1632 ROLE OF PROTEIN KINASE Cδ IN SILICA-INDUCED APOPTOSIS AND AUTOIMMUNITY.

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Epidemiological studies have shown associations between silica exposure and several autoimmune diseases, including scleroderma and systemic lupus erythematosus. We have previously reported that lupus-prone New Zealand mixed mice develop an exacerbated autoimmune disease following exposure to crystalline silica. Fourteen weeks following exposure to silica, NZM mice develop high levels of autoantibodies to nuclear antigen. These mice also develop high levels of proteinuria due to increased immune complex and complement deposition within the glomerulus of the kidney. Silica exposure increases the level of pulmonary fibrosis and alters the numbers of T helper cells, B1a B cells and regulatory T cells. We have also reported that autoantibodies from silica-exposed mice specifically recognize apoptotic alveolar macrophages. Protein Kinase Cδ has been reported to induce apoptosis in many cell types and by several different stimuli. Recently, many reports have implicated apoptosis as playing a significant role in the development of several autoimmune diseases, therefore an increase in apoptotic proteins following silica exposure may provide a possible mechanism for the exacerbation of autoimmunity in NZM mice. In the present study, we are examining the activation of PKCδ in alveolar macrophages following silica exposure. PKCδ RNA and protein is increased 2-fold within the AMs of silica treated NZM mice. Lung sections obtained 14 weeks following silica exposure in NZM mice show increased staining of PKCδ and the staining appears to occur within the AM population as demonstrated by colocalized staining of PKCδ and F4/80. *In vitro*, using bone marrow derived macrophages, silica exposure increases the level of PKCδ within 2 hours following exposure. This increase in PKCδ at 2 hours also correlates with an increase in caspase-9 within 2 hours following silica exposure. Therefore, silica exposure appears to induce apoptosis by the activation of PKCδ which leads to activation of caspase-9 and the subsequent apoptosis may expose excess antigen to the immune system allowing the exacerbation of systemic autoimmune disease.

1633 MECHANISMS OF NORDIHYDROGUAIARETIC ACID (NDGA)-MEDIATED APOPTOSIS IN FL5.12 CELLS.

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NDGA is a general lipoxygenase (LOX) inhibitor that induces apoptosis in a variety of cells, including human Jurkat T lymphocytes and FL5.12 cells (murine pro-B lymphocytes), independent to its role as a LOX inhibitor. Effects on various kinases may be involved in this pro-apoptotic effect. Western immunoblotting experiments showed that treatment of FL5.12 cells with 10 μM NDGA leads to phosphorylation of extracellular signal-regulated kinases (ERK1/2), peaking at 1 h post-treatment. Although ERK1/2 is mainly known as a pro-survival protein there is evidence that activation of ERK is involved in apoptosis [Cancer Res. **61**: 6569-6576 (2001)]. Consistent with this report, pre-treatment of FL5.12 cells with an ERK1/2 inhibitor, PD98059 (50 μM), followed by 10 μM NDGA, attenuated the apoptosis induced by NDGA. Another kinase signaling system involved in cell survival is the PI-3K/Akt pathway. Akt phosphorylation and activation is protective to cells as this kinase then phosphorylates and inactivates various pro-apoptotic substrates including ASK1 and members of the Bcl-2 family. Inhibition of the PI-3K/Akt pathway usually drives cells to apoptosis. NDGA treatments that induced apoptosis inhibited phosphorylation of Akt. In addition, pre-treatment of FL5.12 cells with the PI-3K inhibitor wortmannin (100 nM) enhanced NDGA-mediated apoptosis. These data support the hypothesis that NDGA-mediated apoptosis involves both activation of ERK1/2 as well as inhibition of the Akt survival pathway. These data further suggest a possible crosstalk between the MAP kinase and the PI-3K pathways. (Supported by CA83701 and Center Grant ES07784).

1634 ENVIRONMENTAL STRESS-MEDIATED SENSITIZATION OF B-LYMPHOID CELLS TO PESTICIDE-INDUCED APOPTOSIS AND INDUCTION OF MAP KINASE PATHWAYS.

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Lymphocytes, at different stages of differentiation, can differ in resistance to drugs and environmental chemicals depending on expression level of Bcl-2 and other apoptotic proteins. Furthermore, cell resistance may be altered as a result of exposure to multiple environmental stresses. We have shown previously that a stress resistant B-lymphoid cell line, overexpressing Bcl-2, can be sensitized to arsenic-induced apoptosis following exposure to sub-lethal heat stress. However, it is unknown whether resistant B-cell lines can be sensitized to other classes of environmental chemicals. Therefore, we investigated whether the EW36 resistant B-cell line can be sensitized by heat stress to pesticide-induced apoptosis. The involvement of the c-jun N-terminal kinase (JNK) pathway in regulating enhanced apoptosis induction by the combination treatments was also investigated. EW36 cells were exposed to sub-lethal heat stress for 1 hr and then treated with 0, 5, 10, 25, 50 micromolar antimycin A (piscicide) or pyridaben (miticide) and incubated at 37 C. Cells were harvested at 2 hr post-treatment for studies of JNK activation and at 24 and 48 hours for c-jun expression and apoptosis induction. Exposure of EW36 cells to heat or antimycin individually failed to activate JNK or induce apoptosis. However, the heat plus antimycin combination treatment activated JNK, induced c-jun expression, and induced substantial apoptosis. An AP-1 inhibitor blocked apoptosis induction by the combination of heat plus antimyicn, and c-jun expression was not induced. The heat plus pyridaben combination treatment also induced substantial apoptosis compared to controls, but this effect was independent of activation of the JNK/c-jun pathway. These findings show that heat stress can sensitize resistant EW36 B-cells to pesticide-induced apoptosis, but the particular pesticide exposure determines whether the JNK/c-jun pathway will be activated. This signaling pathway, along with Bcl-2, may play an important role in regulating apoptotic cell death induced by pesticides.

1635 THIOREDOXIN AND TGHQ-INDUCED APOPTOSIS IN HL-60 CELLS.

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2, 3, 5-*tris*-(Glutathion-S-yl)hydroquinone (TGHQ) is present in the bone marrow of rats and mice co-treated with hydroquinone and phenol, hematotoxic metabolites of benzene. TGHQ is also hematotoxic in rats, and induces DNA damage, growth arrest, and apoptosis in human promyelocytic leukemia (HL-60) cells. During TGHQ-induced apoptosis of HL-60 cells reactive oxygen species (ROS) generation occurs, with concomitant deceases in cellular glutathione (GSH). However, although co-treatment of HL-60 cells with catalase protects against TGHQ-induced apoptosis, it does not prevent depletions in cellular GSH. We have now investigated whether other cellular thiol pools, specifically thioredoxin (TRX), modulate TGHQ-induced apoptosis. Western blot analysis and flow cytometry revealed that TGHQ (200 μM) treatment of HL-60 cells decreased TRX levels. Consistent with this finding, c-Jun N-terminal kinase (JNK) and p38 MAPK were activated in response to TGHQ. Interestingly, although catalase treatment did not block TGHQ-induced depletions of cellular GSH, it did prevent decreases in TRX levels. Although cytochrome *c* is released from mitochondria, concomitant with the dephosphorylation of pS70 Bcl-2 and the translocation of Bax to mitochondria 2h after TGHQ treatment, $\Delta\Psi_m$ remained intact for at least 4h after treatment. The data indicate that TGHQ facilitates ROS production, which causes subsequent decreases in cellular TRX, which may be subsequently coupled to the apoptosis signal-regulating kinase 1 (ASK1) - JNK/p38 apoptosis signaling pathway, culminating in the release of cytochrome *c* without interruptions in $\Delta\Psi_m$ (ES09224).

1636 THE FATE OF INSTILLED APOPTOTIC MACROPHAGES IN THE LUNGS.

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Our previous studies of particle exposures have implicated abnormal accumulations of apoptotic macrophages and apoptotic bodies in lung injury from high burdens of low toxicity particles and low burdens of highly toxic particles. To determine how the lungs process abnormal accumulations of apoptotic cells, we have used intratracheal instillation of apoptotic or normal macrophages from autologous donor rats. We characterized the fate of instilled apoptotic and normal macrophages by prelabeling the cells with UV fluorescent beads (0.5 micron, bright blue, polystyrene). One million labeled cells were instilled and the animals sacrificed at 4 hrs, 1 day, 1 week or 4 weeks after instillation. The right lung of each animal was lavaged with PBS and the left lung was inflation fixed with cryopreservative. Cytospins of lung lavage and cryosections of lung were stained for apoptotic cells with yellow-green fluorescence labeled nucleotides by terminal deoxytransferase (TdT). At 4 hours, 50% of the microsphere labeled, apoptotic cells which had been instilled were phagocytized. By 1 day, greater than 90% of the instilled apoptotic macrophages were phagocytized. There were no TdT positive nuclei in microsphere labeled cells at one week. No microsphere labeled, normal macrophages were found to be phagocytized at any time point. TdT positive cells in the lungs receiving normal

macrophages was too low to quantify. The results demonstrate that instilled apoptotic macrophages are rapidly ingested and processed by resident alveolar macrophages while otherwise normal macrophages are not.

1637 APOPTOTIC CELL INSTILLATION RESULTS IN ELEVATED TGF-β AND APOPTOSIS-INDUCED-APOPTOSIS IN RAT LUNG.

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Results from animal inhalation with either high burden or highly toxic particles showed increased apoptosis in the lungs. Previous studies by our group indicated that pulmonary administration of apoptotic lung cells induced inflammatory cell influx and lung fibrosis, indicating a direct linkage between apoptosis and fibrotic lung disorders (Ref.1). In this study, we further characterized the kinetics of pulmonary response to apoptotic cell instillation in rats and investigated the possible role of TGF-β in this process. Rats instilled with apoptotic cells showed a decrease in lung cell apoptosis at 1 week post-treatment, consistent with our previous finding showing the clearance of labeled apoptotic cells in rat lungs after 1 week (Ref.2). However, the level of lung cell apoptosis was higher at 4 and 12 weeks post-treatment compared to the control groups instilled with normal non-apoptotic cells. These results suggest that the apoptotic cells observed at latter time points were not those originally instilled but were subsequently induced after the treatment. Analysis of BAL fluids from treated rats showed an increase in TGF-β expression at 1 and 4 week post-treatment compared to the control groups. TGF-β is a known inducer of apoptosis and a key mediator of lung fibrosis, our results therefore suggest that increased TGF-β by apoptotic cell instillation may be responsible for the increased lung cell death and subsequent development of pulmonary fibrosis. References: 1. Wang L, Antonini J, Rojanasakul Y, Castranova V, Scabilloni JF, Mercer RR. Potential role of apoptotic macrophages in pulmonary inflammation and fibrosis. Am. J. Cell. Physiol. 194:215, 2002 2. Robert R. Mercer James Scabilloni, James Antonini, Vincent Castranova and Liying Wang. The fate of apoptotic macrophages instilled into the lung. American Thoracic Society Annual Meeting, Baltimore, MD, March 2004.

1638 PERTURBATION OF TESTICULAR CELL PROLIFERATION USING SODIUM ARSENITE.

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In this study we report the effects of sodium arsenite exposure on the cell proliferation of the normalized mouse Leydig cell line, TM3. The Leydig cells were grown in culture medium containing 5% horse serum, 2.5% fetal bovine serum. Cell cultures were switched to serum-free medium prior to treatment with test compound. The treatments consisted of 1pg, 10pg, 100pg, 1ng, 10ng, and 100ng per ml sodium arsenite, along with a control. Results indicated a significant ($p \leq 0.05$) increase in cell proliferation in all treatments with growth ranging from 119.79 to 191.80%, with the exception of 100ng/ml when compared to the control. Peak proliferation for sodium arsenite was noted at 10pg/ml (191.80%). The other growth was recorded as follows: 166.88% at 1pg/ml, 163.03% at 100pg/ml, 155.32% at 1ng/ml, 126.80% at 10ng/ml, and 119.79% at 100ng/ml. These results are of concern given that sodium arsenite has previously been reportrd to decrease DNA repair. Hence, when coupled with our findings here, a mechanism for the induction of genomic instability can be postulated. This then may explain the carcinogenic activity of sodium arsenite.

1639 ARSENIC ACTIVATES NADPH OXIDASE THROUGH CDC42 AND THEIR INVOLVEMENT IN ACTIN FILAMENT REMODELING AND CELL MOTILITY IN ENDOTHELIAL CELLS.

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Although arsenic is a human cancinogen, the molecular mechanisms of its action remain to be understood. By stimulating mouse endothelial cell lines (SVEC4-10) with 10 μM arsenic, we found that arsenic induced activation of NADPH oxidase as determined by the nitroblue tetrazolium test. Using confocal microscopy, the generation of $O^{\cdot}_2$ and H_2O_2 was observed to be increased in SVEC4-10 cells after exposure to arsenic. We also found that arsenic activated Small Rho GTPase CDC42. Disruption of CDC42 with a dominant negative CDC42 abolished arsenic-induced activation of NADPH oxidase and subsequent generation of $O^{\cdot}_2$ and H_2O_2, suggesting that CDC42 may mediate the effects of arsenic on NADPH oxidase. Furthermore, it was found that arsenic stimulation induced actin filament remodeling and increased cell motility in SVEC4-10 cells. These changes were abrogated by either disruption of CDC42 or inhibition of NADPH oxidase, indicating that the cell signaling changes induced by arsenic are relevant to these cellular functions. Taken together, the data imply that arsenic may promote motility-dependent metastasis of cancer cells through CDC42, NADPH oxidase and reactive oxygen species.

1640 TRANSGENERATIONAL EFFECTS OF CHROMIUM(III) ON OFFSPRING WEIGHT, SERUM TIIODOTHYRONINE, AND HEPATIC GENE EXPRESSION.

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Exposing male mice to chromium(III) chloride before mating results in increased tumors in offspring. An epigenetic mechanism has been postulated, involving alterations in hormones and in gene expression in the offspring. In pursuit of this hypothesis, male Swiss mice were injected i.p. with 1 mmol/kg chromium(III) chloride, 2 wks before mating. Offspring (10 wks old) were weighed and assessed for serum triiodothyronine (T3)and hepatic gene expression by cDNA microarray analysis. Both male and female offspring of Cr(III)-treated fathers were significantly heavier than those of control fathers: 31.4 +/- 0.4 g (S.E.) (N = 62) vs 30.6 +/- 0.2 (N = 90) (P = 0.035) for males and 24.6 +/- 0.3 (N = 54) vs 23.0 +/- 0.3 (N = 54) (P = 0.0003) for females. Serum T3 levels were also greater in the offspring of Cr(III)-treated fathers: 107.7 +/- 2.0 ng/dl vs 95.6 +/- 3.4 for males (P<0.0001) and 96.4 +/- 2.2 vs 89.3 +/- 2.8 for females (P = 0.054). Microarray analysis was carried out on eight pairs of livers from female offspring of Cr(III)-treated and control fathers, chosen to represent serum T3 ratios over the range 1.04 to 2.68. Linear regression analysis selected for hepatic genes showing microarray ratios correlating with serum T3 ratios with a significance of P <= 0.001. Fifty-eight genes met this criterion, including 25 named genes. Several are known to be regulated by T3, e.g. beta 2 adrenergic receptor and the calcium-regulator phospholamban. Others may pertain to growth: protein tyrosine phosphatase 4a2, pancreatic colipase, and complement component 3a receptor 1. Expressions of several genes associated with tumor suppression were negatively associated with serum T3 ratios: the transcription factors Ikaros, zinc finger protein 161, and Kruppel-like factor 5, as well as single-strand DNA binding protein and natural killer tumor recognition sequence. These results are consistent with hormone-mediated effects of paternal Cr(III)-treatment on offspring physiology, and with an epigenetic mechanism of transgenerational carcinogenesis.

1641 INDUCTION OF THE HYPOXIA MARKERS, CARBONIC ANHYDRASE IX AND Cap43, BY NICKEL OR CELL DENSITY IS RELATED TO ASCORBATE DEPLETION.

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Nickel compounds are known carcinogens and represent potential hazard for environmentally and occupationally exposed people. Mechanisms of nickel-induced carcinogenesis are currently unknown. Recently, we have shown that carcinogenic nickel compounds induced HIF-1 transcription factor and all known hypoxia-inducible genes. HIF-1 activation likely plays important an role in malignant transformation caused by nickel since stimulation of soft agar growth by nickel can be observed in HIF-1 alpha normal but not in knock-out cells. In this study we further investigated mechanisms of nickel-induced activation of hypoxia-inducible genes. We have found that both carbonic anhydrase IX (CAIX) and Cap43, known as intrinsic marker of hypoxia and a tumor marker, are also induced by exposure to soluble nickel compounds in human lung epithelial (HAE) cells. In cells exposed to 0.5 mM nickel(II) sulfate, the induction of both proteins was observed as early as after 2 hrs. The involvement of HIF-1 transcription factor in the induction of these proteins was also confirmed in cells exposed to cobalt(II), desferoxamine and dimethyloxoglycine (DMOG). Additionally, both CAIX and Cap43 were induced in HIF-1 alpha normal but not in knock-out cells. The induction of CAIX and Cap43 by nickel(II), cobalt(II) and desferoxamine, but not by DMOG, can be abolished by 0.1 mM of ascorbic acid added to culture media. These data suggest that nickel exposure may result in depletion of cellular ascorbate, leading to a shift in the iron oxidation status. CAIX and Cap43 could be also induced by high cell culture density. Here we show that the density-dependent induction of Cap43 and CAIX may be associated with the depletion of ascorbate in dense cultures.

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Preface

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