1620 SUPPRESSION OF THE PRIMARY IMMUNOGLOBULIN M (IGM) ANTIBODY RESPONSE BY DELTA^9-TETRAHYDROCANNABINOL (Δ^9-THC) IN HUMAN PRIMARY B CELLS.

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We have previously shown that Δ^9-THC, a plant-derived cannabinoid, significantly attenuated the primary IgM antibody response induced by ligation of CD40 in mouse splenic B cells. However, the effect of Δ^9-THC on humoral immune responses in humans is uncertain. Thus, the objective of this study was to investigate the influence of Δ^9-THC on *in vitro* T cell-dependent antibody response in human B cells using an *in vitro* activation model, which employs cell surface-expressed CD40 ligand (CD40L) and recombinant cytokines [interleukin (IL)-2, IL-6, and IL-10]. Similar to what we observed in mice, pretreatment with Δ^9-THC suppressed the number of IgM antibody forming cells induced by CD40L plus cytokines as determined by ELISPOT. Furthermore, Δ^9-THC suppressed B cell activation induced by CD40L plus cytokines as measured by suppressing the upregulation of the B cell activation markers, CD80, CD86, and CD69, as assessed by flow cytometry. Impairment in B cell activation correlated with suppression of B cell proliferation. Collectively, these studies suggest that Δ^9-THC-mediated suppression of the primary IgM response is due, in part, to impairment of B cell activation and proliferation. (Supported in part by DA07908 and Royal Thai Government Scholarships)

1621 INDUCTION OF MYELOID-DERIVED SUPPRESSOR CELLS BY ENDOCANNABINOIDS REQUIRES MAST CELLS.

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Cannabinoids are a group of compounds that mediate their physiological and behavioral effects by activating specific cannabinoid receptors. Cannabinoid receptor 1 (CB1) is primarily expressed in the CNS. In contrast, cannabinoid receptor 2 (CB2) is predominantly expressed on immune cells. In addition to the exogenous cannabinoids found in the Cannabis plant, there are also endogenous cannabinoids (endocannabinoids), such as 2-arachadonoyl glycerol(2-AG) and N-arachidonoylethanolamine (anadamide, AEA). The endocannabinoids also mediate their effects by activating CB1 and CB2 receptors. Cannabinoids have been shown to act as potent immunosuppressive agents and have been shown to mediate beneficial effects in a wide range of inflammatory diseases. Recently, we showed that endocannabinoids can trigger large numbers of a subset of monocytic precursors called Myeloid-Derived Suppressor Cells (MDSCs) that are highly immunosuppressive and prevent T cells from proliferating in response to antigens. In this study, we investigated the mechanism by which endocannabinoids are able to induce MDSCs. Cytokine analysis of mice treated with endocannabinoids showed that several cytokines were secreted in response to endocannabinoid treatment, including G-CSF and GM-CSF. To investigate the source of these cytokines, we attempted to induce MDSCs in mice that were deficient in mast cells. These studies revealed that mast cell-deficient mice were unable to induce MDSCs at a level consistent with wild type mice. When mast cells were adoptively transferred into deficient mice, the ability to induce MDSCs was restored. Taken together, these studies point to a significant role played by mast cells in the induction of MDSCs by endocannabinoids (Supported in part by NIH grants P01AT003961, R01AT006888, R01ES019313, R01MH094755).

1622 DIFFERENTIAL MODULATION OF NF-κB SUBUNITS BY ACROLEIN.

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Cigarette smoking impairs pulmonary immunity by suppressing T cell responses, resulting in compromised immune surveillance and high risk of respiratory infections. In previous studies, we have identified that the α-β-unsaturated aldehyde acrolein is the major immunosuppressive agent present in cigarette smoke, which reproduces the effects of cigarette smoking on pulmonary immunity. Acrolein inhibits the production of a variety of cytokoines involved in T cell response. Mass Spectrometry analysis revealed that acrolein alkylates Cys61 and Arg307 residues in the DNA-binding domain of the upstream transcription factor NF-κB p50 subunit, which agrees with an inhibition of p50's binding to the *IL-2* promoter by >99%. In contrast, our preliminary study showed that acrolein elicits the production IL-8, macrophage chemotractant protein-1 (MCP-1) and cyclooxygenase 2 (COX 2). Furthermore, acrolein has minimal effect on DNA binding of p65 subunit of NF-κB. The purpose of current study is therefore to test the hypothesis that acrolein activates NF-κB p65 subunit, leading to the upregulation of its target genes, such as *IL-8*, *MCP-1* and *COX 2*. U937 cell line was used to study the effects of acrolein on: (1) the production of cytokines IL-8 and IL-10, (2) the binding capacity of p65 to *IL-8* promoter, and (3) the expression of key NF-κB signaling molecules. Results show that acrolein increases IL-8 production, but inhibits LPS-induced IL-10 production. Acrolein promotes the binding of p65 and phosphorylated p65 to the *IL-8* promoter. As expected, acrolein inhibits constitutive binding of p50 to *IL-8* promoter. Acrolein also induces nuclear translocation of p65 and phosphorylated p65 and increases p38 phosphorylation. Acrolein adduction on the DNA binding domain of p65 is currently under investigation. Conclusion: collectively our data suggest that acrolein promotes IL-8 production by activating NF-κB p65 subunit and thereby alters the activity of NF-κB pathway through differential modulation of its p50 and p65 subunits. *This work is partially sponsored by NIEHS grant ES005673.*

1623 Δ^9TETRAHYDROCANNABINOL (THC) AMELIORATES STAPHYLOCOCCAL ENTEROTOXIN B (SEB)-INDUCED ACUTE LUNG INJURY THROUGH REGULATION OF EPIGENETIC PATHWAYS.

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Acute Lung Injury (ALI), commonly caused by sepsis, leads to respiratory and multiple organ failure and subsequently, Acute Respiratory Distress Syndrome (ARDS). It is characterized by infiltration of inflammatory lymphocytes in the lung that causes damage to alveolar epithelial cells, pulmonary edema and fibrosis. In this study, Staphylococcal Enterotoxin B (SEB) was used to induce ALI in mice. SEB is a superantigen that activates T cells expressing Vβ8, which leads to activation of ~20% of T-cells and massive release of pro-inflammatory cytokines, leading to induction of ALI/ARDS. In the current study, we tested the hypothesis that Δ^9Tetrahydrocannabinol (THC), a cannabinoid, known for its anti-inflammatory properties, can ameliorate the toxicity of SEB. Intranasal administration of SEB caused infiltration of lymphocytes into the lung, which was reduced after THC treatment. While SEB caused an increase in absolute numbers of NK, NKT, Macrophages, and Vβ8+ T cells, THC treatment caused a decrease in their absolute numbers. Cytokine analysis of bronchoalveolar fluid (BALF) showed that SEB induced high expression of Th1 cytokine, IFN-γ. Interestingly, THC treatment led to a switch from Th1 to Th2 phenotype (IL-10, IL-4 and IL-6). Epigenetic studies revealed that SEB caused an up-regulation of miRNA(miR)-155 and THC reduced its expression by half indicating that THC may mediate its effect through down-regulation of miR-155 and consequent suppression of inflammation. Additionally, methylation studies of the IFN-γ, IL-4 and IL-10 gene promoters indicated that the THC-induced switch in cytokine profiles can be explained in part by modifications at the epigenetic level. Together, our data demonstrated that THC can ameliorate SEB-induced ALI through regulation of epigenetic pathways.(Supported in part by NIH grants P01AT003961, R01AT006888, R01ES019313, R01MH094755).

1624 OXYGENATED FATTY ACIDS IN PLASMA OF TUMOR-BEARING ANIMALS STIMULATE THEIR SCAVENGER RECEPTOR A1-MEDIATD UPTAKE BY DENDRITIC CELLS: MASS-SPECTROMETRIC EVIDENCE.

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Dendritic cells (DC) are the most potent antigen presenting cells responsible for the development of immune responses in cancer. The function of DC in tumor-bearing hosts is severely compromised. To a large extent, the defects in DC function in tumor-bearing mice and patients with cancer are due to the accumulation of high amounts of lipids. To identify possible sources of lipids taken-up by the DC, we performed oxidative lipidomics analysis of plasma and DC of tumor-bearing animals. We found that both plasma and DC contained significant amounts of highly oxidizable polyunsaturated free fatty acids (FFA) represented mostly by C18:2, C18:3, C20:4 and C22:6 species as well as their oxygenated (oxFFA) species. Their contents were significantly higher in EL-4 tumor-bearing animals than in control mice. MS analysis revealed that oxFFA were mainly represented by 13-HODE, 9-HODE, 12-HETE, tetranor-12-HETE, and 16-HDoHE. To determine the extent to which scavenger receptor A1 might be involved in the uptake and intracellular transport of oxFFA we assessed their content in DC generated from wt and Msr1−/− HPC in vitro. Markedly reduced levels of all four characterized oxFFA

were found in DC from k/o mice vs those detected in wt animals. Further, we estimated whether oxFFA in DC were esterified into the most abundant class of neutral lipids accumulating in DC of EL-4 tumor bearing animals, triglycerides (TG). We found that oxTG species containing HODE and corresponding to C16:1/C18:2-OH/C15:0 was present only in DC from tumor-bearing mice. Thus, we suggest that the presence of oxygenated species of lipids in plasma of EL-4 tumor-bearing animals may be responsible for their uptake by DC possibly resulting in the loss of their immuno-surveillance function. Supported by NIOSH OH008282; NIH U19 AI068021, HL70755, HL094488.

PS 1625 COMPARING THE IMMUNOSUPPRESSIVE EFFECTS OF CYCLOSPORIN A ON MOUSE SPLENOCYTES *IN VIVO* WITH MOUSE AND HUMAN T-CELLS *IN VITRO* BY TRANSCRIPTOME PROFILING.

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The immunosuppressive drug Cyclosporin A (CsA) is widely used to prevent graft-versus-host disease in humans. In a parallelogram approach we compared the effects of CsA on the transcriptomes of (i) mouse (CTLL-2) and human (Jurkat) T-cell lines (ATCC), in order to determine the degree of interspecies overlap, (ii) splenocytes of C57BL6 mice exposed *in vivo* with CTLL-2 cells exposed *in vitro*, to verify whether CTLL-2 cells are a suitable *in vitro* model for toxicogenomics. Methods: The mice were exposed for 11 days to CsA (3: low, 9: mid, and 27: high mg/kg bw) or to olive oil (Ctrl), respectively. The CTLL-2 and Jurkat cells were exposed for 6 hours to CsA, N=4 biological replicates. CTLL-2 cells were exposed to 7.5 μM (low) or 15 μM (high) CsA, and Jurkat cells to 8 μM CsA or 13 mM DMSO (carrier). Equal amounts of total RNA molecules (800ηg/sample) were hybridized on Affymetrix mouse GeneTitan HT430PM arrays, or on human U133A plus 2.0 arrays. These transcriptomes were analysed at the levels of individual genes and at functional pathway level. Results and Conclusions: We found that the CsA target genes overlapped by 5% between CTLL-2 and Jurkat cells, and by 2% between the mouse *in vitro* and *in vivo* data, respectively. At the pathway level CsA affected (i) metabolism, protein synthesis, and apoptosis in the Jurkat and CTLL-2 cells *in vitro*, and (ii) metabolism, cellular processes, and apoptosis/cell death in the mouse cells, both *in vivo* (splenocytes), and *in vitro* (CTLL-2), respectively (FDR<0.10). In conclusion, at pathway level the immunosuppressive effects of CsA overlap between mouse immune cells *in vivo*, and mouse (CTLL-2) and human T-cells *in vitro* (Jurkat). At the individual gene level these overlaps are more limited. Based on our results CTLL-2 cells are a suitable model for toxicogenomics.

PS 1626 INHIBITORY EFFECTS OF AZOLE-TYPE FUNGICIDES ON INTERLEUKIN-17 GENE EXPRESSION VIA RETINOIC ACID RECEPTOR-RELATED ORPHAN RECEPTORS ALPHA AND GAMMA.

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The retinoic acid receptor-related orphan receptors α and γ (RORα and RORγ), are key regulators of helper T (Th) 17 cell differentiation, which is involved in the innate immune system and autoimmune disorders. However, it remains unclear whether environmental chemicals, including pesticides, have agonistic and/or antagonistic activity against RORα/γ. In this study, we investigated the RORα/γ activity of several azole-type fungicides, and the effects of these fungicides on the gene expression of interleukin (IL)-17, which mediates the function of Th17 cells. In the ROR-reporter gene assays, five azole fungicides (imibenconazole, hexaconazole, triflumizole, tetraconazole and imazalil) suppressed RORα- and/or RORγ-mediated transcriptional activity as the benzenesulphonamide T0901317, a known ROR inverse agonist and a potent liver X receptor (LXR) agonist. In particular, imibenconazole showed RORγ inverse agonistic activity at concentrations of 10-6 M order. However, unlike T0901317, these fungicides failed to show any LXRα/β agonistic activity. Next, five azole fungicides, showing ROR inverse agonist activity, were tested on IL-17 mRNA expression in mouse T lymphoma EL4 cells treated with phorbor myristate acetate and ionomycin. The quantitative RT-PCR analysis revealed that these five fungicides suppressed the expression of IL-17 mRNA without affecting RORα and RORγ mRNA levels. In addition, the inhibitory effect of imibenconazole, as well as that of T0901317, was attenuated in RORα/γ-knocked down EL4 cells. Taken together, these results suggest that some azole-type fungicides inhibit IL-17 production via RORα/γ. This also provides the first evidence that environmental chemicals can act as modulators of *IL-17* expression in immune cells.

PS 1627 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN INDUCES TRANSCRIPTIONAL ACTIVITY OF THE HUMAN POLYMORPHIC HS1, 2 ENHANCER OF THE 3'Igh REGULATORY REGION.

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2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is an environmental toxicant known to inhibit antibody secretion and Ig expression. Inhibition of Ig expression may be partially mediated through repression of the 3'Igh regulatory region (3'IghRR). TCDD inhibits mouse 3'IghRR activation and induces aryl hydrocarbon receptor (AhR) binding to dioxin response elements (DREs) within the 3'IghRR enhancers: hs1,2 and hs4. The human hs1,2 enhancer (hu-hs1,2) is polymorphic due to the presence of one to four invariant sequences (IS), which have been correlated with several autoimmune diseases. The IS also contains a DRE-like site. Therefore, the objective was to determine if hu-hs1,2 activity is sensitive to TCDD. Utilizing a mouse B-cell line (CH12.LX), we compared the effects of TCDD on mouse (mo-hs1,2) versus hu-hs1,2 enhancer activity. TCDD inhibited mo-hs1,2 similar to the inhibitory effect on mouse 3'IghRR activation. In contrast, hu-hs1,2 was activated by TCDD and antagonist studies supported an AhR-dependent activation. TCDD also induced hu-hs1,2 activity in a human B-cell line (IM-9). Absence of a Pax5 binding site is a major difference between the human and mouse hs1,2 sequence. Insertion of a Pax5 site in hu-hs1,2 markedly blunted basal reporter activity but did not alter TCDD's effect. Additionally, deletional analysis demonstrated a significant IS contribution to hu-hs1,2 basal activity but TCDD-induced activity was not strictly IS number-dependent. Taken together our results suggest that hu-hs1,2 is a significant target of TCDD and support species differences in hs1,2 regulation. Therefore, sensitivity of hu-hs1,2 to chemical-induced modulation may influence the occurrence and/or severity of human diseases associated with hu-hs1,2.

PS 1628 EFFECT OF LINDANE ON NITRIC OXIDE AND CYTOKINE RESPONSES IN RAW 264.7 MURINE MACROPHAGES.

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Lindane or gamma hexachlorocyclohexane is a persistent organochlorine pesticide that has been banned for agricultural use in the United States but remains an ectoparasiticide treatment for lice or scabies. Lindane may be present in potentially toxic levels in the environment and has been demonstrated to have immunotoxicity, developmental toxicity and genotoxicity. Our aim is to determine if exposure of RAW 264.7 murine macrophages to lindane has direct effects on their ability to respond to stimulation by bacterial Lipopolysaccharide (LPS) and interferon gamma (IFN). RAW 264.7 cells are maintained in continuous adherent culture with passage every 5-6 days. A concentration of $1x10^6$ cells per milliliter are prepared in a 24-well plate and grown overnight. Cells are pretreated with vehicle (DMSO), or a dose response of lindane (5, 50 or 200 μM) prepared in DMSO (final concentration less than 1%) for 24 hours. Following this exposure, cells are washed four times in culture medium and stimulated with a combination of 100 ng/ml LPS and 0.5 U IFN. After 24 hours of stimulation, supernantants are collected for analysis of nitric oxide (Greiss reaction) and cytokine (ELISA) production to determine macrophage functional responses. Cells are then lysed and proteins isolated for later Western blot analysis. Nitrite concentrations in supernatants were determined for no pretreatment controls (38.1 ± 2 μM) and DMSO vehicle controls (44.3 ± 3.4 μM) demonstrated no significant difference. Nitrite concentrations for lindane treatments of 5 μM (33.6 ± 1.7 μM), 50 μM (30 ± 4 μM) and 200 μM (26 ± 1 μM) treatments showed a significant decrease in nitric oxide response (ANOVA Dunnett's t-Test, P<0.05) in the 50 and 200 μM treatments compared to controls. Each value represents an average of four wells of treatment. Our initial findings suggest lindane has direct immunotoxic effects on macrophages in a model that could be used to understand previously demonstrated immunotoxic effects in the literature.

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