MS Center for Food Safety and Post-Harvest Technology.
CRIS Progress Report (2015)

Title of project: "***Targeting the Endocannabinoid System to Enhance Immunity***"

One-sentence description: The goal will be to identify serine hydrolases in macrophages that can be targeted (i.e. inhibited) by small molecules for the purpose of enhancing endocannabinoid levels during microbial infection, and establish whether the microbicidal activity of the macrophages is concomitantly enhanced by these inhibitors.

Investigators: Matt K. Ross, PhD (PI); Mariola Edelmann, PhD (co-I)

Participants: Matt K. Ross; Mariola Edelmann; Evangel Kumari; Sam Borazjani (Research Associate; 0.08 FTE); Jung Hwa Lee (Postdoctoral fellow; 0.20 FTE)

Year funded: 2014

Target audience: The scientific community via peer-reviewed literature.

Publications in 2015 related to this project:
None yet – one in preparation right now.

Poster presentations in 2015 related to this project:

Characterization of Serine Hydrolases Using Chemoproteomic Profiling Approach in Chicken Macrophages with Salmonella Infection. E. Kummari, J. H. Lee, A. Borazjani, M. Edelmann, M.K. Ross. Presented at the *Annual Society of Microbiology* meeting, New Orleans, LA. May 30-June 2, 2015.

Characterization of prostaglandins released from human macrophages infected with enteric bacteria. Evangel Kummari, Navatha Alugubelly, Jung Hwa Lee, Lauren Mangum, Abdolsamad Borazjani, Matthew Ross, and Mariola J. Edelmann. Presented at the Southeast Institute of Metabolomics at the University of Florida, Gainsville, May 13-14, 2015.

Rationale for study:
Macrophages and neutrophils are front line defenders in the innate immune system. Upon pathogenic challenge, they activate the biosynthesis and secretion of a variety of toxic molecules for the purpose of killing foreign organisms. The endogenous cannabinoid (eCB) system is emerging as an important cell signaling system that might influence host-pathogen interaction. The eCB system is comprised of several components including two distinct G-protein coupled receptors (CB1 and CB2); the endogenous arachidonoyl-containing ligands 2-arachidonoylglycerol (2-AG) and anandamide (AEA); and enzymes responsible for 2-AG and AEA biosynthesis and inactivation. Emerging evidence indicates that eCBs such as 2-AG can regulate host defense by stimulating innate immune cells to combat bacterial and viral invaders, although the precise mechanisms involved in pathogen killing will depend on the cellular and/or tissue context. Strategies that enhance the in vivo levels of eCB ligands, such as 2-AG, might significantly improve host defense mechanisms and combat pathogens that negatively impact animal health. The steady-state levels of 2-AG in a biological context are determined by the balance of biosynthetic and degradation rates, which are regulated by specific serine hydrolase enzymes in cells. Our hypothesis is that the eCB system has an important role in innate immunity and will significantly modulate macrophage-pathogen interactions.

Outputs:
This project began in summer 2014. We established the chicken HD-11 macrophage cell line in our laboratory, with the help of Dr. Edelmann's lab, and have initiated cell infections with *Salmonella enterica* serovar Typhimurium. We began characterizing the serine hydrolase profiles in the HD-11 cells before and after infections with *Salmonella* Typhimurium using a chemoproteomic approach with a specific proteomic probe, fluorophosphonate-biotin, which targets the serine hydrolase superfamily of enzymes and enables activity-based protein profiling (ABPP) of the serine hydrolase class of enzymes. Serine hydrolases in chicken HD11 macrophages were inventoried by ABPP-Multidimensional Protein Identification Technology (MudPIT). On the basis of gel-based activity-based protein profiling, at least 8 different serine hydrolases were detected in the HD-11 cell line, whereas 15 serine hydrolases were identified by ABPP-MudPIT**.** ABHD6 and FAAH were identified. These enzymes can catabolize endocannabinoids by catalyzing their hydrolysis and, thus, can regulate their levels in cells. Using ABPP-MudPIT, the small-molecule inhibitor JZL184 was found to selectively inhibit ABHD6 and FAAH activities in intact cells. JZL184 could increase the levels of 2-AG in HD11 cells due to inactivation of 2-AG hydrolytic enzymes. Inactivating the metabolism of endocannabinoids with JZL184 could also augment the phagocytic activity of HD11 macrophages. Furthermore, infection of HD11 cells with *Salmonella* Typhimurium caused a marked downregulation of ABHD6 and FAAH activity after 18 h, which might be compensatory feedback mechanism to increase the concentrations of endocannabinoids.

Outcomes/Impact:
The U.S. poultry industry provides a commodity that is an important source of a high protein diet and is a vital economic driver in the southeastern U.S. Because chickens are housed in tight proximity to one another, they are very susceptible to major outbreaks of *Salmonella* infection, which can spread to humans. Although antimicrobials are effective weapons against such outbreaks, the rapid rise in drug resistance is a major problem. As a result, a greater understanding of the innate immune system and how the eCB system interfaces with it is crucial for development of next-generation antimicrobials that *Salmonella* cannot evade. More generally, this basic knowledge could also be translated to other host-pathogen interactions that plague other Mississippi agro-industries, such as aquaculture. The impact of the current studies suggest that targeting enzymes involved in 2-AG metabolism might be a novel antimicrobial mechanism to treat *Salmonella* infections.

Project modifications:
Project is currently in a one-year no cost extension.