(primarily evident as decreased expression of CD62L and, to a lesser extent, enhanced cell cycling) in TCDD-treated mice on days 2 and 3 post-antigen. In the studies presented here, we examined three potential mechanisms for the loss of antigen-activated T cells in TCDD-treated mice: enhanced emigration out of the spleen, premature cessation of proliferation, and induction of cell death. TCDD significantly enhanced the number of antigen-specific T cells in the blood on day 3 post-antigen, which is consistent with TCDD promoting T cell activation. However the activated T cells in TCDD-treated mice failed to upregulate expression of CD11a, which is important for the extravasation of activated T cells. Thus, the T cells may have been trapped in the blood. CSFE staining showed that antigen-activated T cells continued to proliferate in vehicle-treated mice on day 4 after antigen exposure, whereas no additional cycling was observed in T cells from TCDD-treated mice after day 3. TCDD also induced an increase in apoptosis of activated T cells but not until days 5 and 6, arguing against enhanced cell death as the precipitating cause of the decline in T cells. Since T cells from D011.10$^{lpr/lpr}$ mice declined in a similar manner as normal T cells, the depletion of antigen-activated T cells by TCDD did not occur by a Fas-dependent process. Gene array analysis on day 3 indicated that TCDD increased the expression of several genes associated with cell survival/death. Taken together, these results suggest that TCDD induces an early but dysregulated activation of T cells, resulting in altered migration, truncated proliferation and reduced survival. Supported by NIH Grants P01ES00040, P30ES00210 and T32ES07060.

480 EXAMINING POSSIBLE MECHANISMS UNDERLYING PULMONARY NEUTROPHILIA IN VIRUS-INFECTED MICE TREATED WITH TCDD.

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The immune system is one of the most sensitive targets of the pollutant 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD). Our laboratory studies this immunosuppression using a murine-adapted model of human influenza virus infection. In addition to impairing the adaptive immune response to influenza virus, exposure to TCDD induces pulmonary neutrophilia. Neutrophils release cytotoxic meditors at the site of infection, and excess mediator release can damage healthy tissue. Neutrophil-mediated tissue insult may explain the enhanced mortality that we observe in virus-infected mice treated with TCDD. In fact, *in vivo* depletion of neutrophils improves host resistance in TCDD-treated mice. To determine the mechanism leading to pulmonary neutrophilia, we examined whether neutrophil chemoattractants are enhanced in the lung, adhesion molecules on neutrophils are up-regulated, or neutrophil apoptosis is delayed. Among neutrophil chemoattractants, we evaluated tumor necrosis factor (TNF)-α, interleukin (IL)-1, macrophage inflammatory protein (MIP)-1α, MIP-2, keratinocyte chemoattractant (KC), and lipopolysaccharide-induced CXC chemokine (LIX). Our results show similar levels of these mediators in both treatment groups. Using flow cytometry, we examined the following adhesion molecules on pulmonary neutrophils: CD11a, CD11b, CD11c, CD49d, CD31, CD38, and CD62L. Treatment with TCDD does not enhance the expression of any of these molecules. We studied neutrophil apoptosis using Annexin V and 7-AAD to distinguish *via*ble, apoptotic, and necrotic neutrophils. We also measured CD16 expression, since low levels of CD16 correlate with neutrophils undergoing apoptosis. Although we observed decreased CD16 expression on neutrophils from TCDD-exposed mice, exposure to TCDD did not alter the percent of apoptotic neutrophils. Collectively, our data indicate that TCDD likely promotes neutrophil recruitment *via* indirect mechanisms, such as changes in adhesion molecules on lung epithelium and endothelium.

481 EFFECTS OF SILICA ON *IN VITRO*-GENERATED MACROPHAGE SUBSETS.

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Inhalation of silica can result in chronic inflammation and fibrosis with a decrease in lung function and an increase in susceptibility to autoimmune diseases. Under normal conditions the lung is considered an immune-suppressed organ; however, following exposure to silica the environment becomes chronically immune-activated. A major component of lung immunology is the pulmonary macrophage. Alveolar macrophages (AM) have been implicated as playing a pivotal role in silicosis, including phagocytic uptake of silica. In general, macrophages have the ability to regulate immune responses by performing the role of intermediary between the innate and adaptive immune systems. Recently macrophage subtypes have been described in the mouse with categories including alternatively activated, classically activated, and type-II activated. The subtypes are defined by surface protein expression (i.e. MHC class II, scavenger receptor) and cytokine profile and have potential counterparts in the human. Using bone marrow-derived macrophages we generated these three subtypes by culturing with interferon (IFN)-gamma, interleukin (IL)-13, or IL-4 with either soluble or complexed antigen. We then examined the effects of silica exposure on the subtypes for phenotypic changes as determined by flow cytometry and functional assays. Our functional assays include macrophage cytokine ELISA and antigen presenting cell (APC) cultures and cytokine ELISA. Results demonstrated differential surface marker expression among the subtypes, as well as APC capacity. The type II, and to a lesser extent the classic and alternative, subsets have presented with an increase in APC function when exposed to silica. Our current studies suggest that an increase in macrophage function as a result of silica exposure could be an explanation as to the chronic inflammatory environment presented in silicosis. This work is supported by NIH grants ES 04804 and RR-017670

482 SILICA STIMULATES PHOSPHORYLATION AND ACTIVATION OF AKT IN MURINE ALVEOLAR MACROPHAGES.

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Silicosis is an occupational lung disease caused by the inhalation of silica particles which results in chronic inflammation and progressive pulmonary fibrosis. Phagocytosis of silica by alveolar macrophages (AM) and subsequent activation is a crucial step in the pathogenesis of silicosis. AM release oxygen free radicals and lysosomal enzymes, as well as proinflammatory mediators that propagate inflammation, induce programmed cell death, and promote aberrant cell survival. However, the participation of specific intracellular signaling pathways in regulating the AM response to silica have not been fully elucidated. The serine/threonine protein kinase Akt is activated downstream of phosphatidylinositol 3-kinase and has been shown to regulate diverse cellular processes implicated in mediating a variety of biological responses that include inhibition of apoptosis, promotion of cellular growth, and control of metabolism. To elucidate the role of Akt in silica induced chronic inflammation, we examined lavage fluid and lung sections from Balb/c mice post-silica exposure. Mice received either no treatment or 500μg silica once a week for four weeks. Lavage fluid was collected using 1ml PBS, while whole lungs were fixed and processed for trichrome and immunostained for activated Akt (n=5). Following chronic silica exposure, significantly increased levels of IL-10 and TNFα ($p<0.05$, $p<0.10$, respectively) were observed in the lavage fluid of silica exposed mice compared to no treatment. Fluorescence immunostaining demonstrated visible increases in the presence of the activated form of Akt between 0 and 8 weeks, with decreased immunostaining for phospho-Akt observed by 16 weeks. These data emphasize the need to examine the Akt signaling cascade in the AM response to silica exposure. Furthermore, a better understanding of the molecular events involved in regulating the AM response to silica exposure may permit targeting of specific biochemical pathways for more effective diagnosis and treatment of occupational lung diseases.

483 REPEATED EXPOSURE TO DIESEL EXHAUST PARTICLES CAUSES SUPPRESSION OF CELL-MEDIATED IMMUNE RESPONSES TO *LISTERIA* INFECTION IN BROWN NORWAY RATS.

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Diesel exhaust particles (DEP) have been shown to alter pulmonary immune responses to bacterial infection. However, the *in vivo* effect of DEP exposure on the T cell-mediated immune responses against bacterial infection has not been clearly demonstrated. Here, we examined the effects of repeated DEP exposure on pulmonary responses to bacterial infection. Brown Norway rats were exposed to DEP by inhalation at 20.62 ± 1.31 mg/m^3, 4 hr/day for 5 days, followed by intratracheal inoculation with 100, 000 *Listeria* at 2 hours after the last DEP exposure. DEP-exposed rats showed a significant increase in lung bacterial load at both 3 and 7 days post-infection. The repeated DEP exposure was shown to suppress both the innate, orchestrated by alveolar macrophages (AM), and T-cell mediated responses to *Listeria*. DEP inhibited production of interleukin-1β (IL-1β), tumor necrosis factor-α, and IL-12 by AM, but enhanced *Listeria*-induced production of IL-10, which has been shown to prolong the survival of intracellular pathogens such as *Listeria*. DEP exposure also suppressed the development of bacteria-specific lymphocytes from lung-draining lymph nodes, as indicated by the decreased numbers of T lymphocytes and their CD4$^+$ and CD8$^+$ subsets. In addition, the DEP exposure markedly inhibited the *Listeria*-induced lymphocyte secretion of IL-2 at day 7, IL-10 at days 3 and 7, and interferon-γ at days 3 to 10 post-infection when compared to air-exposed controls. These results show a sustained pattern of down-regulation of T-cell mediated immune responses by repeated low-dose DEP exposure,

which is different from the results of a single high dose exposure (100 mg/m^3, 4 hours), where the acute effect of DEP aggravated bacteria infection but triggered a strong T cell-mediated immunity.

484 PARTICULATE MATTER IMMUNOMODULATORY EFFECT ON THE PROGRESSION OF AUTOIMMUNE DISEASE IN NEW ZEALAND MIXED MICE.

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In this study we examined whether particulate matter exposures would impact the progression of autoimmune disease in a lupus-prone New Zealand mixed (NZM) model. NZM mice were intranasally instilled with either 30 µl saline or a 30 µl saline suspension of 500 µg acid washed PM1648, PM1648 or PM2.5 collected in Houston, TX, once a week for 4 weeks. Mortality and proteinuria levels did not significantly change among the groups. Levels of anti-nuclear antibodies (ANA) were evaluated 4 and 16 weeks following PM instillation by ELISA. All three particulates significantly suppressed the natural development of ANA in the NZM mice. There were no significant differences in the serum levels of IFN-γ, IL-12, and IL-4 among the groups 17 weeks post-PM instillation except for IL-10, which was significantly decreased in the particulate-instilled mice. IgG serum levels were significantly decreased in the PM1648 and PM2.5 instilled mice at 6 weeks following particulate instillation as compared to the saline and acid washed PM1648 instilled groups. Serum levels of IgG subclasses were also measured; IgG2b serum levels were significantly increased in the PM2.5 instilled mice as compared to the saline instilled group at 4 weeks following PM instillation, whereas the PM1648 and PM2.5 instilled mice showed significant decrease in IgG3 serum levels as compared to the control at the same time point. Significant reduction in the levels of serum IgM as compared to the saline group was observed in the PM1648 instilled mice during the first 6 weeks post-particulate-instillation period. There were also slight increases in the amount of immune cell infiltration and fibrosis within the PM 1648 and PM2.5 groups in comparison with the control group. Taken together, the results support the notion that PM modulates the immune system towards a Th2 response, thereby decreasing the effect of a Th1 mechanism of autoimmune disease in the NZM model. This work was supported by NIH grants ES 11120 and RR-017670.

485 TRICHLOROETHYLENE DOES NOT ACCELERATE AUTOIMMUNE DIABETES IN NOD MICE.

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Trichloroethylene (TCE) is an environmental contaminant suspected of inducing immunological changes in human after exposure *via* the drinking water. Previous studies showed that TCE exposure could be linked to autoimmune diseases in susceptible human beings. Increases in antinuclear auto-antibodies and serum immunoglobulin levels, and activation of CD4+ T cells producing Th1-type cytokines have been observed in TCE-treated female MRL +/+ mice. In order to confirm these findings, the effects of TCE exposure were evaluated in another animal model of autoimmune diabetes, the female non obese diabetics (NOD) mouse. Sixty mice were given 0 or 5 mg/ml TCE *via* the drinking water for 12 weeks starting at 11 weeks of age. Blood glucose levels were measured every two weeks. Blood samples were analysed every 4 weeks for Th1/Th2 cytokine profile including IL-4, IL-5, IL-2, IL-12 and IFN-γ by flow cytometry. Cell surface expression of CD44, CD45RB and CD54 on peripheral and splenic CD4+ T lymphocytes was analyzed to evidence possible T cell activation in TCE-treated mice. The mice were killed after 4, 8 or 12 weeks of treatment and a fragment of the spleen was isolated for lymphocyte subset analysis. There were no treatment-related effects on blood glucose levels or on histopathology findings at any time points. TCE did not affect the Th1/Th2 cytokine balance and peripheral and splenic T lymphocyte analysis did not indicate T cell activation. Our results suggest that TCE exposure does not accelerate autoimmune diabetes in female NOD mice. This finding is in sharp contrast with effects previously reported in female MRL +/+ mice. It is also concluded that the selection of relevant animal species or strains for the prediction of autoimmunity in preclinical immunotoxicity evaluation is not straightforward.

486 ANALYSIS OF TARGET ANTIGENS FOR AUTOANTIBODIES ASSOCIATED WITH ASBESTOS EXPOSURE.

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Environmental exposures to particles such as asbestos have been associated with systemic autoimmune disease. Previous studies on a population in Libby MT that have been significantly exposed to asbestos have found that these individuals appear to have an increased frequency of positive ANA (antinuclear antibodies) tests compared to a matched population in Missoula, MT. The goal of this study was to determine if previous positive ANA tests performed in this population were specific for chromatin and double stranded DNA (dsDNA) through the use of Enzyme Linked Immunosorbent assays (ELISAs) and Crithidia luciliae testing, respectively. Our study also aimed to determine if the anti-ENA (Extractable Nuclear Antigen) levels would also be higher in these individuals measured by ELISA, and addressable bead array (LUMINEX). Additionally, mean autoantibody titer for ANAs were performed to determine if there would be a significant change between exposed individuals and those in the control population. The results showed that the percent ANA positive and the mean autoantibody titer for ANAs were significantly higher in the Libby group than those of the Missoula group. The results of tests for antibodies to chromatin and dsDNA on 40 coded samples proved to be negative suggesting that the target for ANA antibodies in these individuals may be histone. The scl-70 ELISA and ENA addressable bead array indicated that those individuals exposed to asbestos had significantly higher ENA scores, particularly for Scl-70, SS-A and SS-B than those in the Missoula control population. These antibodies are commonly seen in autoimmune diseases such as scleroderma and Sjogrens syndrome. These results demonstrate the upregulation of specific autoantibodies in an asbestos-exposed population, and begin to identify some of the specific targets for these autoantibodies. This work was supported by NSF EPSCoR and CDC Grant CCR-822092.

487 DIFFERENCES IN HEPATOTOXICOLOGICAL PROFILE OF ET-743 BETWEEN SPRAGUE-DAWLEY RATS AND CYNOMOLGUS MONKEYS.

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Yondelis™ (trabectedin, ET-743) is a marine-derived compound under investigation in phase II trials and has shown activity in ovarian, breast and advanced pretreated soft tissue sarcoma. In patients, drug-induced toxicities are non-cumulative neutropenia, reversible increases in transaminases and fatigue. The hepatotoxic potential of Yondelis™ was evaluated in repeated dose studies in rodent (Sprague-Dawley rats) and non-rodent models (Cynomolgus monkeys). The latter were chosen as the preferred non-rodent species due to the similarities in metabolic profile to that of humans. In these studies, ET-743 was administered *via* a 3-hour intravenous infusion every 3 weeks for 3 (rat) or 4 (monkey) cycles, or weekly for 3 weeks followed by 1 week without treatment (3 cycles). After repeated dosing in rats, mortality was observed at 50 µg/kg in females and at 50-75 µg/kg in males. A pronounced dose-dependent and only partially reversible hepatotoxicity (increased transaminases, hepatocytic necrosis, bile duct inflammation and proliferation, cholangitis) was noted, and toxicity was cumulative and more pronounced in female rats. The difference in gender sensitivity in rats is likely to be linked to differences in the metabolic profile, biliary excretion and/or liver retention. Administration of a weekly 30 µg/kg-dose was better tolerated by Cynomolgus monkeys than dosing 70 µg/kg every 3 weeks. Hepatotoxicity was less pronounced than in rats and non-cumulative (increased transaminases, hepatocellular hypertrophy, hepatocytic degeneration/necrosis and inflammatory cells in the sinusoids and portal tracts). At 50 µg/kg, exposure to ET-743 was higher in monkeys (23-28 µg.h/L) than in rats (1.7-8.6 µg.h/L), without gender difference. The similar profile of hepatic changes induced by chronic ET-743 treatment in humans and monkeys suggest that the Cynomolgus monkey is a more relevant model for human Yondelis™ hepatotoxicity than the rat.

488 PROTECTION OF FUMONISIN B_1 hepatotoxicity by silymarin and myriocin in female balb/c mice.

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Fumonisin B_1 (FB_1) is a toxic and carcinogenic mycotoxin produced by *Fusarium Verticillioides* found on corn. The biological effects of FB_1 are mainly due to disruption of sphingolipid metabolism resulting from its inhibition of ceramide synthase. Silymarin has a strong protective effect against numerous hepatotoxicants. Myriocin is a selective inhibitor of serine palmitoyltransferase (SPT), the first key enzyme in *de novo* synthesis of sphingolipids. Both silymarin and myriocin protected porcine renal epithelial cells from FB_1 toxicity. In the present study we examined the protective effects of silymarin and myriocin on FB_1 hepatotoxicity in female Balb/c mice. Mice were treated daily with 750 mg/kg silymarin by gavage 16 hr prior or 1 mg/kg myriocin intraperitoneally 30 min before subcutaneous injections of either vehicle or FB_1 at 2.25 mg/kg.day for 3 consecutive days. Silymarin, to a greater extent than myriocin, attenuated FB_1-induced elevation of plasma alanine aminotransferase and aspartate aminotransferase, and hepatocellular apopto-

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