Completed Research Projects

Project Title: Superantigen-induced T Regulatory Cells as a Cell-based Treatment for EAE
Sponsor: NIH, Center for Biomedical Research Excellence in Pathogen-Host Interactions, P20
Investigators: B.L. Kaplan, Junior Investigator

The goal of this project is to determine if low dose superantigens derived from *S. aureus* will induce regulatory T cell populations in mice**.**

Project Title: Brain-penetrating Acetylcholinesterase Reactivators for Several Organophosphates
Sponsor: NIH/NINDS/CounterACT U01
Investigators: J.E. Chambers, PI; R.L. Carr; H.W. Chambers

This project will establish data on novel chemicals invented to be more effective antidotes to counteract the toxicity induced by nerve agents or highly toxic insecticides. Such poisons could be used by terrorists against civilian populations. Our novel chemistries have the potential to not only save lives but also penetrate the blood-brain barrier and protect the brain from long-term damage caused by these poisons.

Project Title: Pilot Study on Human Erythrocyte Ghost Preparation
Sponsor: Exponent Health & Environmental
Investigators: J.E. Chambers, PI

The project will characterize the erythrocyte ghost (cell membrane) preparation from human blood for subsequent studies on the inhibition kinetics of several organophosphate insecticides or their active metabolites.

Project Title: Lipid Glyceryl Ester Homeostasis in Macrophages and Perturbation by Environmental Toxicants
Sponsor: NIH
Investigator: M.K. Ross, PI

This renewal application will elucidate the mechanisms by which endogenous toxins (oxidized low-density lipoproteins) and exogenous toxicants (pesticides) can together dysregulate the endocannabinoid system in macrophages, thus enhancing foam cell development. Combination therapies that utilize both cannabinoid (CB) 1 receptor antagonists and CB2 receptor agonists could be developed to restore homeostasis within the vessel wall, thereby promoting human health. This study will help to determine the feasibility of this approach.

Project Title: Role of Endocannabinoids in Atherosclerosis
Sponsor: NIH F31
Investigators: A.T. Matthews, PI; M.K. Ross

This is a pre-doctoral fellowship to study whether endocannabinoid biosynthesis is enhanced following ligation of the macrophage scavenger receptor CD36 by oxidized low-density lipoprotein as part of a compensatory mechanism to counteract inflammation and oxidative stress. Specifically, this project will

determine whether diacylglycerol lipase β (DAGLβ), the rate-limiting biosynthetic enzyme of the endocannabinoid 2-arachidonoylglycerol, is activated via transduction of NADPH oxidase-derived reactive oxygen species.

Project Title: Effect of Exposure to Persistent Organic Pollutants on the Development of Hepatic Steatosis and Steatohepatitis
Sponsor: MSU CVM Office of Research and Graduate Studies
Investigators: G.E. Howell, III, Co-PI

The present study seeks to determine the effects of exposure to the widely utilized organophosphate pesticide, chlorpyrifos (CPS), on both basal and insulin-induced alterations in hepatic lipid metabolism. These studies will determine the effects of direct CPS exposure on molecular mediators of insulin-induced hepatic *de novo* lipogenesis at the cellular level utilizing both immortalized McArdle-RH7777 hepatoma cells as well as primary hepatocytes. CPS induced alterations in both metabolic phenotype and regulation of molecular mediators of hepatic lipid metabolism will be determined at the systemic level in both normal and diet-induced, type 2 diabetic mice. Successful completion of these studies will reveal the mechanisms governing CPS-induced hepatic *de novo* lipogenesis and determine if exposure to CPS exacerbates hepatic steatosis and hypertriglyceridemia in high fat fed mice, a widely utilized model of type 2 diabetes.

Project Title: Survey of Biomarkers Related to Health Disparities in the Mississippi Delta Population
Sponsor: MSU/ORGS
Investigators: J.E. Chambers, PI; J.A. Crow; R.W. Wills

Project Title: Development of Paraoxonase (PON) Enhancers to Accelerate the Efficiency of PON-Mediated Nerve Agent Hydrolysis
Sponsor: DoD/DTRA
Investigators: J.E. Chambers, PI; H.W. Chambers; S.R. Gwaltney

Paraoxonase (PON) is an enzyme associated with the serum high density lipoprotein particle and its activity is protective of cardiovascular health. PON also has a limited ability to destroy organophosphates, such as nerve agents. This project seeks nucleophilic small molecules that can enhance the PON-mediated destruction of organophosphates. Such nucleophiles may have potential as antidotes to protect against nerve agent intoxication.

Project Title: New Environmental Public Health Indicator Linking Organochlorine Compounds and Type 2 Diabetes
Sponsor: EPA/STAR
Investigators: J.E. Chambers, PI; J.A. Crow; M.K. Ross; R.W. Wills

This project seeks to identify an Environmental Public Health Indicator, which will link environmental levels of a contaminant with levels in people that are associated with a specific disease. The approach is to determine the levels of legacy organochlorine pesticides (or their metabolites) in soils and in human

serum, and to determine whether there is an association between these levels of chemicals and the presence of type 2 diabetes.

Project Title: Targeting the Endocannabinoid System to Enhance Immunity
Sponsor: USDA/Mississippi Food Safety Initiative
Investigators: M.K. Ross, PI; M. Edlelmann, J.H. Lee, S. Borazjani

The goal of this project was the identification of serine hydrolases in chicken macrophages that can be targeted (i.e. inhibited) by small molecules for the purpose of enhancing endocannabinoid levels during microbial infection, and whether the microbicidal activity of the macrophages is concomitantly enhanced.

Project Title: Effects of Leukoreduction on Eicosanoid Biosynthesis in Stored Canine Packed Red Blood Cells
Sponsor: Morris Animal Foundation
Investigators: M.K. Ross, Co-PI; J. Thomason, PI

This project examines whether storage of canine packed red cells leads to the increased production of bioactive eicosanoids.

Project Title: The Role of Exposure to Bioaccumulative Organochlorine Compounds on the Occurence of Obesity and Type 2 Diabetes
Sponsor: NIH/NIEHS
Investigators: G.E. Howell, III, PI; J.E. Chambers

Analysis of recent epidemiological data gathered in the National Health and Nutrition Examination Survey from 1999-2001 (NHANES) determined that there is a strong correlation between serum concentrations of organochlorine compounds including oxychlordane and p',p-DDE and the occurrence of insulin resistance and type 2 diabetes. These organochlorine compounds are persistent organic pollutants that are stored in fat tissue and bioaccumulate through the food chain. Almost all Americans over the age of twelve have detectable levels of these compounds in their bloodstream. Thus, the goal of my current research is to delineate the mechanism by which these compounds may elicit or exacerbate insulin resistance and type 2 diabetes. Currently, I am utilizing NIH3T3-L1 adipocytes as a model to determine the effect of these organochlorines on adipogenesis and expression of cytokines/adipokines that have been determined to cause insulin resistance. In addition, the effect of these compounds on insulin resistance in the two major sites of systemic glucose disposal, the adipose tissue and the skeletal muscle is being explored using NIH3T3-L1 and L6 myotubules, respectively. The overall goal of my present studies is to determine if exposure to these compounds can elicit insulin resistance in these target tissues and to determine the mechanisms by which this occurs.

Project Title: A Longitudinal Study of Paraoxonase 1 (PON1) in Relationship to Type 2 Diabetes and Aging
Sponsor: Institute of Medicine of the National Academies/Air Force Health Study
Investigators: J.E. Chambers, PI; J.A. Crow; R.W. Wills.

This pilot project will obtain 250 human serum samples and selective health and demographic information from veterans of the Vietnam conflict who were sampled at 10, 20 and 30 years ago. One objective of the study are to investigate the possible age-related decline in the serum enzyme paraoxonase 1 (PON1) in the same individuals. The second objective is to determine whether lower activities of PON1 may serve as a predictive biomarker for risk of type 2 diabetes.

Project Title: In Vitro Sensitivity Study of Rat Tissue Cholinesterase to Chlorpyrifos-Oxon Inhibition
Sponsor: Dow AgroSciences LLC
Investigators: J.E. Chambers, PI

This project determines the potency of an organophosphate insecticide active metabolite to inhibit the target enzyme, acetylcholinesterase, in several tissues.

Project Title: Survey of Biomarkers Related to Health Disparities in the Mississippi Delta Population
Sponsor: MSU/ORGS
Investigators: J.E. Chambers, PI; J.A. Crow; M.K. Ross; R.W. Wills

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Project Title: Molecular Biology of Mesocortical and Mesoaccumbens Dopamine Neurons
Sponsor: NIH/NIEHS
Investigators: J.B. Eells, PI; J.E. Chambers

Aberrant dopamine neurotransmission has been implicated in schizophrenia with elevated mesoaccumbens (or subcortical) dopamine neurotransmission contributing to positive symptoms of schizophrenia (paranoia, hallucinations, delusions, and bizarre behavior) and attenuated mesocortical dopamine neurotransmission affecting negative symptoms (social withdrawal, blunted emotions, and cognitive deficits). Targeting the dopamine system has proven effective at treating positive symptoms of schizophrenia as all currently approved antipsychotics block the dopamine D2 receptor but the efficacy of antipsychotic at alleviating negative symptoms is limited. The mesocortical and mesoaccumbens dopamine neurons have distinct afferent innervation, pharmacology, neurochemistry and electrophysiology properties; however, there is an important gap in information about the differential genes expression that underlie the functional differences of these two dopamine neuron populations and how they are regulated. The specific aims of the current proposal are to elucidate differential gene expression between mesocortical and mesoaccumbens dopamine neurons and the effect of inactivation of the prefrontal cortex on regulation gene expression of these dopamine neurons. To achieve these aims, the combination of rapid tyrosine hydroxylase fluorescent immunocytochemistry and a fluorescently labeled retrograde tracer will be used to visualize specific populations of dopamine neurons and laser capture microdissection will be used to capture these neurons and isolate RNA. These experiments are expected to elucidate unique gene expression profiles and differential regulation of gene expression. Once we understand the features that distinguish these dopamine neuronal populations and how they are regulated, we can begin to investigate strategies to specifically target either of these systems to control abnormal dopamine neurotransmission in pathological conditions such as schizophrenia.

Project Title: Organophosphate-mediated Inhibition of Cholesteryl Ester Hydrolase
Sponsor: NIH/NIEHS
Investigators: M.K. Ross, PI; J.A. Crow

Cardiovascular disease (CVD) in its various forms is the leading cause of death in the United States. Reverse cholesterol transport is a mechanism by which cholesterol present in atherosclerotic plaques within arterial walls is transported to the liver via high density lipoprotein particles for excretion in bile. Recent studies have suggested that human cholesteryl ester hydrolase (CEH), an enzyme that metabolizes cholesteryl esters, plays an important role in the regulation of reverse cholesterol transport. Our long term goal is to understand the role that environmental toxicants such as agricultural chemicals play in human disease. Three commonly used organophosphate (OP) insecticides will be used in this study. The goal of this project is to determine if exposure to OP insecticides will inhibit the CEH-catalyzed metabolism of cholesteryl esters, which could therefore increase the risk of developing atherosclerosis. The results from this study will provide preliminary insights into whether OP oxon metabolites can directly alter the structure-function of an enzyme involved in cholesterol metabolism, thus leading to an increased probability of a pathological outcome (i.e. atherosclerosis).

Project Title: Effect of Organophosphate Exposure on Cholesteryl Ester Hydrolase (R15 Administrative Supplement)
Sponsor: NIH/NIEHS
Investigator: M.K. Ross, PI

This administrative supplement will extend the aims of our parent grant to study the effects of organophosphate (OP) pesticides on other genes and proteins besides CES1 that participate in cholesterol metabolism. The effects of OP pesticides on the abundance and activities of these proteins in cholesterol-loaded human THP1 macrophages using RT-PCR, western blotting, and functional assays (e.g., cholesterol efflux and cholesterol mass determination) will be examined.

Project Title: Effect of Organophosphates on Cholesteryl Ester Hydrolase (R15 Competitive Supplement)
Sponsor: NIH/NIEHS
Investigators: M.K. Ross, PI; J.A. Crow

It will be determined if the endocannabinoid tone of vessel wall macrophages can be significantly perturbed by chronic exposure to bioactive OP metabolites, thus resulting in an activated endocannabinoid system that modulates cholesterol metabolism in macrophages.

Project Title: Regulation and Function of Nurrl in Adult Nigrostriatal Dopamine Neurons
Sponsor: NIH/NIEHS
Investigator: J.B. Eells, PI

Cardiovascular disease (CVD) in its various forms is the leading cause of death in the United States. Reverse cholesterol transport is a mechanism by which cholesterol present in atherosclerotic plaques within arterial walls is transported to the liver via high density lipoprotein particles for excretion in bile. Recent studies have suggested that human cholesteryl ester hydrolase (CEH), an enzyme that metabolizes cholesteryl esters, plays an important role in the regulation of reverse cholesterol

transport. Our long term goal is to understand the role that environmental toxicants such as agricultural chemicals play in human disease. Three commonly used organophosphate (OP) insecticides will be used in this study. The goal of this project is to determine if exposure to OP insecticides will inhibit the CEH-catalyzed metabolism of cholesteryl esters, which could therefore increase the risk of developing atherosclerosis. The results from this study will provide preliminary insights into whether OP oxon metabolites can directly alter the structure-function of an enzyme involved in cholesterol metabolism, thus leading to an increased probability of a pathological outcome (i.e. atherosclerosis).

Project Title: Synthesis and Assay of Novel Pyridinium Oximes with Potential Activity in the Central Nervous System for Reactivating Phosphorylated Acetylcholinesterase
Sponsor: DoD/DTRA
Investigators: J.E. Chambers, PI; H.W. Chambers

The objective of this grant is to synthesize and test, both *in vitro* and *in vivo*, novel oximes that can cross the blood-brain barrier and therefore will be capable of reactivating brain acetylcholinesterase (AChE) that has been inhibited by surrogates for the nerve agents sarin and soman. The synthesis will produce a series of phenoxyalkyl pyridinium oximes that are sufficiently lipophilic to cross the blood-brain barrier. These experiments will be performed at MSU within the Center for Environmental Health Sciences. This research at MSU will complement the on-going intramural research on physiologically-based pharmacokinetic/pharmacodynamic (PBPK/PD) modeling occurring at the AFRL/HEPB, Wright Patterson Air Force Base, Ohio. In summary, this project will synthesize novel lipophilic phenoxyalkyl pyridinium oximes, will compare these newly synthesized oximes to the currently approved oxime reactivator 2-PAM in *in vitro* and *in vivo* studies, and will perform PBPK/PD modeling to gain a better understanding of the disposition of the oxime and chemical warfare agents.

Project Title: Synthesis and Assay of Novel Pyridinium Oximes with Potential Activity in the Central Nervous System for Reactivating Phosphorylated Acetylcholinesterase
Sponsor: DoD/DTRA
Investigators: J.M. Gearhart, PI*; J.E. Chambers; H.W. Chambers

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Project Title: Relationship of Blood Esterases, Pesticide Exposure and Cardiovascular Disease
Sponsor: NIH/NIEHS
Investigators: J.E. Chambers, PI; H.W. Chambers; J.A. Crow; M.K. Ross; R.W. Wills

This R21 grant is designed to solidify an interdisciplinary team of basic and , and to position this team for participation in larger-scale on-going multi-institutional epidemiological studies. This health disparity in CVD is logically related to risk factors more prevalent in the South (which has a higher proportion of African Americans than other regions in the country). The South is rural and highly agricultural, so the Southern populations would be expected to be routinely exposed to higher levels of pesticides than populations in many other regions. The following hypothesis will be studied: The effects of pesticide exposure contribute to the development of CVD and are more pronounced in the African American population.

Project Title: The Effect of a Mixture of Pesticides on the Rat Cardiac Proteome
Sponsor: NIH/NIEHS
Investigator: J.A. Crow, PI

Cardiovascular disease is the leading cause of death in the U.S. today and has been so for the last century. Approximately 70 million Americans suffer from some form of cardiovascular disease and many require ongoing pharmacological therapy. Mississippi has the highest age-adjusted death rate from cardiovascular disease of any state in the U.S. Mississippi has an agricultural based economy and much of the population resides in rural settings close to where crops are grown. This proximity results in the contact of the population with current use pesticides as well as legacy pesticides, those pesticides used in the past but persistent in the environment today. This grant proposes to examine the effects of two environmentally relevant pesticides, dieldrin and chlorpyrifos, on the rat cardiac proteome. The goal of this grant is to identify proteins whose levels of transcription and/or post-translational modifications are altered by exposure to these two pesticides. Our long-term objective is to better understand the extent to which commonly used pesticides and other environmental chemicals influence heart disease and its treatment.

Project Title: Molecular Modeling to Develop Better Reactivators
Sponsor: DoD/DTRA
Investigator: S.R. Gwaltney, PI

Nerve agents are a continuing threat, both in wartime and as potential terrorist weapons. Currently, a number of oximes have been deployed or are under consideration as treatment for nerve agent exposure. But one of the primary problems with developing new treatments is a lack of knowledge of how these compounds interact with their target protein on the atomic level and of the atomic-scale forces and interactions that determine the efficacy of an oxime. This project uses molecular modeling to provide a better understanding of the reaction by which oximes reactivate cholinesterases that have been inhibited by nerve agents. The goal is to aid in developing new therapeutics via rational drug design using the information generated by this project.

Project Title: An Integrated Computational Framework for the Interpretation of Organophosphorus Pesticide Biomarkers
Sponsor: EPA/STAR
Investigators: B. Reisfeld, PI; J.E Chambers; R.S.H. Yang; A.N. Mayeno; M.A. Lyons

Although various biomarkers have been used to assess exposure to and poisoning from organophosphorus (OP) pesticides/insecticides, the complexity of OP absorption, distribution, metabolism and elimination, especially for mixtures of these chemicals, warrants integration of computational modeling tools with the biomarker data for more accurate quantitation and assessment of actual whole body exposures and target tissue dosimetry. The objective of this project is to create a computer-assisted framework to aid in the identification, characterization, and understanding of biomarkers resulting from human exposure to mixtures of OP insecticides, using chlorpyrifos and diazinon as the initial test compounds. The framework will use existing human biomarker data, along with information about population and exposure variability and uncertainty, to reconstruct absorbed dose and exposure scenarios, as well as to predict levels of biomarkers resulting from known exposures to one or multiple OP insecticides. The software tool developed, and targeted data acquired, will be

useful in the interpretation of biomarkers indicative of exposure to OP insecticide mixtures, including the effects of population and dose variability and uncertainty. Therefore, the outcome of this project will be a more accurate approach to the assessment of exposure from these mixtures in the cumulative risk assessment process, and therefore the overall benefit will be an improvement of EPA's ability to protect public health.

Project Title: Association of Plasma Organochlorine Levels with Cardiovascular Disease and Diabetes
Sponsor: MSU/ORED
Investigator: J.A. Crow, PI

In 2007, 23.6 million Americans or 7.8% of the population was estimated to have either diagnosed or undiagnosed diabetes (Centers for Disease Control and Prevention[CDC]). The incidence and prevalence of diabetes has been increasing in the U.S. for at least 10 years. Type 2 diabetes (T2D), previously called non-insulin dependent diabetes mellitus (NIDDM), comprises between 90% to 95% of all cases of diabetes and is responsible for most of the increased prevalence. In addition to those diagnosed with diabetes, as many as 57 million Americans were estimated to have prediabetes which frequently progresses to T2D. Diabetes was the seventh leading cause of death in the U.S. in 2006 and increases the risk of developing numerous other diseases such as heart disease and stroke. Within the U.S. certain populations and geographic regions are at higher risk for diabetes than the population as a whole. Minority groups in general and especially African Americans are at higher risk for T2D and its complications. Currently, 3.7 million or 14.7% of African Americans over the age of 20 are estimated to have diabetes. In addition, Mississippi has had the highest prevalence of diabetes of any state in the U.S (CDC). Studies have shown an association between the serum level of persistent organic pollutants (POPs) and diabetes. Interestingly, the POPs identified in these studies included several organochlorine (OC) pesticides or their metabolites. Prior to 1970, OC pesticides were applied in large amounts in Mississippi especially in the highly agrarian Delta region of the state where a large proportion of the African American population resides. OC pesticides and their metabolites are still present in the soil in these regions. The role that POPs and more specifically OC pesticides play in the high prevalence of diabetes in Mississippians in general and in African Americans in particular is unknown. *Given these findings, our central hypothesis is that high plasma levels of OC pesticides and/or their metabolites in Mississippians will be associated with the development and progression of T2D and prediabetes and contribute to the elevated prevalence of cardiovascular disease.* We are currently investigating the association of T2D, prediabetes, and cardiovascular disease with the plasma levels of OC pesticides and/or their metabolites in a group of Mississippians recruited from a local cardiology clinic.

Project Title: Effect of Organochlorine Exposure on Adipose Tissue Insulin Sensitivity and Inflammatory Status
Sponsor: MSU/CVM/ORGS
Investigator: G.E. Howell, III, PI

Analysis of recent epidemiological data gathered in the National Health and Nutrition Examination Survey from 1999-2001 (NHANES) determined that there is a strong correlation between serum concentrations of organochlorine compounds including oxychlordane and p',p-DDE and the occurrence of insulin resistance and type 2 diabetes. These organochlorine compounds are persistent organic pollutants that are stored in fat tissue and bioaccumulate through the food chain. Almost all Americans

over the age of twelve have detectable levels of these compounds in their bloodstream. Thus, the goal of my current research is to delineate the mechanism by which these compounds may elicit or exacerbate insulin resistance and type 2 diabetes. Currently, I am utilizing NIH3T3-L1 adipocytes as a model to determine the effect of these organochlorines on adipogenesis and expression of cytokines/adipokines that have been determined to cause insulin resistance. In addition, the effect of these compounds on insulin resistance in the two major sites of systemic glucose disposal, the adipose tissue and the skeletal muscle is being explored using NIH3T3-L1 and L6 myotubules, respectively. The overall goal of my present studies is to determine if exposure to these compounds can elicit insulin resistance in these target tissues and to determine the mechanisms by which this occurs.

Project Title: Chemical Knockdown of Carboxylesterases
Sponsor: MSU/CVM/ORGS
Investigator: M.K. Ross, PI

The goal of this study is to use small-molecule inhibitors of carboxylesterases (CEs) to study their physiologic function in mice and to identify endogenous substrates of this hydrolytic enzyme.