the immunoglobulin superfamily that amplifies the inflammatory response by triggering secretion of proinflammatory mediators, was upregulated in hepatic macrophages following administration of endotoxin to C3H/HeOuJ and C3H/HeJ mice. C3H/HeJ mice have point mutations in the toll-like-4 receptor, a critical component of endotoxin-mediated cell signaling, and are resistant to endotoxin-induced toxicity when compared to C3H/HeOuJ mice. Hepatic macrophages were isolated from livers following perfusion with collagenase, centrifugal elutriation and density gradient centrifugation. Macrophages from the livers of either C3H/HeOuJ or C3H/HeJ control mice expressed very low levels of TREM-1 mRNA as determined by semi-quantitative RT-PCR. Treatment of mice with endotoxin (3 mg/kg, ip) caused a time-dependent induction of hepatic macrophage TREM-1 mRNA expression which was maximal after 20 hr. Significantly less TREM-1 mRNA was induced in C3H/HeJ mice when compared to C3H/HeOuJ mice. In macrophages from both strains of endotoxin-treated animals, tumor necrosis factor-α (20 ng/ml) or interleukin-1β treatment (50 ng/ml, 24 hr) *in vitro* caused a further 2-10 fold increase in TREM-1 mRNA expression. Lipopolysaccharide (LPS) decreased TREM-1 mRNA expression 2-3-fold in macrophages from C3H/HeOuJ mice but did not alter its expression in cells from C3H/HeJ mice. Taken together, these data demonstrate that endotoxin is a potent inducer of TREM-1 mRNA in hepatic macrophages and that expression of the protein may be an important mechanism for amplifying the inflammatory response to hepatotoxicants. Endotoxin-resistance in C3H/HeJ mice may be due to limited expression of the TREM-1 protein. Support: NIH GM34310 and ES06897.

1599 IMMUNOMODULATION BY DIETHYLSTILBESTEROL IS DOSE AND GENDER LINKED: INFLUENCE ON THYMIC APOPTOSIS AND MITOGEN-INDUCED PROLIFERATION IN CD-1 MICE.

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It is perceived, but not shown, that the immunomodulatory influences of diethylstilbesterol (DES) may vary based on dose or gender. To address this, DES was subcutaneously administered to female and male CD-1 mice as four injections over 1 week at 0, 5, 15 and 30 μg/kg bw dose with immunological and reproductive parameters evaluated 24hr post last injection. Although female thymuses were significantly larger than male thymuses, short-term DES administration neither induced thymic atrophy nor altered relative percentages of thymic subsets. However, DES treatment did induce dose-related apoptosis in the CD4+8+, CD4+8- and CD4-8+ subsets using 7-amino-actinomycin D (7-AAD). The CD4-8- showed significant apoptosis only at the highest dose (30 μg/kg bw). Mitogen-induced proliferation of splenic lymphocytes also varied with hormonal doses and gender. In the females, splenic lymphocytes from low dose DES (5 μg/kg bw)-treated mice showed an increase in proliferative response to Con A, LPS or PMA/ionomycin compared to controls. Conversely, cultures from mice treated with the higher DES doses (15 or 30 μg/kg bw) showed suppressed proliferation, especially with Con A. In the males, DES appeared to produce minimal effects with the exception of increased proliferation to Con A in the 15μg/kg bw. Interestingly, the changes in mitogen-induced proliferation were not paralleled by similar changes in relative expression of CD90+ or CD45+ cells or ratios of anti-apoptotic Bcl-2 to apoptotic Bax proteins. Con A-activated splenocytes from DES-treated mice, specifically in the females, secreted less interferon-γ compared to controls. Collectively, these findings suggest that short-term exposure to DES generates a disparity in the immunological effects depending upon the dose of hormone and sex.

1600 THE GENERATION OF REACTIVE OXYGEN SPECIES DURING EXPOSURE OF PESTICIDE MIXTURES TO IMMUNE CELLS, *IN VITRO.*

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Recent reports suggest that pesticides pose potential health risks to the humans and animals by affecting their immune system. We have observed earlier that endosulfan and permethrin cause immune cell cytotoxicity mainly *via* apoptosis. We hypothesized that these chemicals induce immunotoxicity through Reactive Oxygen Species (ROS) formation. In an attempt to test this hypothesis, we have studied the production of ROS in splenocytes of C57Bl/6 adult male mice exposed to endosulfan and permethrin, *in vitro*. The generation of intracellular hydrogen peroxide (H_2O_2) and superoxide anion was monitored using a flow cytometer in combination with dichlorofluorescin diacetate (DCFH-DA) and hydroethidine (HE) dyes, respectively. Results of these analysis revealed that individual pesticides increased the production of both H_2O_2 and superoxide anion in a dose- and time- dependent manner. The mixtures of pesticides elicited a synergistic effect on the generation of H_2O_2. However, exposure to mixtures of pesticides had little effect on the generation of superoxide anion radicals as compared to individual pesticides. These findings suggest that the pesticide-induced immunotoxicity observed earlier may, at least in part, be associated with the generation of ROS.

1601 DEOXYNIVALENOL-INDUCED APOPTOSIS MEDIATED BY P38 MAPK-DEPENDENT P53 GENE INDUCTION IN RAW 264.7 MACROPHAGES.

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Deoxynivalenol (DON, vomitoxin) and other trichothecene mycotoxins cause immunosuppression by inducing leukocyte apoptosis. Upstream signaling transduction mechanisms contributing to DON-mediated apoptosis were investigated in RAW 264.7 cells, a murine macrophage model. PP1, a Src-family-tyrosine kinase inhibitor selective for Hck, and 2-AP (2-aminopurine), the chemical inhibitor of dsRNA-dependent protein kinase (PKR), additively inhibited DON-induced caspase-3 activity and apoptosis as well as phosphorylation of the mitogen activated protein kinases p38, ERK and JNK. PP1 and 2-AP also inhibited DON-induced p53 binding activity and subsequent phosphorylation of its substrate p21. Pretreatment with PFTα, an inhibitor of p53, abrogated DON-induced caspase-3 and apoptosis. The p38 inhibitor, SB 203580, abrogated DON-induced p21 phosphorylation as well as reduced DON-induced p53 binding activity, whereas ERK and JNK inhibitors were partially inhibitory. Finally, p38 inhibition blocked DON-induced apoptosis, ERK inhibition promoted DON-induced apoptosis, and JNK inhibition had no effect. The results suggest that the principal pathway for DON-induced apoptosis in the macrophage involves the sequential activation of Hck/PKR, p38, p53, caspase-3. (Supported by NIEH Grants ES-09521 and ES-03358).

1602 JP-8 JET FUEL DOES NOT ALTER SERUM CYTOKINE LEVELS IN B6C3F1 MICE FOLLOWING 7-DAY ORAL OR DERMAL EXPOSURE.

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The US Air Force uses approximately 2.5 billion gallons of jet propellant-8 fuel per year. As a result, a considerable number of military personnel are exposed to JP-8 fuel during refueling and servicing of aircrafts, and maintenance of fuel storage tanks. Those exposed have increased incidence of headaches, blocked nasal passages, ear infections, skin irritation, or fatigue. In rodent studies, we have previously reported that 7-day dermal or oral exposure to JP-8 suppresses humoral immunity and decreases thymus weight and cellularity. Additionally, other published studies have demonstrated that a single, dermal exposure to JP-8 modulated serum cytokine levels in mice, thereby accounting for a possible mechanism of immunosuppression. To determine if alterations in serum cytokines occurred after 7 days of exposure to JP-8, the following study was performed. B6C3F1 female mice aged 7-10 weeks of age were exposed to JP-8 dermally (50 uL applied to the clipped dorsal thorax of mice with an average weight of 20 g) or orally (2000 mg/kg/day) for a duration of 7 days. Serum was collected 24 hours after the last exposure to JP-8. Using ELISA and Cytometric Bead Array methods, it was determined that levels of IL-2, IL-4, IL-5, IL-6, IL-10, and TNF-alpha were not significantly altered after exposure to JP-8 *via* the oral or dermal route. It was also learned that, when used as a negative control in the dermal study, acetone as compared to olive oil induced a suppressive effect on IL-4 and IL-6 serum cytokine levels. Consequently, interpretation of immunological dermal studies utilizing acetone should be made with caution. Overall, these findings indicate that serum cytokine levels were not elevated after a 7-day exposure to JP-8, regardless of the route of administration. These observations indicate that serum cytokine levels are not sustained above normal levels following repeat exposure to JP-8.

1603 HEPATIC PHASE I AND II ENZYME PROFILES AFTER 7-DAY DERMAL OR ORAL EXPOSURE TO JP-8 JET FUEL.

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Many military and industrial personnel are exposed to JP-8 jet fuel during service and refueling of aircrafts, and maintenance of storage tanks. In toxicological studies using mice, it has been demonstrated that JP-8 can induce immunosuppression fol-

lowing dermal, oral, or inhalation exposures. After dermal or oral exposure to JP-8, we have previously demonstrated that thymus weight and cellularity are significantly diminished and specific IgM antibody production is suppressed by 50% or more as compared to controls. To further evaluate the effects of JP-8, this study presents comparative metabolic enzyme profiles obtained after oral or dermal exposure to JP-8. Western blotting was performed to determine the protein expression of Phase I and Phase II hepatic enzymes at 24 hours or 7 days after a 7-day exposure to JP-8. Female B6C3F1 mice were exposed to JP-8 either orally (2000 mg/kg/day) or dermally (50uL neat application). Twenty-four hours post-exposure, protein expression of CYP2E1, 2B1, GSTmu, and GSTpi, but not CYP1A1, were significantly increased in mice exposed orally to JP-8. Following a week recovery period, these enzymes returned to constitutive levels. In dermal studies, despite the presence of immunosuppression comparable to orally exposed mice, there was minimal to no induction of CYP2B1, GSTmu, and GSTpi. Current studies are underway to confirm the dermal exposure profile for CYP2E1 and CYP1A1. Additionally, histological examination of the livers from these same mice exposed orally or dermally, indicated that there was no increase in the amount of fat, hydropic degeneration or necrosis in the liver. These data suggest that the metabolism of JP-8 may not be required for immunotoxicity.

1604 EFFECT OF CYCLOOXYGENASE (COX) INHIBITORS ON HUMAN LEUKOCYTE MIGRATION THROUGH ENDOTHELIAL CELL MONOLAYERS.

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Previous studies have shown that traditional NSAIDs such as ibuprofen inhibit human leukocyte migration through endothelial cell monolayers. Leukocyte-endothelial cell interactions play a central role in various inflammatory processes. Upon signaling from chemokines, cytokines and adhesion molecules during inflammation, leukocytes first attach, roll and migrate through the vascular endothelium to the inflammation site. The current study using an *in vitro* double chamber cell culture system, compared the effect of an *in vitro* non-selective COX inhibitor (ibuprofen) and *in vitro* selective COX-2 inhibitors (SC-236, -791, -872 and -236) on human leukocyte migration through endothelial cell monolayers. Human umbilical vein endothelial cells were cultured on fibronectin coated 3m microporous membranes until an endothelial cell monolayer formed. Monolayers were treated with 10ng/ml recombinant human TNF for 4 hours before the migration assay began. 1x106 freshly isolated human leukocytes and/or cultured endothelial cell monolayers were preincubated with test agents at 1x, 10x and 50x multiples of their therapeutically relevant concentrations. Migration assays were carried out for 3 hours in a 37oC 5% CO2 incubator. Leukocytes that migrated through the endothelial cell monolayer to the lower chamber were quantitated. Each set of experiments was performed with peripheral blood from ten different normal human subjects. Our results confirmed inhibitory effects of ibuprofen on human leukocyte migration starting at the therapeutically relevant concentration. No effect on leukocyte migration was seen with the *in vitro* selective COX-2 inhibitors at 1X and 10X concentrations indicating that COX-2 inhibition does not affect leukocyte migration. At extremely high concentrations (50x), leukocyte migration was decreased with *in vitro* selective COX-2 inhibitors. This may be related to loss of COX selectivity and inhibition of COX-1 at these concentrations. In conclusion, *in vitro* selective COX-2 inhibitors do not affect leukocyte-endothelial cell interactions during the inflammatory process.

1605 ROLE OF IL-1BETA IN LPS POTENTIATION OF DEOXYNIVALENOL-INDUCED LEUKOCYTE APOPTOSIS IN MICE.

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LPS and Deoxynivalenol (DON) co-exposure induces corticosterone-dependent apoptosis in thymus, Peyers patches, and bone marrow in mice after 12 hr. We investigated the role of endogenous and exogenous IL-1beta in stimulating corticosterone and in inducing leukocyte apoptosis in this model. LPS (0.1 mg/kg, ip) and DON (12.5 mg/kg, po) co-exposure induced splenic IL-1beta mRNA significantly compared to vehicle or each of the toxins alone. Mice deficient in IL-1 receptor did not exhibit LPS + DON-induced leukocyte apoptosis whereas toxin co-treatment induced apoptosis in corresponding wild-type mice. Plasma corticosterone levels in LPS + DON-treated IL-1 receptor deficient mice were significantly lower at 12 hr than wild-type mice. In B6C3F1 mice, intraperitoneal injection of IL-1 receptor antagonist (100 microg/mouse, twice at 3 hr intervals) also significantly inhibited LPS + DON-induced apoptosis in thymus, Peyers patches and bone marrow compared to LPS + DON-treated mice. Three injections of IL-1beta protein (500 ng/mouse, ip at 2 hr intervals) induced apoptosis in thymus (4.7%) and Peyers patches (16.0) whereas single injection of equivalent amount of IL-1beta (500 or1500 ng/mouse) did not induce apoptosis in any of the organs in B6C3F1 mice. In parallel to apoptosis, corticosterone levels in multiple IL-1beta injected mice were significantly higher (404.0 ng/ml) at 12 hr than control or single injected mice (90.4 ng/ml). ACTH levels in LPS + DON-treated mice were not correlated with the induction of plasma corticosterone or leukocyte apoptosis. Taken together, the results indicate that IL-1beta is a critical mediator of LPS + DON-induced leukocyte apoptosis and that it possibly acts through ACTH-independent corticosterone upregulation (supported by NIH Grants DK 58833 and ES 03358).

1606 ALTERATIONS OF MATERNAL/FETAL CYTOKINE CONCENTRATIONS IN SMOKERS AND NON SMOKERS AT CHILDBIRTH.

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Plasma cotinine concentrations are the benchmark for smoking status assessment. Smoking has been shown to elicit an inflammatory response associated with respiratory dysfunctions such as asthma and emphysema. As expected, maternal smoking during pregnancy has a deleterious effect on the fetus. Using commercially available ELISA kits, we measured cytokine concentrations in the plasma of mothers and matched cord blood samples collected at birth. Maternal/fetal pairs were placed in smoking and nonsmoking categories based on plasma cotinine concentration. Tumor Necrosis Factor-α, and the Interleukins 1β, 6, 8 and 10 were measured as markers of elevated inflammatory status. Preliminary studies show that with the exception of Il-1β, fetal cytokine concentrations were lower in the plasma of the babies whose mothers smoked. We attribute this difference to the suppression of the immune response caused by cigarette smoke exposure. These alterations may also be attributed to the stress of and the events leading up to delivery. We hypothesize that this altered immune response may account for the predisposition of children whose mothers smoked throughout pregnancy to respiratory ailments such as asthma as well as childhood allergy problems. Future studies will clarify the role of these individual cytokines in the spectrum of immune system maturity.

1607 TARGETED DELETION OF CD44V7 EXON LEADS TO DECREASED IL-2-INDUCED ENDOTHELIAL CELL TOXICITY AND VASCULAR LEAK SYNDROME.

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Endothelial cell (EC) toxicity is widely reported in a number of clinical settings including autoimmunity, transplantation, graft versus host disease and cytokine therapy. Administration of IL-2 activates cytolytic lymphocytes to mediate EC injury and trigger vascular leak syndrome (VLS). In the current study, we investigated the nature of CD44 variant isoforms involved in EC injury and the mechanism of VLS induction. Administration of IL-2 into CD44 wild-type (WT) mice led to increased gene expression of CD44 variant isoforms containing the v6 and v7 exons and to significant induction of VLS in the lungs. In contrast, CD44v6/v7 KO and CD44v7 KO mice showed markedly reduced levels of VLS. The decreased IL-2-induced VLS in CD44v7 KO mice did not result from differential activation and expansion of CD8+ T cells, NK and NK-T cells or altered degree of perivascular lymphocytic infiltration in the lungs. Interestingly, IL-2 activated cytolytic lymphocytes from CD44v7 KO mice showed a significant decrease in their ability to mediate lysis of EC *in vitro*. Furthermore, adherence and conjugate formation of cytolytic lymphocytes from CD44v7 KO mice to EC isolated from the lungs of TIE2-GFP transgenic mice was markedly reduced when compared to LAK cells from CD44 WT mice. Finally, culturing of LAK cells with Abs against CD44v7 led to a significant reduction in the adherence to and killing of TME endothelial cells. The current study demonstrates that CD44 isoforms containing v7 play a key role in the adhesion of cytolytic lymphocytes to EC leading to injury of EC. This work was supported in part by grants from National Institutes of Health (HL 10455, HL 058641, DA 0114885, and ES 09098).

1608 DIFFERENTIAL REGULATION OF IL-2 GENE TRANSCRIPTION BY TGF-β1 IN NAIVE AND EFFECTOR/MEMORY $CD4^+$ T CELLS.

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TGF-β1 signaling in T cells plays a role in the maintenance of T-cell homeostasis and self-tolerance as illustrated by autoimmune disease in CD4-promoter dnTGF-βRII transgenic mice. The cellular and molecular mechanisms underlying homeostatic control of $CD4^+$ T cell populations remain elusive, but may be mediated, in