cancer in children. In light of these data, the National Institute for Occupational Safety and Health (NIOSH), together with Colorado State University (CSU) are investigating the effects of putative occupational carcinogens on cancer-linked molecular events in appropriate cellular systems. We are currently interested in the biological plausibility of EMFs as a putative carcinogen in SF767 human glioma cells. Here we study the effect of short-term environmental-level EMF exposure using cDNA microarrays. Following a 3 hr exposure we investigate changes in gene induction to determine appropriate genetic biomarkers of effect of exposure to EMF. At 70% confluence SF767 are exposed in the presence or absence of either epidermal growth factor (EGF) or melatonin, to a 12 mG, 60 Hz EMF. Ambient field strength in the cell culture incubator is maintained at 2 mG, 60 Hz. Total RNA was then isolated for use on the PerkinElmer oncogene array. Computer analysis and comparison of gene up and down regulation is performed across groups. These data suggest a limited yet complicated response of glioma cells to environmental field exposure. The number of oncogenes induced by EMF alone and in the presence or absence of melatonin and/or EGF range from 8 to over 40. The number of suppressed oncogenes ranges from 7 to more than 20. Current cluster analysis of this data may offer preliminary evidence of the possible role of EMF as a carcinogen in human glioma cells.

65 ORAL EXPOSURE TO INORGANIC MERCURY ALTERS T-LYMPHOCYTE PHENOTYPES AND CYTOKINE GENE EXPRESSION IN BALB/C MICE.

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Mercury is a well recognized health hazard and an environmental contaminant. Mercury is known to suppress immune responses, but the mechanisms responsible for this effect are still unclear. The aims of this study were to investigate the effect of mercury on immune parameters, such as hematology, lymphocyte phenotypes and cytokine gene expression. Male BALB/c mice were exposed continuously to 0, 0.3, 1.5, 7.5, or 37.5 ppm of mercury in drinking water for 14 days. Food and water consumption decreased in a dose-dependent manner in mice exposed to mercury. Body weight was reduced at the highest dose of mercury whereas the relative kidney and spleen weight were significantly increased. The dose-range of mercury used did not cause hepatotoxicity as indicated by circulating alanine aminotransferase and aspartate aminotransferase. Circulating blood leukocytes were elevated in mice treated with the highest dose of mercury. Single-cell splenocyte cultures were used to determine the effects of mercury treatment on mitogen-induced lymphocyte blastogenesis. Mercury at 1.5 ppm increased the PHA and LPS stimulation indices for T and B lymphocytes, respectively reflecting the observed decrease in basal splenocyte proliferation in mercury-treated mice. Exposure to 7.5 and 37.5 ppm of mercury decreased the CD8+ T lymphocyte population in thymus, whereas double positive CD4+/CD8+ and CD4+ thymocytes were not altered. Mercury ranging from 1.5 to 37.5 ppm dose-dependently decreased CD3+ T lymphocytes in spleen; both CD4+ and CD8+ single positive lymphocyte numbers were decreased. The population of CD45+ B lymphocytes was not changed. Mercury altered the expression of cytokines (tumor necrosis factor α, interferon γ, interleukin-12), c-myc, and major histocompatibility complex II in various organs. Results indicated that decreases in T lymphocyte populations in immune organs and altered cytokine gene expression may contribute to the immunosuppressive effects of inorganic mercury.

66 INDUCTION OF HEPATIC METALLOTHIONEIN BY VANADIUM.

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We have previously shown that the induction of hepatic metallothionein (MT) in mice by manganese (Mn) administration is entirely dependent on the production of inflammatory cytokine, interleukin-6 (IL-6). It is known that IL-6 is one of the mediators of MT induction. In the present study, we investigated the induction mechanism of MT synthesis by vanadium (V) in mice. Male ICR mice were injected subcutaneously with ammonium metavanadate (V[5]; 0.05-0.3 mmol/kg) and sacrificed 24 h after the injection. Although hepatic total vanadium concentration was lower than that in kidney, MT concentration was increased dose-dependently in the liver by V[5] administration, but not in the kidney. HPLC/ICP-MS analysis on the distribution of the metals in hepatic cytosol of V[5]-treated animals showed that the major metal bound to MT-I and MT-II was not vanadium, but zinc. A time-course study showed that hepatic total vanadium content, serum alanine aminotransferase (ALT) activity and serum IL-6 concentration reached the peak at 4-6 h after V[5] (0.3 mmol/kg) injection, and then declined quickly. Concentration of serum amyloid A (SAA), an acute-phase protein that is induced by IL-6, increased at 24 h after the injection. To confirm the involvement of IL-6 in MT induction by V[5], IL-6 null and control mice were administered with V[5]. In IL-6 null animals, no increase in SAA was observed, and MT induction by V[5] was significantly decreased to about 55 % of control animals. These data suggest that both IL-6-dependent and independent mechanisms are involved in MT induction by V[5] in mice.

67 ENHANCED GENOTOXICITY BY DIMETHYLARSINIC ACID IN METALLOTHIONEIN-I/II NULL MICE.

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To elucidate the protective role of metallothionein (MT) in the genotoxicity of dimethylarsinic acid (DMA), we examined the sensitivity of MT-I/II null mice to the genetic damage and the induction of apoptosis caused by DMA. Eight-week-old female MT-I/II null mice and wild-type mice were given p.o. injection of DMA (188, 375, 750 mg/kg). The blood, urine and liver were removed from each mouse under ether anesthesia at 24 hr after the injection. The DNA strand breaks in the peripheral blood cells and the 8-hydroxy-deoxyguanosine (8-OHdG) in the serum and urine were determined as indicators of genetic damage. The production of DNA strand breaks in both MT-I/II null mice and wild-type mice was elevated by DMA treatment in a dose-dependent manner. The increased the production of DNA strand breaks in MT-I/II null mice at 750 mg/kg DMA was significantly higher than that of wild-type mice. Moreover, 8-OHdG levels in the serum and urine of MT-I/II null mice were also increased by DMA treatment and they were significantly higher than those of wild-type mice. On the other hand, the DMA-induced apoptosis in the liver was not different between MT-I/II null mice and wild-type mice. MT concentrations in the liver of wild-type mice were increased by DMA treatment in a dose-dependent manner. There were no detectable amounts of hepatic MT in untreated MT-I/II null mice, and they could not be induced by DMA treatment. These results suggested that MT plays an important role in defense of DMA-caused genotoxicity.

68 METAL INDUCED ACTIVATION OF METALLOTHIONEIN GENE EXPRESSION.

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Metallothionein (MT) transcription is induced following exposure to elevated concentrations of a variety of transition metals. Metal-activated transcription is controlled *via* interactions between metal response elements (MREs) and the metal response element-binding transcription factor, MTF-1. It has been proposed that metals can initiate intracellular signaling cascades that result in altered states of MTF-1 phosphorylation, ultimately leading to the activation of MT transcription. COS-7 cells were transfected with one of three fusion chloramphenicol acetyltransferase (CAT) reporter genes: p-42-CAT (minimal mouse MT-1 promoter); pMREd5'-CAT (5 tandem copies of MREd' inserted upstream of the TATA box in p-42-CAT); or p-153-CAT (intact mouse MT-1 promoter). Cells were co-transfected with a β-galactosidase reporter plasmid, pSV-β-gal to control for transfection efficiency. Following a 4 hr metal exposure, the level of CAT protein was determined by a CAT-ELISA. Results were normalized with respect to the level of β-galactosidase activity. Of metals tested, only cadmium, copper, mercury, arsenic, antimony, silver, gold, and zinc were able to activate MT transcription *via* the MRE, while aluminum, indium, selenium, beryllium, tin, iron, cobalt, manganese, bismuth, molybdenum, lead, titanium, vanadium, chromium(III and IV), and nickel were not. CAT assay results were verified using reverse transcription PCR (RT-PCR) with MT primers and total RNA isolated from COS-7 cells exposed to the various metals for 4 hours. All real-time MT data was normalized to an actin standard. Of metals tested, cadmium, copper, zinc, mercury, silver, and arsenic exposures caused an increase in the steady state levels of MT mRNA. Inhibition studies were completed in order to test the hypothesis that phosphorylation of MTF-1 is involved in activation of MT gene transcription. These studies involved pre-treating cells for 30 minutes with 100μM of a broad range protein kinase C (PKC) inhibitor, H7. Inhibition results suggest that MT transcription induced by these metals occurs *via* a convergent signal transduction pathway involving PKC.

69 MT-3 OVEREXPRESSION INCREASES CHEMOTHERAPEUTIC RESISTANCE AND AFFECTS THE GROWTH OF BREAST CANCER CELL LINES.

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The third isoform of the human metallothionein (MT) gene family is unique compared to the 1 and 2 isoforms in that it has a more restricted pattern of tissue distribution. This laboratory has previously shown that overexpression of MT-3 in the