Project Leader (PI): Matt K. Ross, PhD

Co-PI(s): Mariola Edelmann, PhD

Collaborator(s): Chinling Wang, PhD

Objective(s): ***Targeting the Endocannabinoid System to Enhance Immunity***

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

Milestones for FY 2016-17:

1. *Project Title: Targeting the Endocannabinoid System to Enhance Immunity*
2. *Project Milestones:*

   Aim 1. Using a chemoproteomic profiling approach, determine the profile of serine hydrolase enzymes and identify the 2-arachidonoylglycerol (2-AG) hydrolytic enzymes in a chicken macrophage cell line (HD-11).

   Aim 2. Determine whether phagocytosis of *Salmonella* by chicken macrophages alters the activities of serine hydrolases responsible for 2-AG biosynthesis and degradation.

Progress Report:

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 in order to kill foreign organisms. The endogenous cannabinoid (eCB) system is emerging as an important cell signaling system that can influence host-pathogen interactions. 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 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.

We established a chicken macrophage cell line (HD-11) in our laboratory and initiated cell infections with *Salmonella enterica* serovar Typhimurium. Using a chemoproteomic approach, we characterized the serine hydrolase profiles in the macrophages before and after infection with *Salmonella* Typhimurium. An activity-based probe, termed fluorophosphonate-biotin, which targets the serine hydrolase superfamily and enables activity-based protein profiling (ABPP) of these enzymes, was utilized**.** The serine hydrolases in chicken 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 by this profiling study. Both enzymes can catabolize endocannabinoids by catalyzing their hydrolysis; thus they regulate the cellular concentrations of these bioactive lipids. Using the ABPP-MudPIT approach, a small-molecule inhibitor, JZL184, was discovered to potently and selectively inhibit both ABHD6 and FAAH activities in intact cells. In addition, treatment of macrophages with JZL184 caused the levels of the endocannabinoid 2-arachidonoylglycerol (2-AG) to increase due to inactivation of the 2-AG hydrolytic enzymes. Moreover, we discovered that inactivating the cellular metabolism of endocannabinoids with JZL184 could augment the phagocytic activity of HD11 macrophages. Furthermore, on the basis of gel-based ABPP, infection of HD11 cells with *Salmonella* Typhimurium appeared to induce a marked downregulation of ABHD6 and FAAH activity by 18 hours. This suggested a host-mediated compensatory feedback mechanism to increase the local concentration of endocannabinoids to help clear the bacterial infection. This hypothesis was incorporated into a USDA-NIFA grant application that was submitted in 2016.

In collaboration with Dr. Chinling Wang (Basic Sciences, CVM), we performed an in vivo study in which young chickens were treated with JZL184 (intraperitoneal injection; 0, 10, or 40 mg/kg bw; n=6 birds per group) followed by subsequent oral challenge to *Escherichia coli (E. coli)*. The extent of gross lesions in the air sacs (i.e. airsacculitis) was evaluated at three time points over a 2.5-day period. Dr. Wang has significant experience with this type of challenge model and we reasoned it might provide preliminary data for a USDA grant application. We hypothesized that JZL184 would indirectly enhance the clearance of the bacterial infection (via its effects on the host's endocannabinoid system), which would manifest in reduced airsacculitis caused by *E. coli*. Birds that received JZL184 exhibited a greater proportion of air sac gross lesions than those birds that received vehicle (control). In addition, it appeared that the drug vehicle (PEG300:Tween80) had exacerbated the inflammatory reaction in the air sacs following *E. coli* challenge when compared to birds that received saline and *E. coli*. Nonetheless, gross air sac lesions in JZL184-treated birds were more severe than those in vehicle-treated birds. It should be noted, however, that *E. coli* colony forming units (CFUs) in air sacs were not enumerated following bacterial challenge. This is a limitation of the study because this endpoint might be more relevant for evaluating the efficacy of JZL184 on bacterial clearance. Serum cytokine levels (e.g. IL-6 and IL-1β) are currently being measured to determine whether JZL184 affected these pro-inflammatory molecules. Future in vivo studies using a different drug vehicle and with longer evaluation time points (>2.5 days) might be considered.

Accomplishments:

A USDA-NIFA grant application, which used preliminary data generated under this project, was submitted in August 2016 to the "Understanding Antimicrobial Resistance" program (Proposal 2016-10253, Project Director: Ross; Targeting the Endocannabinoid System to Enhance Innate Immunity). The proposal was placed in the ***Medium Priority*** category. To give context, the disposition of all applications to this program was as follows:

Recommended for Funding:	
Outstanding %	0
Not Recommended for Funding:	
High Priority %	15
Medium Priority %	42
Low Priority %	15
Do Not Fund %	27

I plan to respond to the critiques and resubmit the grant.

Significant Activities that Support Special Target Populations: None

Technology Transfer: None

International Cooperation / Collaboration: None

Publications: Jung Hwa Lee, Evangel Kummari, Abdolsamad Borazjani, Mariola J. Edelmann, and Matthew K. Ross. (2017) Characterization of serine hydrolases in a chicken macrophage cell line (HD-11) and stimulation of the endocannabinoid system following infection with *Salmonella.* In preparation for submission to *Poultry Science.*

Presentations: *J.H. Lee, A. Borazjani, E. Kummari, M.J. Edelmann, and M.K. Ross, Targeting the Endocannabinoid System to Enhance Innate Immunity Using Chemoproteomics. American Society for Mass Spectrometry, San Antonio, TX. June 7-10, 2016.*

Please attach a photo or figure:

Activity-based protein profiling of serine hydrolases in a chicken macrophage cell line (HD-11) and selective inhibition of endocannabinoid-hydrolytic enzymes (ABHD6 and FAAH) by small molecule inhibitor JZL184.