IIT UPDATE

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Tewari-Singh Lab Investigating the Immune Mechanism of Mustard Gas Toxicity

IIT-affiliated faculty member Neera Tewari-Singh recently began work on a Department of Defense Tier 1 Discovery award focused on identifying a novel immune mechanism of mustard gas (sulfur mustard; SM) toxicity, which could also be applicable to other chemical exposures in Gulf War Illness (GWI). The grant, "Mast cells in sulfur mustard exposure: novel targets for modulation to develop therapies against the long-term health effects in Gulf War Veterans," is funded through the Gulf War Illness Research Program in the Department of Defense Congressionally Directed Medical Research Programs.

It is reported that during the Gulf War (1990-1991) U.S. troops could have been exposed to a number of chemicals including the low levels of chemical warfare agents (CWAs) like SM. Studies in the U.S. and other locations have dependably established that approximately 25-32% of Gulf War veterans suffer from a disorder with variable symptoms including fatigue, headaches, cognitive dysfunction, musculoskeletal pain, and respiratory, gastrointestinal and dermatologic complaints. These symptoms of GWI relate closely to the long-term consequences observed with SM exposure. Mast cells are well known to contribute to allergic inflammatory diseases, but also have wide ranging effects on many physiological systems that are affected in GWI including pulmonary, dermal, gastrointestinal and nervous systems when activated (e.g. degranulation). Importantly, a role for mast cells has been suggested in the mechanism of vesicating chemical agents like SM-induced inflammatory response and tissue damage.

Tewari-Singh, under this project, will embark on understanding the role of mast cell-induced immune responses in vesicant inhalation and skin exposures using nitrogen mustard [NM; bis(2-chloroethyl) methylamine] as a surrogate for SM. Tewari-Singh and her team are conducting cell culture studies to elucidate mast cell-induced immune responses following mustard vesicating agent exposure. Further, in vivo studies in mast cell and wild-type mice are also planned, to confirm the role for mast cells in NM-induced skin and lung toxicity as well as inflammation.

For over a decade, Tewari-Singh has worked with the National Institutes of Health's Countermeasures Against Chem-

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Wu Joins Team Awarded $9.8 Million to Study Low-Moisture Food Safety

IIT-affiliated faculty member, **Felicia Wu**, joins a team of researchers awarded a five-year, $9.8 million grant from the U.S. Department of Agriculture National Institute of Food and Agriculture (USDA NIFA) that takes a holistic look at reducing threats of pathogens in low-moisture foods. NIFA has designated the project as a Center of Excellence, meaning it has high merit value and meets criteria for broad impact. The project is led by Dr. Bradley Marks, professor and chair in the Michigan State University Department of Biosystems and Agricultural Engineering. Dr. Wu leads the team on a theme devoted to integrated risk, economic, and sustainability analyses for low-moisture food systems to improve food safety, which take into account reduced foodborne disease outbreaks, costs of technologies, behavioral change, and energy and water use.

Low-moisture foods, like cereals and flour, dried fruit and nuts, have been recalled repeatedly in the last few years, posing health risks to consumers and economic threats to businesses.

The grant team of economists, engineers, microbiologists, consumer educators and risk modelers, is working to reduce the risk of Salmonella, E. coli and Listeria from harvest to consumer.

In addition to John A. Hannah Distinguished Professor, Felicia Wu, MSU researchers Sanghyup Jeong, assistant professor in BAE, and Elliot Ryser, professor in the Department of Food Science and Human Nutrition (FSHN), are part of the grant team. Other members are from Purdue University, Ohio State University, Washington State University, University of California-Davis, the University of Arkansas, the Illinois Institute of Technology, and the U.S. Food and Drug Administration (FDA). Additionally, North Carolina State University will be conducting the external evaluation of the project impacts.

Low-moisture foods are used as ingredients in a variety of products, so if one supplier faces a recall, numerous items could be affected. One recall or outbreak could put a small operation out of business.

E. coli, Salmonella and Listeria can't be completely eliminated from dried fruits, nuts, flour and cereals. However, their occurrence can be reduced — and Marks and his team are looking forward to developing solutions to do just that.

Marks and his team will look at wheat flour, dried apples and almonds as case studies that can be applied to other low-moisture foods.

A major component of the grant is creating a food safety culture, or an established understanding of the importance of food safety, as a measure to reduce outbreaks.

"You really have to make sure everybody from harvest to the consumer understands their role in ensuring food safety," Marks said. "We will develop training and educational resources that help advance that goal."

Read more of this article that originally appeared on CANR: https://www.canr.msu.edu/news/michigan-state-university-researcher-awarded-9-8m-to-study-low-moisture-food-safety.

Upham Awarded R21 Grant to Develop High-Throughput Toxicity Screening Assay

IIT-affiliated faculty member, **Brad Upham**, was recently awarded a $445,403 National Institutes for Environmental Health Sciences R21 grant for his project, "High-throughput toxicity screening of environmental contaminants and drug candidates using a novel gap junction intercellular communication bioassay in lung and liver cells."

In recent decades, new "emergent" chemicals used in industry, transportation, agriculture, and urbanization are entering the environment at increasing levels as hazardous wastes that are often nonbiodegradable. Many of these emerging contaminants have been discovered and quantified in living organisms, including humans; however, the adverse health effects to environmental exposure on wildlife and the general population are largely unknown. With this grant, Upham will be developing a new screening system to identify environmental contaminants or drug candidates that can cause adverse health effects. The development of this new screening system will provide additional and critical new information to assess the potential toxicity of these emergent chemicals in our environment.

In vitro high-throughput screening (HTS) assays have been widely applied as an alternative to animal-based testing in determining the toxicity of environmental contaminants and drug candidates. Current HTS studies provide the community with rich toxicology information and the biological endpoints chosen for HTS determines the toxicological relevance. Although many biological endpoints exist for HTS, there is still a need to incorporate further endpoints to achieve a more comprehensive assessment on the potential adverse health effects of environmental contaminants and potential drug candidates.

The novelty of the assay being developed by Upham relies on a molecular endpoint responsible for coordinating normal functions in tissues by integrating cell-to-cell signaling through gap junctions. Gap junctional intercellular communication (GJIC) is essential for maintaining normal tissue function by allowing the cells to coordinate their biological roles in a tissue. Interruption of this intercellular signaling can result

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Two IIT-Affiliates Receive Awards at MI SOT Annual Fall Meeting

The Michigan Regional Chapter of the Society of Toxicology recently held their annual fall meeting, "New Approach Methodologies and the Future of Toxicology," on October 16, 2020. Two IIT-affiliated students received awards at this meeting.

EITS graduate student **Jenna Strickland** received the Best Graduate Student Platform Presentation Award for her presentation, "Hepatoprotective role of Axl in acetaminophen overdose." Strickland is mentored by **Dr. Bryan Copple**.

Strickland is currently working on elucidating the mechanism(s) underlying macrophage dysfunction that occur in acute liver failure patients with the poorest prognosis. Elucidation of this mechanism could inform development of drugs to reverse macrophage dysfunction leading to liver repair and ultimately reversal of acute liver failure in patients. Additionally, the Copple laboratory has developed a high-throughput assay that can detect differentiation of proinflammatory macrophages into pro-repair macrophages for use as a drug screening platform to identify chemicals/drugs that stimulate this process. Drugs identified from this screen could ultimately be used to restore macrophage function and liver repair in patients with acute liver failure and fibrosis.

Lich Dan Rajasinghe, postdoctoral research associate in the laboratory of IIT-affiliated faculty member **Dr. James Pestka**, received 1st place in the Postdoctoral Research category for his research, "Docosahexaenoic acid (DHA) suppresses broad spectrum of inflammatory proteins elicited in a murine model of silica-triggered lupus flaring."

Rajasinghe's postdoctoral research has contributed to explore and elucidate molecular mechanisms for the ameliorating effects of dietary lipids on silica-induced pulmonary autoimmune disease in mice/cells genetically prone to develop to autoimmune effects similar lupus.

Occupational airway exposure of crystalline silica/dust (cSiO2) leads to development of pulmonary inflammation, which play a significant role in the development of autoimmune diseases such as lupus. The Pestka lab previously established that airway exposure of cSiO2 to lupus-prone mice markedly decreases onset time and increases disease. Remarkably, consuming omega-3 polyunsaturated fatty acids (PUFAs) found in fish oil, most notably docosahexaenoic acid (DHA) resolve inflammation, systemic autoimmunity, and glomerulonephritis. Rajasinghe's postdoctoral work has taken a step beyond autoimmunity disease prevention, to identifying the underlying mechanism of prevention of cSiO2-triggered autoimmunity development with dietary DHA.

Tewari-Singh Lab cont.

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ical Threats (CounterACT) program, to understand the mechanisms related to the inflammation/injuries from exposure to chemical threats including vesicating agents. Her goal is to further use this information to identify targeted therapies.

"I am extremely excited about this project," said Tewari-Singh, an assistant professor in the Department of Pharmacology and Toxicology, "because the proposed studies will have a strong potential to aid in understanding the mechanism of the inflammatory process and immune response following low exposures to alkylating warfare agent SM. The identification of applicable markers for therapeutic approaches will have a strong translational impact to potentially develop targeted treatments against chronic SM exposure. These treatments will be highly valuable in GWI, and for future veterans as well as vulnerable civilian populations, who are at risk to be exposed to similar chemical agents."

Upham R21 Grant cont.

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in tissue dysfunction. The new screening tool developed by Upham will be able to process large numbers of samples.

The new screening system developed by Upham can help identify potential adverse health effects at the early stages of drug development, thus potentially reducing the cost to the supplier and the consumer. "Today, drug industries face unprecedent productivity challenges due to financial pressures driven by the increasing cost of bringing a drug to market. Identifying potential adverse health effects at the early stages of drug development will significantly drive down the cost of potential new drugs," commented Upham.

Groundbreaking Research in the Robison Lab Illuminates the Opaque Pathways of Depression

Groundbreaking research in the lab of **A.J. Robison**, IIT-affiliated faculty member and associate professor in the Department of Physiology and MSU's Neuroscience Program, is directing some new rays of light onto the molecular, cellular and circuit-level mechanisms underlying depression-like diseases.

The results were recently published in Nature Communications.

"In this paper, we perform the first ever CRISPR-based gene editing [a genetic engineering technique in molecular biology by which the genomes of living organisms may be modified] in a single circuit between two areas of the mouse brain," explained Robison about the culmination of five years of research funded by the National Institutes of Mental Health. "We can reach into the mouse brain and manipulate specific genes in a circuit involved in depression and anxiety-like behaviors — a critical advance on the road to genetic medicine for psychiatric diseases."

Scientists estimate there are roughly 80-100 billion neurons connecting regions of the brain. To accomplish the feat of locating and manipulating a single gene in a single circuit required new and sophisticated technology. With the expertise of co-author Rachael Neve, director of the Gene Transfer Core at Massachusetts General Hospital, they developed it.

"The key advance is that we designed a dual-vector system to manipulate a specific gene in the connections between two brain areas, and that has never been done before," Robison said.

The neurons that Robison and his team zeroed in on originate in the ventral hippocampus (vHPC), a deep-seated structure that projects to regions in the brain important in stress susceptibility, mood and social avoidance. Neurons rooted in the vHPC reach out with branch-like structures called axons to connect with the nucleus accumbens, or NAc. The completed circuit is regulated by the star of the pioneering paper, the transcription factor known as DFosB.

Using the viral vector technology specifically designed and packaged by Neve, the team split the CRISPR system in half. Half of the system, inert on its own, was an enzyme that can mutate DNA in the vHPC. The other half, a guide RNA, was sent to all cells that project to the NAc and tells the enzyme where to bind and the specific gene to mutate. Only those cells specific to the circuit from the vHPC to the NAc got both halves, triggering the enzyme to bind with and turn off a single gene: FosB.

"When the FosB gene was turned off in the neurons, we were able to get a circuit-specific behavioral effect relevant to a disease like depression," said Robison about the landmark discovery. "When we put it back, or rescued it within the circuit, the effect was erased."

"One of the most exciting findings from our investigations was the circuit-specific role of the ΔFosB protein in conferring resilience to stress," Eagle said. "We also discovered that ΔFosB altered the excitability of hippocampal circuit neurons and may be affecting long-term downstream changes that lead to changes in the activity of this circuit." But removing DFosB permanently altered the expression of a suite of genes, in effect removing the conductor from the orchestra. To that end, the paper goes on to report in-depth experiments on DFosB largely done by the members of the Robison Lab including co-first authors Claire Manning, a 2019 neuroscience graduate, now a postdoc at Stanford University; and Andrew Eagle, a former postdoctoral researcher, now an assistant professor in the MSU Department of Physiology.

Based on the findings in the paper, the Robison Lab will continue to develop highly collaborative and cutting-edge techniques, accelerated by MSU's newly completed Interdisciplinary Science and Technology Building. "This work is important because it elucidates a potential mechanism, namely ΔFosB, for how stress may contribute to depression," Eagle continued. "Future clinical work may find ways to directly manipulate ΔFosB, or more likely one of its gene targets, to provide resilience to stress and decrease the incidence of depression in vulnerable people."

"The end of this paper, which shows us measuring the changes of expression in hundreds of genes when we remove DFosB, is only the beginning of years of work for our lab," Robison said. "Which genes are important and what are they doing in the brain? This is the challenge of a lifetime for me and my lab."

This article was originally featured on the College of Natural Sciences website and on MSU Today.

Recent EITS Graduates

Robert Freeborn

Pharmacology and Toxicology
Mentor, Cheryl Rockwell

Dr. Robert Freeborn received his Ph.D. after completing the dual major program in Pharmacology and Toxicology and Environmental Toxicology. His dissertation was, "*The Role of NRF2 Activation on the Murine T Cell Response to Influenza Infection.*"

Freeborn is now a postdoctoral student at Stanford University in the laboratory of Maria Grazia Roncarolo in the department of Pediatrics/Stem Cell and Regenerative Medicine. Freeborn is working on identifying the molecular mechanisms that drive the differentiation of Tr1 cells, a type of regulatory T cell, while also exploring the potential of these cells as a curative therapy for pediatric AML using humanized mouse models.

Strakovsky & Team Awarded $2.1 Million to Study Chemical Effects on Women's Health Post-Pregnancy

A team of researchers from Michigan State University and the University of Illinois, Urbana-Champaign (UIUC) led by IIT-affiliated faculty member **Rita Strakovsky**, has been awarded a five-year, $2.1 million grant from the National Institutes of Health (NIH). They will be studying the long-term effects of phthalate exposure on mothers four to seven years after giving birth.

According to the U.S. Food and Drug Administration, phthalates are a class of chemicals that increase the durability and flexibility of many common plastic products, including food packaging materials. Phthalates are also used in personal care products as scent and color stabilizers.

In previous studies, phthalates have been shown to disrupt endocrine system function, which is responsible for the production of hormones that regulate growth, development, metabolism and several other important physiological processes.

The new project will expand on the Illinois Kids Development Study (I-KIDS), a large pregnancy cohort study that began at UIUC in 2014 and evaluates the effects of environmental chemical exposure on children's development from birth to age 5. Susan Schantz, a professor and environmental neurotoxicologist at UIUC, leads the I-KIDS project.

Strakovsky's involvement with I-KIDS began while she was a postdoctoral researcher at UIUC before joining MSU. She was previously awarded an NIH grant to investigate phthalate exposure and hormonal disruption in pregnant women who participate in I-KIDS.

"Up to this point, we have been primarily concerned with endocrine disruptors and child development," Strakovsky said. "We have been focusing on the developmental origins of health and disease, a concept that suggests a person's health throughout life is heavily influenced by environmental exposures in utero and in early childhood.

"The question we have now is, what about the mother's health? Estrogen and other reproductive hormones are important for women's metabolic and cardiovascular health, so our goal is to understand the potential consequences of hormonal disruption."

Roughly 350 mothers from I-KIDS will be reenrolled for the new project and will meet with researchers four to seven years after giving birth.

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Read more of this story by Cameron Rudolph on the MSU CANR website: https://www.canr.msu.edu/news/msu-awarded-2-1-million-grant-to-study-chemical-effects-on-women-s-health-post-pregnancy.

Three IIT-Affiliated Faculty Awarded by College of Human Medicine

Three IIT-affiliated faculty members were recently selected for prestigious awards by the College of Human Medicine at their annual awards ceremony. **Dr. Masako Morishita** received the Early Career Research Excellence Award, **Dr. Eran Andrechek** received the Research Excellence Award, and **Dr. James Trosko** received the William B. Weil Jr., MD, FAAP, Endowed Distinguished Pediatric Faculty Award.

Early Career Research Excellence Award - **Masako Morishita**, PhD, is an associate professor in the Department of Family Medicine and an environmental health scientist whose research focuses on exposure science. Her expertise is in the health effects of indoor and outdoor airborne particle pollution and of metals found in soil. Dr. Morishita conducts multidisciplinary studies to better understand the adverse impacts of these exposures on human health.

Research Excellence Award - **Eran Andrechek**, PhD, is an associate professor and a recognized leader with many outstanding contributions to the field of breast cancer research, as well as an exceptional mentor and teacher in the Department of Physiology.

William B. Weil Jr., MD, FAAP, Endowed Distinguished Pediatric Faculty Award - **James E. Trosko**, PhD, is a professor emeritus in the Department of Pediatrics and Human Development and is also recognized as an MSU Distinguished Faculty.

"To be recognized as a recipient of the Dr. William B. Weil, Jr. Award is received with genuine humility," commented Dr. Trosko. "Together with my colleague, Dr. Andre Bachmann, this helps me realize that Dr. Weil's vision is still alive under the current leadership of Dr. Keith English."

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