the percentage of CD80+ DCs and monocytes, whereas FD stimulation increased the percentage of CD80+ cells. The percentage of ILT4+ cells was decreased in all cell types after PM stimulation. Similar phenomenon was seen after FD stimulation in monocytes and pDCs, but not in mDCs. Although PM samples induced parallel immune reactions, the strength of the effects was determined by the PM size-fraction. Conclusions: Studied environmental samples were able to modulate the immunogenicity and tolerogenicity of peripheral DCs and monocytes. The observed effects were partly opposite, suggesting that high risk and protective environments have differing capacities to influence properties of these cells.

1980 Hydraulic Fracking Sand Dust Produces a Pro-Inflammatory Response in Murine Macrophage Cells

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Hydraulic fracturing is used in the majority of natural gas wells across the United States. Water, sand, and chemicals are delivered at high pressure to drilled wells to cause fractures in the shale formations, allowing for the release of natural gas. Fracking sand, comprised mainly of silica dioxide (SiO_2), along with water and chemicals, is used to keep these fissures open. Silicosis is a pulmonary disease that affects workers exposed to inhaled silica and is characterized by inflammation and fibrosis, causing a decrease in lung capacity. Fracking sand dust (FSD) is generated during preparation of fracking fluid for injection. In this study, murine macrophage cells (RAW 264.7) were used to better understand the mechanisms of toxicity associated with inhaled FSD (< 10 µm). We hypothesized that the soluble and non-soluble components present in the FSD would each play a unique role in observed pro-inflammatory responses and cytotoxicity. FSD was washed in PBS twice, 5 days each time, allowing for any soluble material to be released (1st and 2nd washes, respectively). On the 10th day, sand that was twice washed was re-suspended in PBS (10mg/ml) so that comparisons could be made to a freshly prepared, unwashed mixture. Compared to similarly treated silica controls, the viability of cells (measured with a fluorogenic substrate) exposed to PBS from the 1st and 2nd washes were decreased by 40%, whereas unwashed sand decreased viability by 60% over a 24 h period. Intracellular ROS generation from sand washed once was significantly higher compared to sand that was washed twice. Unwashed FSD sand generated the most intracellular ROS and was significantly higher than sand re-suspended after two washes. Production of the hydroxyl radical (·OH) was also the highest in unwashed sand, followed by PBS from the first and second washes. Sand re-suspended in PBS after 10 days generated the least amount of ·OH. Finally, production of the pro-inflammatory cytokines IL-1β and TNFα were measured using ELISA. While IL-1β production decreased with washing, TNFα production remained elevated. Our results indicate that FSD is cytotoxic to RAW 264.7 cells, as evidenced by decreases in viability, and stimulates intracellular ROS and ·OH production. The stark differences in production between IL-1β and TNFα stimulated by the dust warrants future studies into the pro-inflammatory effects of its soluble and insoluble components.

1981 Effects of Cyanobacteria *Oscillatoria* sp. Lipopolysaccharide on B Cell Activation and Toll-Like Receptor 4 Signaling

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Background: Cosmopolitan Gram-negative cyanobacteria may contaminate freshwater by releasing toxins, such as lipopolysaccharide (LPS), thus affecting human and animal health. We recently reported that cyanobacterium *Oscillatoria* (OSC) sp. LPS elicited classical and alternative activation of rat microglia (BMG) *in vitro* (The Toxicologist, 144: Abstract 137, 2015). LPS activates cells both from the innate and adaptive immune system. LPS from other Gram-negative bacteria binds to toll-like receptor 4 (TLR4) on B cells to increase B cell activation. Due to the likely exposure of OSC sp. LPS to B cells found in the gut associated lymphoid tissue, we tested whether OSC LPS would stimulate TLR4 on murine B cells (MuB) *in vitro* to proliferate, phagocytose antigen, and produce IgM in a manner similar to LPS from another Gram-negative bacteria, *E. coli*. Methods: OSC LPS was prepared by hot phenol/water extraction. *E. coli* LPS (Ec LPS) 026:B6 from Difco Lab, Detroit, MI was used as a positive control. MuB were isolated from c57/Bl6 mice and treated *in vitro* with either OSC LPS, or Ec LPS in a concentration-dependent manner. Proliferation was determined using MTT assay, flow cytometry determined increased phagocytic ability, and IgM production was determined by ELISA. Western blot analysis was used to analyze OSC sp. TLR4 signaling. Results: Ec LPS, and OSC LPS stimulated a concentration-dependent increases in proliferation, phagocytosis, and IgM production, but OSC sp. was less potent than Ec LPS. Further experiments indicate that these changes were due to differences in TLR4 signaling. Conclusions: Our data indicate that OSC sp. LPS acts as an agonist to enhance B cell activation, albeit at a lower efficacy than other gut commensals such as Ec LPS. Nevertheless, this agonistic ability of OSC sp. LPS to activate B cells could significantly impact the mechanism by which *Oscillatoria* sp. cyanobacteria cause disease in humans and animals. Support by the Biomedical Sciences Program, College of Health Science, Midwestern University and the University of Hawaii at Manoa is gratefully acknowledged.

1982 Effect of Short-Term and Long-Term Exposure of Asbestos on Human T Cell Line MT-2

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Asbestos fibers cause mesothelioma and lung cancer. We propose that asbestos suppress anti-tumor immune system in addition to transformation of mesothelial and lung epithelial cells. It is considered that regulatory T cells, Treg produce inhibitory cytokines to suppress immune reaction against tumor cells. We employed human T cell line MT-2 cell as a model of Treg and cultured them with low concentration of asbestos for 8 months. MT-2 cells exposed with low-concentration asbestos for long term showed higher viability after treatment with high lethal dose of asbestos than original MT-2 cells, and they were designated as MT-2Rst. However, it is still unclear how asbestos induces apoptosis in MT-2 cells and molecular basis of resistance of MT-2Rst cells to high concentration of asbestos. Recently, we found that forkhead transcription factor FoxP3 plays an important role in regulation of apoptosis induced by asbestos. In this study, we analyzed the regulation of FoxP3 transcription in MT-2Org and MT-2Rst cells using luciferase reporter plasmids containing promoter of FoxP3 gene. However, there was no difference in FoxP3 reporter activity between MT-2Org and MT-2Rst. These results indicate that asbestos suppressed FoxP3 transcription through the epigenetic mechanisms. On the other hand, we examined the effect of asbestos on mitochondrial activity and found that asbestos induces mitochondrial dysfunction. Further studies are needed to clarify molecular basis of asbestos-induced apoptosis and modification of MT-2 cells by long-term treatment with asbestos.

1983 Triclosan Suppresses RBL-2H3 Mast Cell Function via Inhibition of Phospholipase D Activity

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Triclosan (TCS) is a synthetic antimicrobial agent used in many types of consumer products, resulting in detection in human various specimens. Mast cells are ubiquitous in the human body and play crucial roles by secreting myriad effectors (degranulation), such as histamine and other mediators of numerous physiological processes and diseases. Using rat basophilic leukemia, clone 2H3 (RBL-2H3) mast cells as well as human HMC-1 cells, we previously demonstrated that TCS inhibits mast cell degranulation. Using calcium ionophore stimulation to bypass early signaling events, we have found that TCS's suppressive effects are enhanced. We also found that TCS strongly inhibits membrane ruffling of antigen (Ag)-stimulated RBL-2H3 cells. These findings suggest that TCS's target in mast cells lies downstream of Ca^{2+} influx. Thus, we investigated downstream signaling molecules of Ca^{2+} rise, such as phospholipase D (PLD) and protein kinase C (PKC). PLD is expressed in RBL-2H3 cells in two isoforms, both of which are essential to the degranulation process. PLD1 is expressed within the cytoplasmic granules that undergo membrane fusion during degranulation whereas PLD2 is continually expressed along the cell surface. Here, using fluorescent-based PLD activity ELISA, we demonstrate that TCS inhibits the activity of PLD in Ag-stimulated RBL-2H3 mast cells. We also found that the PLD inhibitor, FIPI (5-Fluoro-2-indolyl des-chlorohalopemide), along with TCS exposure, produces additive inhibition of PLD activity. We are now investigating the ability of TCS to affect PKC, which works closely with PLD, to stimulate degranulation. These data provide a mechanism underlying TCS inhibition of mast cell degranulation.


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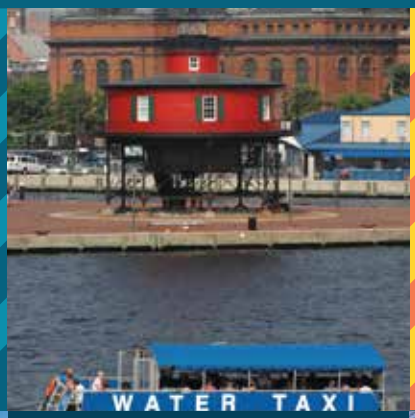

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