PS 2022 Elucidating the Role of the Polymorphic Human hs1, 2 Enhancer in the Effects of TCDD

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a potent environmental toxin known to inhibit immunoglobulin (Ig) gene expression in various animal studies. We have identified the mouse 3'Ig heavy chain regulatory region (3'IghRR) as a sensitive transcriptional target of TCDD, which may mediate the inhibitory effect of TCDD on Ig expression. Interestingly, the human hs1,2 enhancer is polymorphic and has been associated with a number of autoimmune diseases. Suggesting a species difference, TCDD inhibited mouse hs1,2 enhancer activation and activated basal human hs1,2 (hs-hs1,2) enhancer activity. The objective of this study was to determine the effect of stimulation and TCDD on hu-hs1,2 enhancer activity using a human B-cell line (CL-01) and luciferase reporter constructs regulated by each of the human hs1,2 alleles. Our results verity that TCDD alone activates each of the hu-hs1,2 alleles. Surprisingly, B-cell stimulation through TLR 7 and 8 by R848 inhibited basal activity of the hu-hs1,2 alleles and TCDD co-treatment reversed this inhibition. In contrast, R848 induced Ig secretion and activated a 3x NF-κB luciferase reporter, therefore confirming functional signaling through the TLRs and activation of the CL-01 B-cell line. R848 also induced class switch recombination from IgM to IgG. Furthermore, TCDD inhibited both IgM and IgG secretion in cells stimulated with R848. These results suggest that the hu-hs1,2 enhancer may be a negative regulator of 3'IghRR activity and Ig expression. Alternatively, the hs1,2 enhancer may function differently when studied in isolation as compared to its function in the intact 3'IghRR. Future studies will evaluate the effect of TCDD in the absence or presence of stimulation on the activity of the entire human 3'IghRR and its other enhancers, hs3 and hs4. Elucidating the role of the polymorphic hs1,2 enhancer in 3'IghRR activity and the effect of TCDD on the enhancers of the 3'IghRR may provide insights into the etiology of autoimmune diseases associated with the hs1,2 polymorphism. (Supported by NIEHS R01ES014676)

PS 2023 Benzo(a)pyrene Exposure Suppresses FcγRII (CD32)-IgG Antibody Complex Binding by Disruption of Lipid Raft Membrane Integrity

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The fundamental requirement in activation of macrophage effector functions is the binding of immunoglobulins to Fc receptors. The FcγRIIa (CD32) immunoreceptors have been reported to either constitutively reside in or associate with detergent resistant membranes (DRMs) upon binding to IgG, the most abundant Ab class in circulation. Previous research suggests that exposure to benzo(a)pyrene, B(a)P, an environmental toxicant, suppresses macrophage effector functions but the mechanism remains undefined. The purpose of this study was to elucidate the mechanism of B(a)P-induced suppression by examining the effects of B(a)P exposure on CD32-lipid raft interactions in the regulation of IgG binding to CD32. B(a)P exposure altered lipid raft integrity by depleting membrane cholesterol at over a 50% depletion rate (577.8 ± 204.6, $p < 0.001$). The exposures also lead to a 30% (216.2 ±, 142.7 $p < 0.05$) reduction in affinity or an exclusion of CD32 from lipid rafts. The 50% diminution in membrane cholesterol as well as the 30% exclusion of CD32 from lipid rafts caused significant suppression of CD32-mediated IgG binding by 60% (486.2 ± 166.7, $p < 0.001$) which suggests that intact lipid rafts are required for IgG complex binding to CD32. Future studies are directed at establishing whether B(a)P-induced suppression increases macrophage susceptibility to microbial infection.

PS 2024 Wear Particles Derived from Metal Hip Implants Generates Multinucleated Giant Cells in a 3-Dimensional Peripheral Tissue-Equivalent Model

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Multinucleate giant cells (MGCs) are created by the fusion of 5 to 15 monocytes or macrophages. MGCs can be generated by hip implants at the site where the metal surface of the device is in close contact with tissue. MGCs play a critical role in the inflammatory processes associated with adverse events like aseptic loosening of the prosthetic joints and bone degeneration as observed in some patients. Upon interaction with metal wear particles, endothelial cells can upregulate bioactive molecules and pro-inflammatory cytokines that can enhance a localized immune response. However, the role of endothelial cells in the generation of MGCs has not been completely investigated. We first developed a three-dimensional peripheral tissue-equivalent model (PTE) consisting of collagen gel, with a monolayer of endothelial cells on gel surface and human peripheral blood mononuclear cells (PBMCs) on top, which mimics peripheral tissue under normal physiological conditions. The cultures were incubated for 14 days with Cobalt chromium alloy (CoCr ASTM F75, 1-5 microns) wear particles, PBMC were allowed to transit the endothelium and harvested cells were analyzed for MGC generation using propidium iodide (PI) by flow cytometry. Increase in forward scatter (cell size) and in the PI uptake (DNA intercalating dye) was used as a measure of MGCs. Our results show that endothelial cells induce generation of MGCs 4 fold higher in 3-dimentional PTE system as compared to traditional 2-dimensional culture plates. Furthermore, we also checked whether increase in particle to cell ratio affects MGC generation. We found that increase in dose of particle to cell ratio decreases MGC generation compared to control, indicating that cells underwent apoptosis or necrosis with higher particle concentration. In sum, we have established a robust and relevant model to follow MGCs formation using flow cytometry. We observed a consistent generation of metal wear particle-induced MGCs which herald MoM hip failure.

PS 2025 Dermal Exposure to Triclosan Induces Changes in Expression of Innate and Adaptive Immune Genes in a Mouse Model

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Triclosan has had widespread use in the general population as an antimicrobial agent and is commonly found in consumer products such as soaps, deodorants, toothpastes, shaving creams, mouth washes, and cleaning supplies. Triclosan has recently attracted the attention of the scientific community, regulatory agencies and the general public because of its high production volume, widespread applications and reports of endocrine-disrupting effects. A positive association between urinary levels of triclosan and diagnosis of allergies, hay fever, and sensitization to aeroallergens and foods has been identified. While not generally considered to be a sensitizing chemical, work by our group has recently shown that dermal exposure to triclosan at concentrations similar to those in consumer products augmented the allergic response to a known allergen in a mouse asthma model. However, the specific mechanism of this augmentation has yet to be elucidated. These studies were conducted to investigate the mechanism responsible for the augmented allergic response following dermal triclosan exposure. BALB/c mice were exposed dermally on the ears to concentrations of triclosan ranging from 0.75-3% (0.375-1.5mg/mouse/day) for up to 9 consecutive days. Expression of immune genes in the ears and lymph nodes of mice following exposure was analyzed using quantitative polymerase chain reaction. Robust thymic stromal lymphopoietin (TSLP), IL-1 beta, TNF-alpha increases and modest CCL22 & IL-22 dose responsive increases in gene expression were observed in the ears. In the lymph node draining the exposure site, dose responsive increases in CCL22 & IL-4 and decreases in T-bet, IFN-gamma, TNF-alpha & IL-1beta gene expression were observed. A decrease in expression of RORgammat was observed in both the ear and lymph nodes following exposure to 3% triclosan. These results suggest that triclosan may augment allergic responses by modulating both innate and adaptive genes.

PS 2026 The α7 Nicotinic Acetylcholine Receptor Agonist GTS-21 Improves Bacterial Clearance in Mice by Restoring Hyperoxia-Compromised Macrophage Function

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Mechanical ventilation with supraphysiological concentrations of oxygen (hyperoxia) is routinely used to treat patients with respiratory distress. However, prolonged exposure to hyperoxia compromises macrophages' ability to phagocytose and clear bacteria. Previously, we have shown that hyperoxia induced the release of nuclear protein, high mobility group box-1 (HMGB1), into both the airways of hyperoxia-exposed mice and the extracellular milieu of cultured macrophages. Extracellular HMGB1 can impair macrophage phagocytosis and increase mortality of mice infected with *Pseudomonas aeruginosa* (PA). GTS-21, an α7nAChR agonist, can inhibit endotoxin-induced HMGB1 release. The aim of this study was to determine whether GTS-21 can inhibit hyperoxia-induced HMGB1 release into the extracellular milieu, enhance macrophage function, and improve bacterial clearance in mice under hyperoxic conditions. GTS-21 (0.04, 0.4, and 4 mg/kg)

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