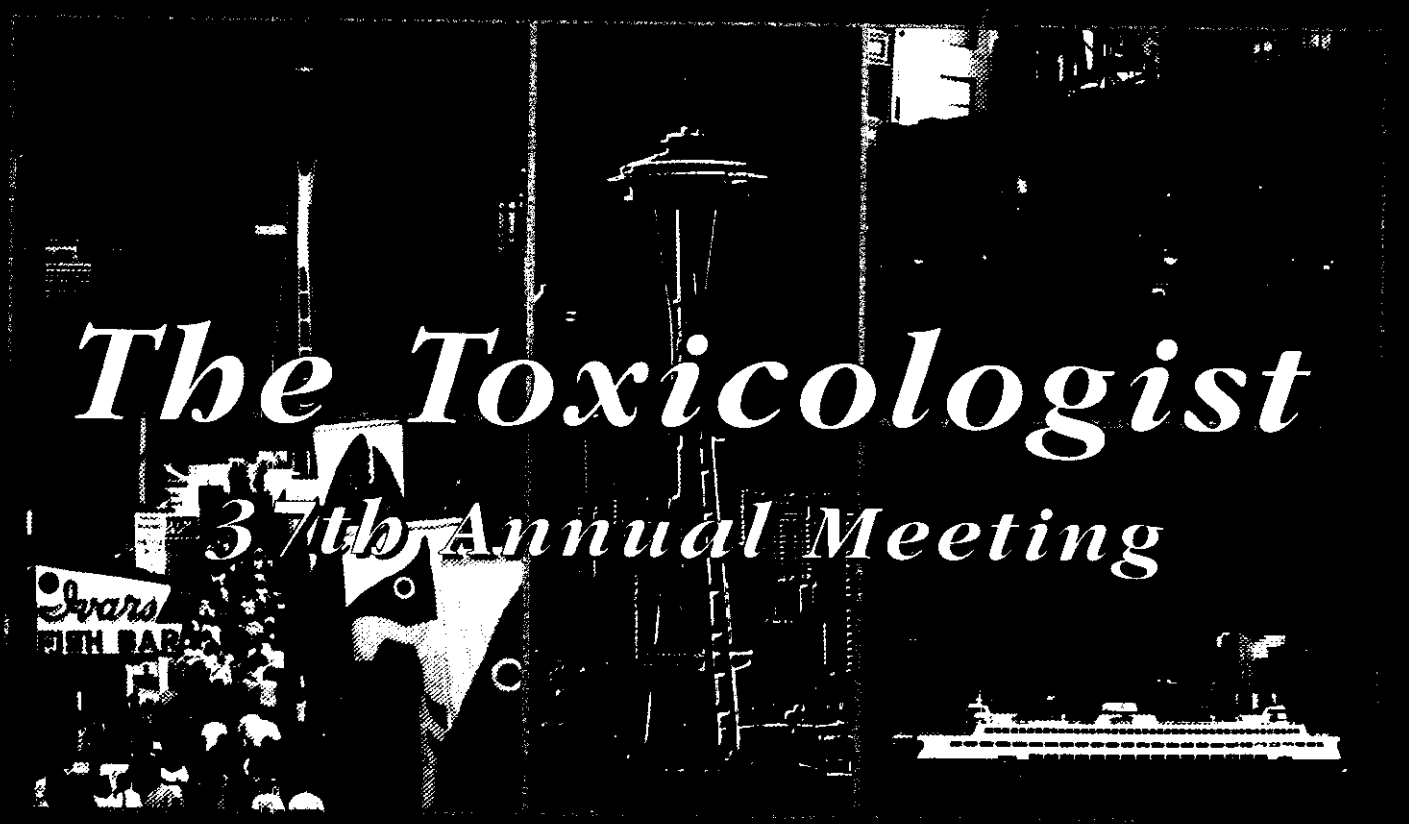

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Preface

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 37th Annual Meeting of the Society of Toxicology, held at the Washington State Convention Center, Seattle, Washington, March 1-5, 1998.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 407.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 433.

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