GWVI-RAC TOXICOGENOMICS

- NIEHS – Star Projects
- Chlorpyrifos fetal exposure
- Sarin Toxicogenomics
- Organophosphate and Neurotrophic Factors
- Epigenetics and Environmental Lung Disease
- Toll-Like Receptors in Human Disease
- Mitochondrial DAMPs Inflammatory Resp
- Cytokines in Soman status epilepticus

Toxicogenomics of Rat Brain Early Following Acute Sarin Exposure

Damodaran, et al at Duke University

Early post exposure global gene expression patterns revealed statistically significant alterations:

Ion channel, calcium channel, binding proteins, growth factors, G-protein coupled receptor path molecules, neurotransmitter transporters, cytoskeletal/cell adhesion molecules, mitochondrial assoc. proteins, myelin proteins, cytokines, gabanergics, and others.

These involve both protective and degenerative paths.

Toxicogenomics of Rat Brain Early Following Acute Sarin Exposure

- Altered mRNA expression of: Neurotransmitter transporters (SynaptoJanin 1, Syntaxin 6), Receptors of signaling systems (GABA-A), Receptor gated Ion channels (Cl,K), and Voltage dependent Ion channels (Ca).
- Over-expression of Arrestin (CAMKII) assoc with OPIDN and chronic desensitization/hyper-phosphorylation.
- Altered lipophil mRNA leading to myelin degradation.
- CREB transcription factor- persistent phosphorylation- Delayed Neurotoxicity.

Acute Phase Resp. (Cytokine) in Soma Induced Status Epilepticus Rat Brain

- Johnson et al – USAMRICD
- Measured levels of 10 Cytokines in rat piriform, hippocampus, and thalamus , 0-72 hours after seizure onset.
- Increased IL-1Alpha and IL-1 Beta in microglia producing neuronal damage and death.
- Increased IL-6 which is potentially neuroprotective seen in neurons and hypertrophic astrocytes.

Acute Phase Resp. (Cytokine) in Soman Induced Status Epilepticus Rat Brain

- Peaks at 12 hours, with IL-6 a plateau 12-24 hours.
- Other studies have shown increases in the corresponding MRNA of some of these Cytokines.

Toxicogenomic Profile in Maternal/Fetal Rat Brain After Gestational Chlorpyrifos

- Moreira- University of Washington, at Seattle
- Explored dose dependent alterations in transcriptional response in fetal and maternal C57BL/6(mice brain) after daily exposure.
- Performed gene ontology analysis.
- Gene expression dose-effect relationship was not necessarily related to AchE inhibition..

Toxicogenomic Profile in Maternal/Feta Rat Brain After Gestational Chlorpyrifos

Gene expression examined involved:

Cell adhesion, translation, synaptic transmission, neurogenesis, and long term potentiation.

Disturbance of Ubiquitin Proteasome System was also observed; this controls many cellular processes.

Organophosphate Exposure Effects on Fibroblast Growth Factor in Rat Brain

Slotkin et al – Duke University

Exposures to Chlorpyrifos/Diazinon on neonatal days 1-4 below systemic toxicity threshold but spanning threshold for barely detectable cholinesterase inhib.

Used a microarray to measure mRNA for FGF and FGF receptors.

Both chemicals suppressed FGF-20 in forebrain and FGF -2 & 22 in brainstem, while increasing FGF R -4 in brainstem, however FGF -2 and FGF R-4 effects were greater for Diazinon, and there were dissimilar results for FGF -1, 14, and FGF R – 1.

Organophosphate Exposure Effects on Fibroblast Growth Factor in Rat Brain

Robust effects were seen even at doses which did not inhibit cholinesterase.

Differences between the agents effects were unrelated to the degree of cholinesterase inhibition.

Note- FGF-2 & 20 are involved in hippocampus and striatum development and are targets of organophosphate neuro-developmental effects as well as assoc with loss of Dopamine neurons in Parkinson's Dx.

Epigenetics and Environmental Lung Disease

Schwartz – National Jewish Health, Denver

Epigenetic mechanisms mediate the effect of the environment on the human genome by controlling the transcriptional activity of specific genes, at specific points in time, in specific organs.

3 Mechanisms: DNA methylation, Non-coding RNAs, and Histone modification.

In COPD alveolar macrophages have increased histone acetyl-transferase and decreased histone de-acetylase affecting transcriptional regulation of inflammatory mediators

Epigenetics and Environmental Lung Disease

- These findings are the opposite in steroid treated asthmatics.
- In a murine model using fetal DNA methylation produced T-cell phenotypes predisposing to airway disease which was reversed by de-methylating agents postnatally.
- Smoking exposure in utero causes DNA hyper-methylation associated with childhood asthma.

Effect of Toll-Like Receptors/Genetics in Human Disease

Garantziotis et al – Duke University / NIEHS

- TLRs enable immune recognition of exo/endogenous prototypic ligands while orchestrating innate/adaptive immune response.
- Genetic variations affect this response through polymorphism creating variability.
- TLRs have been genetically conserved through evolution/across diverse species.
- Pathogen Associated Molecular Patterns (PAMPS) are recognized by TLRs which initiate a response.

Effect of Toll-Like Receptors/Genetics in Human Disease

TLR signaling is mediated through myeloid differentiation factor 88 and some independent pathways, via IL-1 receptor associated kinase and TNF receptor assoc factor 6.

TLR-4 ligand exposure on farm decreases asthma risk.

TLR-4 mediates inflammatory response to atherogenic stimuli in C-V disease.

TLR-4 hypo-responsive polymorphisms of NOD2 which eliminates salmonella is assoc with Crohn's dx

TLR-5 in SLE late phase amp. of adaptive response.

Effect of Toll-Like Receptors/Genetics in Human Disease

Risk Stratification

(certain polymorphisms associated with increased susceptibility).

Therapeutic modification of TLR signaling.

Mitochondrial DAMPs Cause Inflammatory Responses to Injury

Zhang et al - Harvard Medical School and Queen Mary's University Hosp of London.

Injury produces a Systemic Inflammatory Response Syndrome (SIRS) just as PAMPs in infection produce an immune response- Damage Associated Molecular Patterns (DAMPs) produce an immune response to trauma.

Mitochondrial DAMPs (formyl peptides and mitochondrial DNA) released by injury produce immune stimulation.

Mitochondrial DAMPs Cause Inflammatory Responses to Injury

Activate PMNs by formyl peptide receptor-1 and TLR-9- promoting PMN calcium flux and phosphorylation of mitogen activated protein (MAP) kinases causing PMN migration and degranulation resulting in organ damage.

Mitochondrial DAMPs(MTDs) are similar to bacterial PAMPs creating a sepsis like state and collateral organ damage.

MTDs increase pulmonary albumin permeability in rat lung, IL-6 and TNF-Alpha with PMN infiltration.

NIEHS – STAR Project: Genetic Mod. of Hydrocarbon Solvent Neurotoxicity

- Spencer et al- OHSU
- 2,5 Hexanedione (and 1,2 Diacetly Benzene) cause axonal degeneration by reacting with neuro-filament Lysine forming pyrrole polymerization accumulating in neurofilaments.- Blocked by Glutathione.
- Glutamate –Cysteine ligase and Glutathione Sythetase are rate limiting steps in Glutathione biosynthesis.

NIEHS – STAR Project: Genetic Mod. of Hydrocarbon Solvent Neurotoxicity

- Mice and rats up-regulate GCL and GSH synthesis in response to pr0-oxidant chemical exposures.
- Plan to use inducible GCL transgenic mice exposed to 2,5 HD and 1,2 DAB (as well as non-neurotoxic 1,3 DAB and 1,4 HD) and observe results.

NIEHS – STARS Project: Comparative Genomic Responses to Env. Toxicants

Freedman et al – DUKE University/NIEHS

Organisms respond to toxins by activating transcription of genes encoding proteins that repair, defend, remove or metabolize toxins.(such as superoxide dismutase, glutathione transferase, DNA repair enzymes and others).

Plan to test Candida elegans, Zebrafish embryo, mice.

Exposure to Endo/mycotoxin, Cadmium, Diquat, and N-Methyl-Nitro-Nitroso-Guanadine (MNNG)

Comparative Genomic Responses to Env. Toxicants

Identify the expression profiles for toxicants, the genes whose transcriptional responses are conserved, and define the regulatory pathways controlling the response.