4-1-2019

Propionate Enhances the Antimicrobial Defenses in Macrophages Against Listeria monocytogenes

John Weis
University of Dayton

Propionate Enhances the Antimicrobial Defenses in Macrophages Against *Listeria monocytogenes*

Honors Thesis

John C. Weis

Department: Biology

Advisor: Yvonne Sun, Ph.D.

April 2019

Propionate Enhances the Antimicrobial Defenses in Macrophages Against *Listeria monocytogenes*

Honors Thesis

John Weis

Department: Biology

Advisor: Yvonne Sun, Ph.D.

April 2019

Abstract

Propionate is a short chain fatty acid produced by the bacteria in the human gut. It has a wide range of nutritional functions in the human body. Several studies have also reported the effects of propionate on immune cell activation. In this study, we used *Listeria monocytogenes* as a model pathogen to determine how and what the effects of propionate on immune cells influence cell susceptibility to infections. *Listeria* is a dangerous intracellular pathogen that can replicate inside immune cells such as macrophages. Therefore, we can assess the impact of propionate on infection susceptibility by performing cell culture infections. We previously showed that propionate treatment on *Listeria* does not impact its ability to grow inside macrophages. However, when macrophages were treated with propionate prior and during infections, I was able to observe a significantly decreased *Listeria* intracellular growth compared to non-treated macrophages. These results suggest that propionate may strengthen the antimicrobial mechanisms in macrophages to restrict the intracellular growth of *Listeria*. Results from my study will help establish the role of propionate in regulating our immune defense mechanisms during host-pathogen interactions.

Dedication

I would like to dedicate this to my parents who I wouldn't be here without.

Contents

Chapter 1

Personal Reflection

I am a Pre-medicine major with a minor in medical humanities from Columbus, Ohio. I first started my research experience at The University of Dayton my sophomore year in 2016. Prior to my research at the University of Dayton, the summer of my freshman year I worked at The Ohio State University Biomedical Research Tower. There I assisted in conducting research on lung Cancer in Dr. Nana-sinkam's lab. My experience that summer propelled me to push myself forward as I learned how valuable research can be to both the experimenter, and what the research is being applied too. That next summer I came to UD very interested in joining a Biology laboratory. My future aspirations are to become a physician, and receive a Master of Public Health. I have always been very interested in infectious disease. When I discussed this interest with my Advisor Dr. DeBeer, she directed me to Dr. Yvonne Sun and her Microbiology Lab. After talking with her and learning more about what her research goals and interests are, I decided that this was the laboratory I wanted to join.

I first began my research experience by shadowing Elizabeth Abrams and Erica Rinehart. Both of them have been very influential and helped me out tremendously along the way here at Dayton. With the help of the Szabo Grant, awarded by the University of Dayton Premedical Department, I then spent the summer of 2017 in Dayton and began to work on my first project. This project consisted of investigating the relationship between *Listeria monocytogenes* and propionate. This summer experience was monumental for my future as I grew tremendously academically and as a person. My biggest take away from working in a research laboratory every day was how to deal with failure and turn it into success. Working with cell culture, I often had to deal with contamination for a number of different reasons. This problem, along with many others that I

would encounter on a day to day basis, forced me to exercise my problem-solving and critical thinking skills. Being able to critically think and problem solve to find that solution is crucial. I also learned that summer to ask questions and think deeper about what I really was doing while conducting each experiment. At the end of the summer I presented my first poster at the University of Dayton Summer Science Research Symposium. This presentation showed me how important it is to learn how to effectively communicate research findings. Speaking to someone who may not understand scientific terms is something many physicians have to handle every day. I credit this summer experience as the reason I continued to conduct research under Dr. Yvonne Sun's guidance.

With the help of the University of Dayton Dean's Summer Fellowship I was then able to return again to continue my research in the summer of 2018. I continued my original project, started a new multidisciplinary project with the Chemistry Department, and was able to act as a mentor to younger students and help them begin their own projects. I was also able to travel to the ASM Microbe and present my research at a major national conference. Today I am very thankful that University of Dayton, and especially Dr. Sun, have been so supportive of undergraduate researchers. I believe that research has significantly enhanced my academic and professional learning while my time at the University of Dayton.

My Initiation into Dr. Sun's Research

In the short time that Dr. Sun's laboratory has operated on the University of Dayton's campus, she has lead the lab to many very interesting findings. Previous research from Dr. Yvonne Sun's laboratory, specifically from undergraduate student Elizabeth Abrams and graduate student Erica Rinehart, found that *Listeria* grown anaerobically possess different surface morphology and decreased growth.[20] This finding influenced my decision to investigate how and if propionate would also affect *Listeria* differently aerobically and anaerobically. Dr. Sun's laboratory found that this was the case and propionate's effect on *Listeria monocytogenes* growth and Listeriolysin O (LLO)

production is modulated by anaerobicity. Specifically the main findings were that propionate resulted in increased adherent growth but decreased planktonic growth. This research also found that *Listeria monocytogenes*' central carbon metabolism and LLO production are both altered by the presence and absence of oxygen.[16] Seeing the results from Erica and Elizabeth promoted Dr. Sun and I to further investigate if propionates has a significant effect on *L. monocytogenes* virulence. Another Graduate student who recently received his Ph.D. found the importance of respiratory activity on *Listeria monocytogenes* production of LLO, cell to cell spread, and phagosomal escape.[21] This research together prompted me to investigate *Listeria*'s ability to infect macrophages in both anaerobic and aerobic conditions, as well as with and without the addition of propionate.

The structure of the thesis will contain four chapters. Chapter 1 was an introduction to my background, and personal research goals. Chapter 2 will be an introduction to *Listeria* and Pathogenesis. Chapter 3 will contain materials, methods, and the discuss of my propionate project. Chapter 4 will contain my acknowledgements and appendix.

Chapter 2-Introduction/Background

Overall Thesis Research Goal

Propionate is a short chain fatty acid produced by the bacteria in the human gut. It has a wide range of nutritional functions in the human body. Several studies have also reported the effects of propionate on immune cell activation. In this study, we used *Listeria monocytogenes as* a model pathogen to determine the effects of propionate on immune cells. Specifically how propionate influences cell susceptibility to infections. *L. monocytogenes* is a dangerous intracellular pathogen that can replicate inside immune cells such as macrophages. Therefore, we can assess the impact of propionate on infection susceptibility by performing cell culture infections. We previously showed that propionate treatment on *L. monocytogenes* does not impact its ability to grow inside macrophages. However, when macrophages were treated with propionate prior and during infections, I was able to observe a significant decrease in *Listeria* intracellular growth compared to non-treated macrophages. These results suggest that propionate may strengthen the antimicrobial mechanisms in macrophages to restrict the intracellular growth of *L. monocytogenes*. For my thesis proposal, I plan on further identifying which antimicrobial functions of macrophages are enhanced by propionate. I will perform cell culture infections using commercially available inhibitors that separately block the antimicrobial functions in macrophages. If a specific antimicrobial mechanism of macrophages is enhanced in the presence of propionate, then the use of the corresponding inhibitor will eliminate the enhanced antimicrobial functions. Results from my study will help establish the role of propionate in regulating our immune defense mechanisms during host-pathogen interactions.

Introduction to *Listeria* pathogenesis

Listeria monocytogenes is a gram positive pathogenic bacterium that is the number 3 pathogen contributing to domestically acquired foodborne illness resulting in death.[29] Interestingly, *L. monocytogenes* is an intracellular pathogen. This means that *L. monocytogenes* invades and thrives physically inside human cells. Unique to *L. monocytogenes* are certain virulence mechanism that allow the pathogen to be successful at invasion, including Listeriolysin O (LLO). LLO allows *L. monocytogenes* to escape the phagosome after intracellularly invading human cells such as macrophages.[33] ActA is another virulence factor that allows *Listeria* to be motile once escaping the phagosome, this is crucial for the survival of *Listeria* and avoidance of defense mechanism of the human body.[34] It is also known that there are two other proteins associated with virulence, p60 and surface protein 104. Surface protein 104 is associated with cell adhesion and p60 is associated with finishing replication.[34] The combined virulence of this pathogen often results in casualties. *L. monocytogenes* primarily affects the elderly or those who are immunocompromised, and is a major threat to pregnant women.[30] In 2018 alone, the organism was responsible for 2 multistate outbreaks, and resulted in a death of an individual.[31] Deaths by *L. monocytogenes* are often associated with meningitis or stillbirths, infection outcomes requiring the bacteria to cross the intestinal epithelium.[32] Therefore, to help these vulnerable populations from *L. monocytogenes* infections, it is important to identify ways of preventing bacterial crossing the epithelium.

Introduction to Short Chain Fatty Acids

An extremely under researched way of combating *L. monocytogenes* infection is looking towards the recent explosion of research on short chain fatty acids (SCFAs). When *L. monocytogenes* first enters our intestines, it is exposed to SCFAs, which are fermentation end products by our intestinal microbes. In humans, SCFA's have been known to alter chemotaxis and phagocytosis, activate reactive oxygen species (ROS), and change cell proliferation and function.[25] Furthermore

they have been found to possess anti-inflammatory, anti-tumorigenic, and antimicrobial effects in the human body.[25]

The most common SCFA's found in the body are Acetate, propionate, and butyrate.[26] These SCFA's have been found to both change the gut microbial composition in humans, as well as directly be toxic to bacteria such as salmonella.[26] Propionate is the three carbon SCFA and appears naturally in the human body at a concentration of 0.31 mM in mammals.[4] Previous research has specifically found that propionate based ingredients have inhibited *L. monocytogenes* in a factory setting on turkey deli meat.[7] This study was also supported by another study that found that 0.2% propionate was able to inhibit growth on ham and turkey deli style meat.[6]

Introduction to the Biological Functions of Propionate

Propionate has also been found to induce physiological changes within the body. Previous research has shown that Propionate effects many of our immune cells in different and similar ways. Polymorphonucleocytes (PMN's) are a group of cells in our immune system that include Neutrophils, Basophils, and eosinophils. These granulocytic cells often come in contact with propionate as they are constantly flowing throughout the body, and respond quickly to areas of infection. In one study, propionate stimulated PMN polarization, F-Actin Localization, and cytoplasmic pH oscillation.[4] Propionic Acid was also found to increase calcium mobilization in human neutrophils, which has the potential to have multiple physiological effects during an infection as neutrophils are the first responders.[11] Another study found that Short-chain fatty acids generated by microbes within the gut cause global impairment of the microbicidal activity of neutrophils.

In the human body monocyte derived cells include Macrophages, Dendritic Cells (DC's) and Mast cells. Macrophages are the main motile phagocytic cell within the body and are often responsible for clearing foreign invaders. They are also very important in the activation of the

acquired immune system activity of T and B cell lymphocytes.[27] In a study, short chain fatty acids resulted in modulation of gene expression in both immature and mature human Dendritic cells as well as modulation of macrophage biology in the bone marrow.[12] Phagocytosis is just one defense mechanism of macrophages, they also have the ability to send signals to cells to increase proliferation, enhance cellular repair, and also kill foreign invaders with reactive oxygen species (ROS). There are two known types of macrophages that metabolize arginine differently leading to different cellular processes. M1 macrophages express nitric oxide synthase and metabolize arginine into nitric oxide (NO).[15] M2 macrophages metabolize arginine into ornithine or urea.[15] Ornithine and Urea are used in further pathways that increase cell proliferation and cellular repair mechanism. NO is used as a reactive oxygen species that can be toxic to foreign invaders and human cells. Macrophages are also very crucial in the defense against intracellular pathogens, such as *L. monocytogenes*. Besides the use of ROS, macrophages also have been known to deprive the invaders of essential nutrients such as iron, fatty acids, or amino acids.[28]

Chapter 3 Propionate & *Listeria monocytogenes* Pathogenesis

Experimental Methods

Bacterial Strains and culture conditions

The strains of *Listeria monocytogenes* used in this study were the wild type 10403s (serotype 1/2A) and a mutant that did not contain the Hly gene which encodes for the virulence factor LLO. For all experiments in this study, the strains were grown overnight in filter-sterilized brain heart infusion (BHI) media for between 14-18 hours. For aerobic growth, bacteria were grown in a 37 C incubator with shaking at 250 rpm. For anaerobic growth, bacteria were grown statically in a 37 C anaerobic chamber (Type A, Coy Laboratory, Grass Lake MI, USA). The Chamber contains a nitrogenous atmosphere with 2.5% Hydrogen. Optical density was measured in a 96 well plate at 600 nm with a volume of 200 ul per well using a 96 well plate reader (Synergy4, Biotek, Winooski, VT, USA). Sodium propionate stock solutions were prepared at 1 M in deionized water, filter sterilized, and stored in a -20 C freezer.

Infections

For all infections, the cell line RAW264.7 macrophages were used. The macrophages are kept in Dulbecco's Modified Eagle Medium (DMEM) with 10% Fetal Bovine Serum (FBS). The infection procedure consisted of a standard gentamicin protection assay to monitor *L. monocytogenes* intracellular growth at MOI of 10 after 30 minutes of infection. Cultures were inoculated overnight for 14-18 hours. Optical density was then taken the following morning to determine the correct amount of *L. monocytogenes* needed to ensure an MOI of 10. When passaging macrophage cell line, the cells were scraped off the bottom of a petri dish using a lifter. The liquid was then transferred to a 50 ml conical tube and then spun at 1500 RPM for 3 minutes. The liquid was then removed and

the cells were suspended in 10 ml's of DMEM with 10% FBS. Depending on when the experiment was to take place, 1-3 ml of macrophages in the media was put into the fresh petri dish. Cells were seeded for the infection 48 hours prior to the infection which enabled them to grow to confluence in their designated well. 10 ul's of suspended cells were pipetted into a cell counter. To ensure a MOI of ten, the number of cells identified in an area of 4x4 was multiplied by 10 to the fourth.

Experiment 1

RAW 264.7 macrophages were seeded 48 hours prior to infection with *L. monocytogenes*. The *Listeria* strain was grown aerobically and anaerobically in filter-sterilized BHI media for 14-18 hours without the presence of propionate. This set for the conditions that had only the *L. monocytogenes* encounter propionate, and never the Raw 264.7 Macrophage cell line. The macrophages with lysed with a 0.01% Triton X solution and then 50uL of *Listeria* was plated on standard Luria Broth (LB) plates. The plates were shaken by hand, and then placed in a 37 C aerobic incubator to grow for 48 hours. After 48 hours the plates were measured by a colony counter, Acolyte 3, and the data was statistically analyzed.

Experiment 2

This experiment differs from experiment 1 in a few different ways. First there are 2 separate conditions of the macrophages. In condition 1, 5 mM of sodium Propionate was added to the RAW 264.7 macrophages 24 hours prior to infection. In condition 2 the macrophages were seeded without the addition of propionate similar to experiment one. Both condition 1 and 2 of the macrophages were originally seeded 48 hours prior to infection. The second difference is that the *Listeria* WT strain was grown aerobically and anaerobically in filter-sterilized BHI media for 14-18 hours without the presence of propionate. This set for the conditions that had only the Cell line encountering propionate, and never the *L. monocytogenes*. Each experiment, the macrophages were monitored visually to access their viability. They were noted under the microscope as well as the

assessment of their media. The macrophage cell line was then infected with *L. monocytogenes* in a standard gentamicin protection assay at an MOI of 10 and growth of L. Monocytogenes was evaluated. The macrophages with lysed with a 0.01% Triton X solution and then 50uL of *Listeria* was plated on standard Luria Broth (LB) plates. The plates were shaken by hand with beads to spread the *Listeria* equally throughout the plate, and then placed in a 37 C aerobic incubator to grow for 48 hours. After 48 hours the plates were measured by a colony counter, Acolyte 3, and the data was statistically analyzed.

Experiment 3

This experiment contained 2 separate conditions of the macrophages and also contained separate conditions of the *L. monocytogenes*. Like experiment 1, there are 2 conditions of macrophages. In the first condition 5 mM of sodium Propionate was added to the RAW 264.7 macrophages 24 hours prior to infection and in the second condition the macrophages were seeded without propionate. Both conditions of the macrophages were originally seeded 48 hours prior to infection. However, In this experiment I used two different strands of *L. monocytogene*s. WT strain and the Delta *hly* strain were grown aerobically and anaerobically in filter-sterilized BHI media for 14-18 hours without the presence of propionate. Again, this set for the conditions that had only the Cell line encountering propionate, and never the *L. monocytogenes*. Each experiment, the macrophages were monitored visually to access their viability. They were noted under the microscope as well as the assessment of their media. The macrophage cell line was then infected with *L. monocytogenes* in a standard gentamicin protection assay at an MOI of 10 and growth of *L. monocytogenes* was evaluated. The macrophages with lysed with a 0.01% Triton X solution and then 50uL of *Listeria* was plated on standard Luria Broth (LB) plates. The plates were shaken by hand with beads to spread the *L. monocytogenes* equally throughout the plate, and then placed in a 37 C aerobic

incubator to grow for 48 hours. After 48 hours the plates were measured by a colony counter, Acolyte 3, and the data was statistically analyzed.

Experiment 4

This experiment contained 2 separate conditions of the macrophages. All macrophages seeded were given 5 mM of sodium Propionate was added to the RAW 264.7 macrophages 24 hours prior to infection. The Nitric Oxide (NO) production inhibitor L-NMMA was also inserted into half of the macrophages 48 hours prior to infection and during the infection of the macrophages with *L. monocytogenes.* The WT strain of *L. monocytogenes* were grown aerobically and anaerobically in filter-sterilized BHI media for 14-18 hours without the presence of propionate. Again, this set for the conditions that had only the Cell line encountering propionate, and never the *L. monocytogenes*. Each experiment, the macrophages were monitored visually to access their viability. They were noted under the microscope as well as the assessment of their media. The macrophage cell line was then infected with *L. monocytogenes* in a standard gentamicin protection assay at an MOI of 10 and growth of *L. monocytogenes* was evaluated. The macrophages with lysed with a 0.01% Triton X solution and then 50uL of *Listeria* was plated on standard Luria Broth (LB) plates. The plates were shaken by hand with beads to spread the *Listeria* equally throughout the plate, and then placed in a 37 C aerobic incubator to grow for 48 hours. After 48 hours the plates were measured by a colony counter, Acolyte 3, and the data was statistically analyzed.

Experiment 5

Two conditions of seeded macrophages were used. In Condition 1 the macrophages seeded were given 5 mM of sodium Propionate was added to the RAW 264.7 macrophages 24 hours prior to infection. In condition 2, propionate was not added to the RAW 264.7 macrophages prior to infection. Both conditions were seeded 48 hours prior to the infection with *L. monocytogenes*. In each condition stated above, half of the macrophages were activated with the known macrophage

activator and cytokine, IFN-Gamma (LPS) during seeding. The WT strain of *L. monocytogenes* were grown aerobically and anaerobically in filter-sterilized BHI media for 14-18 hours without the presence of propionate. Again, this set for the conditions that had only the Cell line encountering propionate, and never the *L. Monocytogenes*. Each experiment, the macrophages were monitored visually to access their viability. They were noted under the microscope as well as the assessment of their media. The macrophage cell line was then infected with *L. monocytogenes* in a standard gentamicin protection assay at an MOI of 10 and growth of *L. monocytogenes* was evaluated. The macrophages with lysed with a 0.01% Triton X solution and then 50uL of *Listeria* was plated on standard Luria Broth (LB) plates. The plates were shaken by hand with beads to spread the *L. monocytogenes* equally throughout the plate, and then placed in a 37 C aerobic incubator to grow for 48 hours. After 48 hours the plates were measured by a colony counter, Acolyte 3, and the data was statistically analyzed.

Bone Marrow-Derived Macrophages

Bone marrow was taken from mice femur housed in the University of Dayton Vivarium. The cells were cultured for a week in DMEM allowing the cells to grow to confluency. The cells were then cultured with L929-conditioned media. This is a fibroblast cell line that secretes M-CSF which allows the macrophages to proliferate into mature bone marrow-derived macrophages.

Experiment 6

As in experiment 2, there are two different conditions of macrophages, except the macrophages are bone marrow-derived macrophages (BMM). In condition 1, 5 mM of sodium Propionate was added to the BMM macrophages 24 hours prior to infection. In condition 2 the macrophages were seeded without the addition of propionate similar to experiment one. Both condition 1 and 2 of the macrophages were originally seeded 48 hours prior to infection. The *L. monocytogenes* WT strain was grown aerobically and anaerobically in filter-sterilized BHI media for 14-18 hours without the

presence of propionate. With these conditions, only the BMM are encountering propionate, and never the *L. monocytogenes*. The macrophages were then monitored visually to access their viability. They were noted under the microscope as well as the assessment of their media. The macrophage cell line was then infected with *L. monocytogenes* in a standard gentamicin protection assay at an MOI of 10 and growth of *L. monocytogenes* was evaluated. The macrophages were lysed with a 0.01% Triton X solution and then 50uL of *Listeria* was plated on standard Luria Broth (LB) plates. The plates were shaken by hand with beads to spread the *Listeria* equally throughout the plate, and then placed in a 37 C aerobic incubator to grow for 48 hours. After 48 hours the plates were measured by a colony counter, Acolyte 3, and the data was statistically analyzed.

Results

Elizabeth Abrams performed a set of cell culture infection experiments and found that propionate treated *L. monocytogenes* is not compromised in intracellular growth (**Figure 1A, 1B**). She treated *Listeria* with propionate and then used a cell culture model to experiment on how the supplementation of propionate to a *L. monocytogenes* culture would impact their survival and viability. There was no significant difference in infection outcomes between aerobically grown *Listeria* treated with 15mM propionate and *Listeria* without propionate. However, anaerobically grown *Listeria* grown overnight treated with 15mM propionate had a significantly higher intracellular CFU at 2 hours post infection (HPI) than *Listeria* grown overnight now treated with propionate (**Figure 1A**) There was no significant difference at any other time point throughout the infection with anaerobically and aerobically grown *L. monocytogenes* treated with or without the supplementation of propionate.

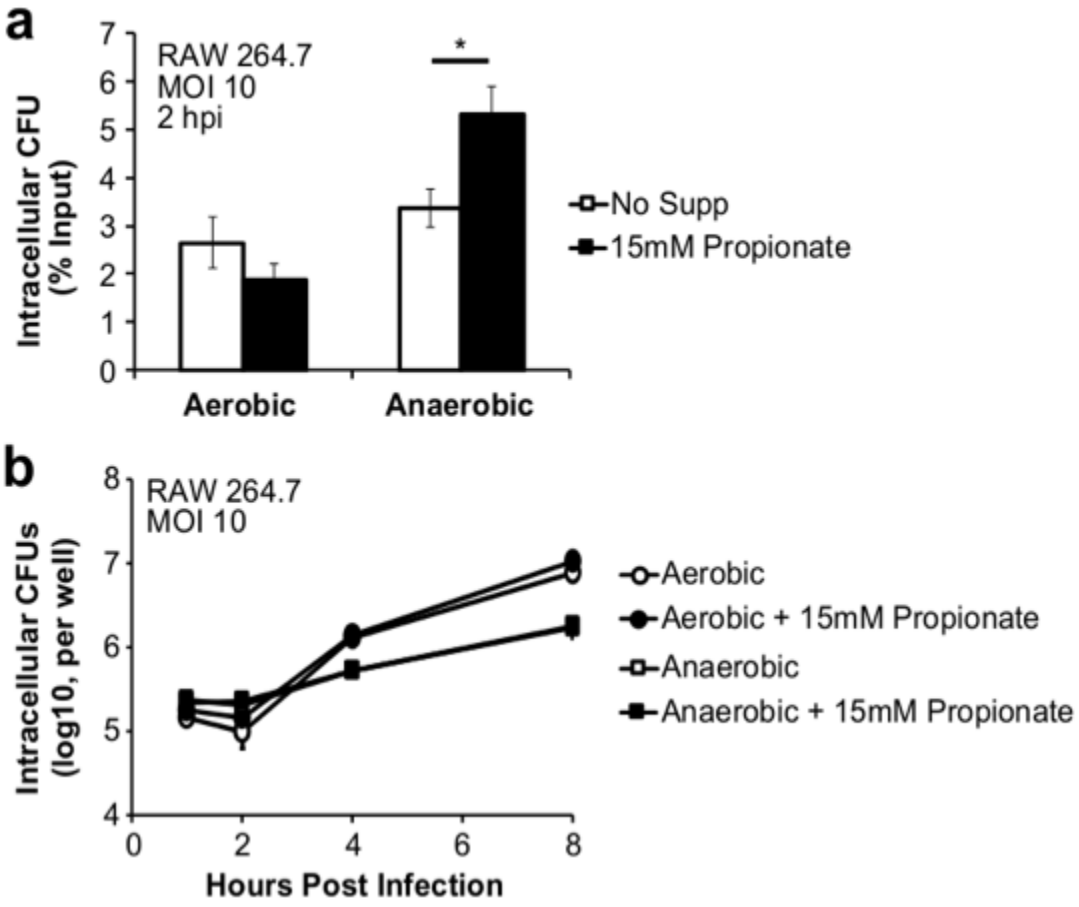
a
RAW 264.7
MOI 10
2 hpi
*
Intracellular CFU
(% Input)
0
1
2
3
4
5
6
7
Aerobic
Anaerobic
No Supp
15mM Propionate
b
RAW 264.7
MOI 10
Intracellular CFUs
(log10, per well)
4
5
6
7
8
0
2
4
6
8
Hours Post Infection
Aerobic
Aerobic + 15mM Propionate
Anaerobic
Anaerobic + 15mM Propionate

Figure 1

I then performed another cell culture infection, but this time supplemented the macrophages with propionate instead of supplementing *L. monocytogenes* with propionate. I found that propionate treated Macrophages are more restrictive to *L. monocytogenes* intracellular growth **(Figure 2**). At 8 hpi, there was a statistically significant decrease of aerobically grown *Listeria* CFU's in the macrophages that had been treated with 5mM of propionate prior to the infection. This decrease was also statistically significant decrease at the 1 hour post infection time point (**Figure 3**).

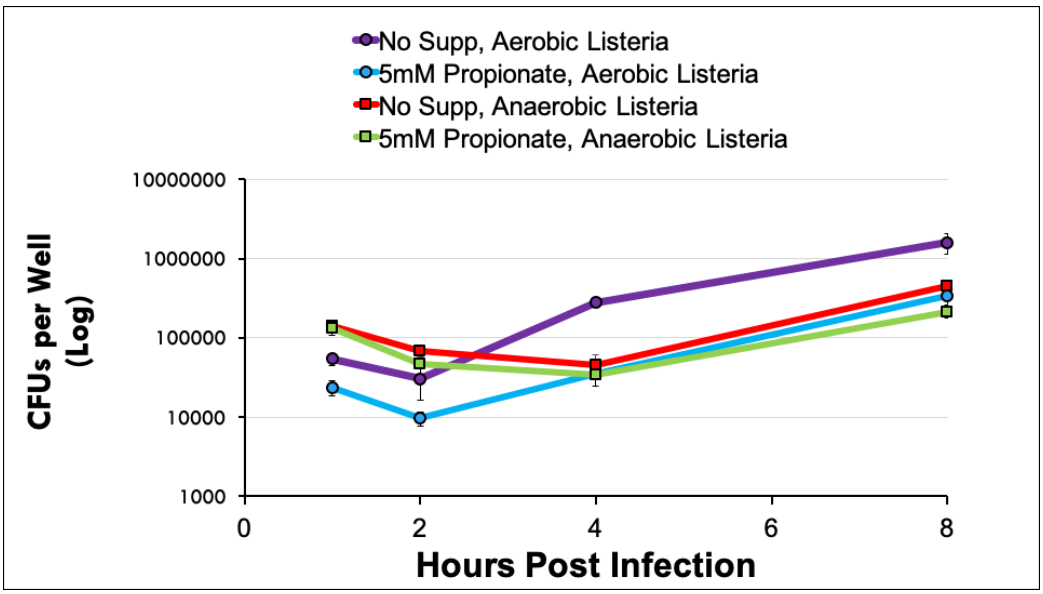

Figure 2

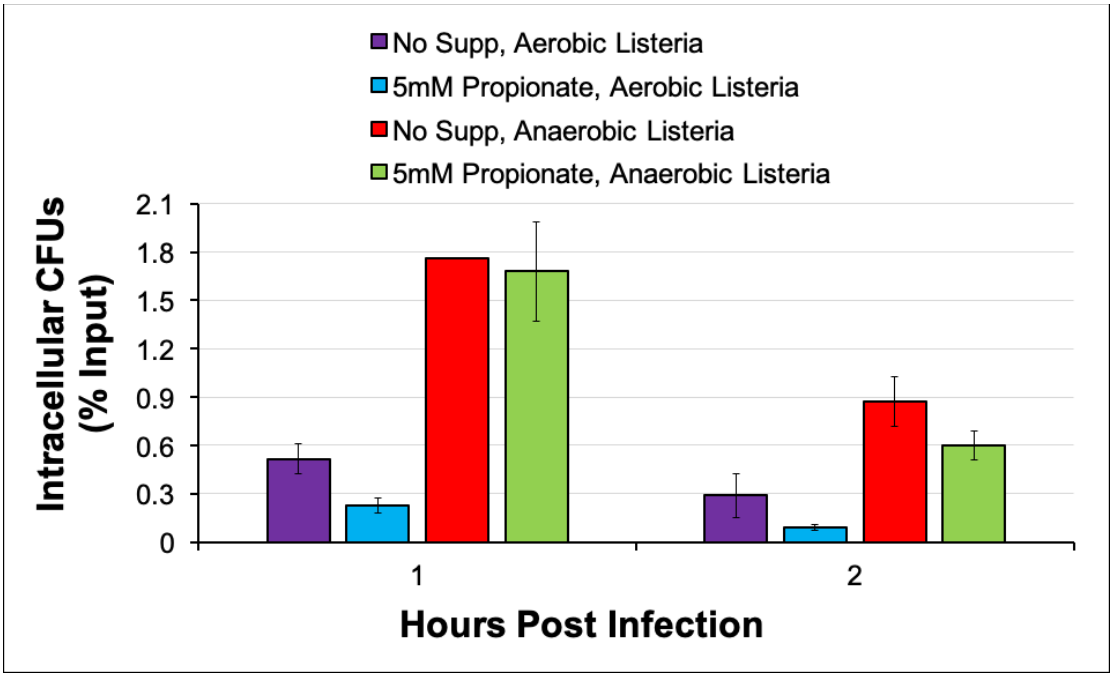

Figure 3

I then found that propionate treated macrophages have reduced amounts of intracellular *L. monocytogenes* in their phagosomes. In both aerobically and anaerobically grown *Listeria* without the *hly* gene, macrophages treated with propionate saw significantly less *L. monocytogenes* inside the phagosomes (**Figure 4, 5**).

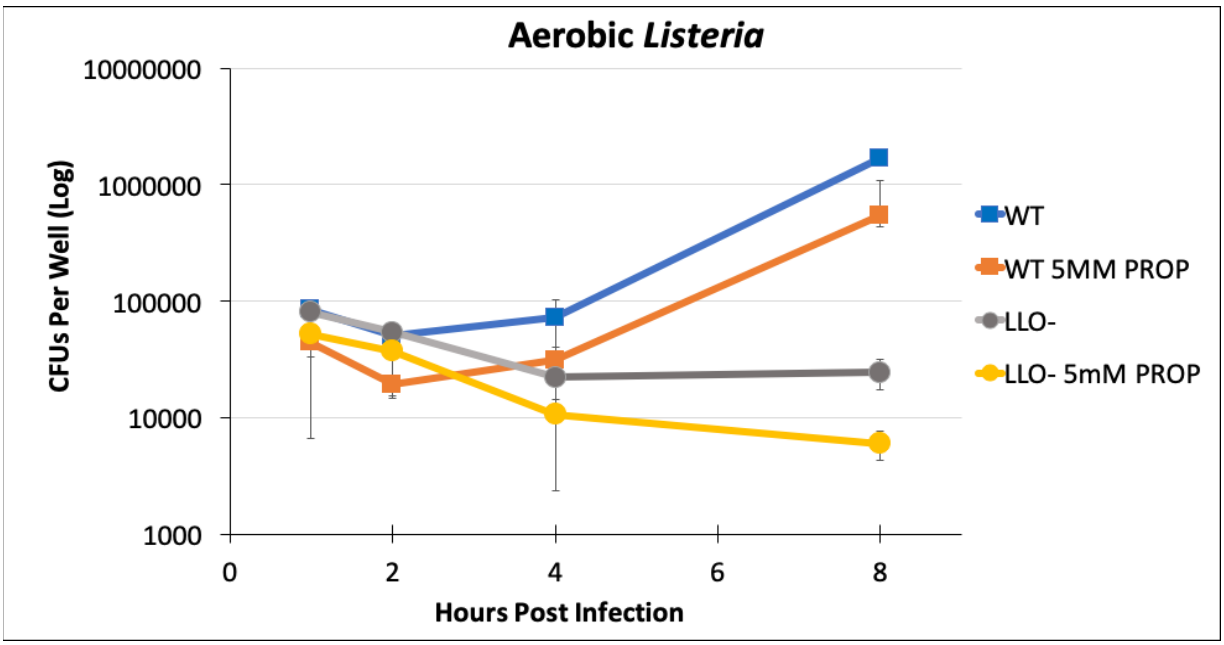

Figure 4

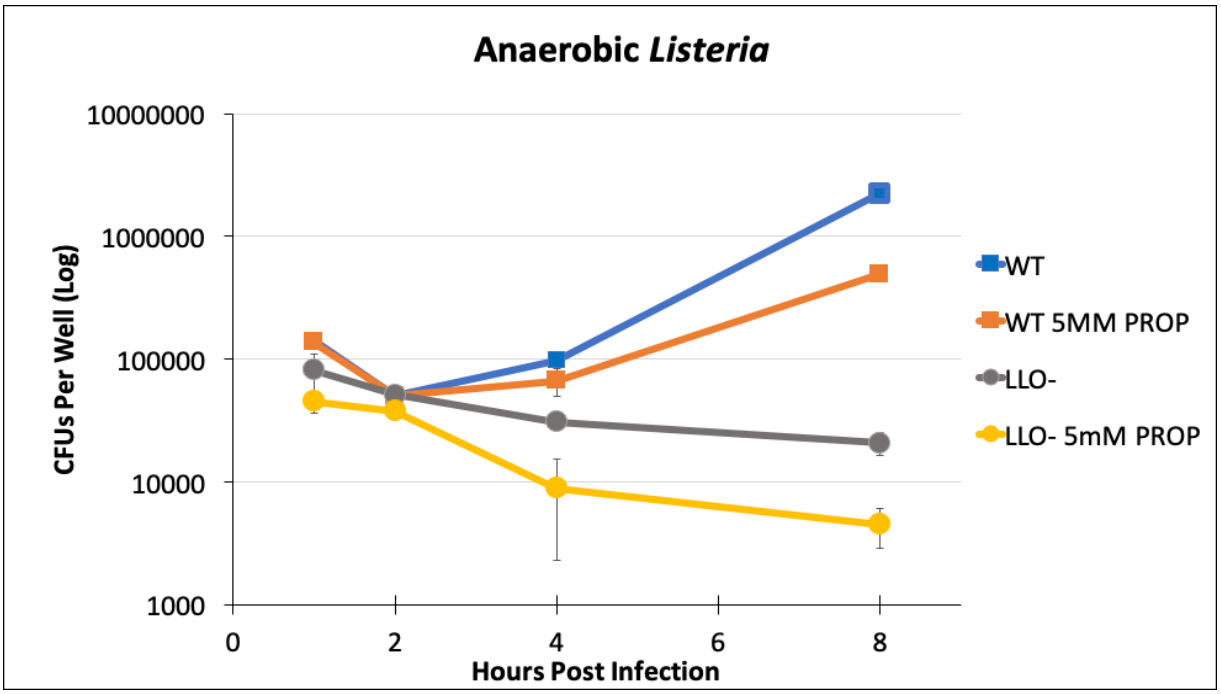

Figure 5

In aerobically grown *Listeria* there was no significant difference of intracellular *Listeria* growth in macrophages between no supplementation of propionate to the macrophages and supplementation of propionate to macrophages when the inhibitor was applied (**Figure 6**). However, interestingly enough when anaerobically grown *L. monocytogenes* was used there was a significant decrease in intracellular *L. monocytogenes* inside the macrophages that were treated with propionate and the Nitric Oxide Inhibitor (**Figure 7**).

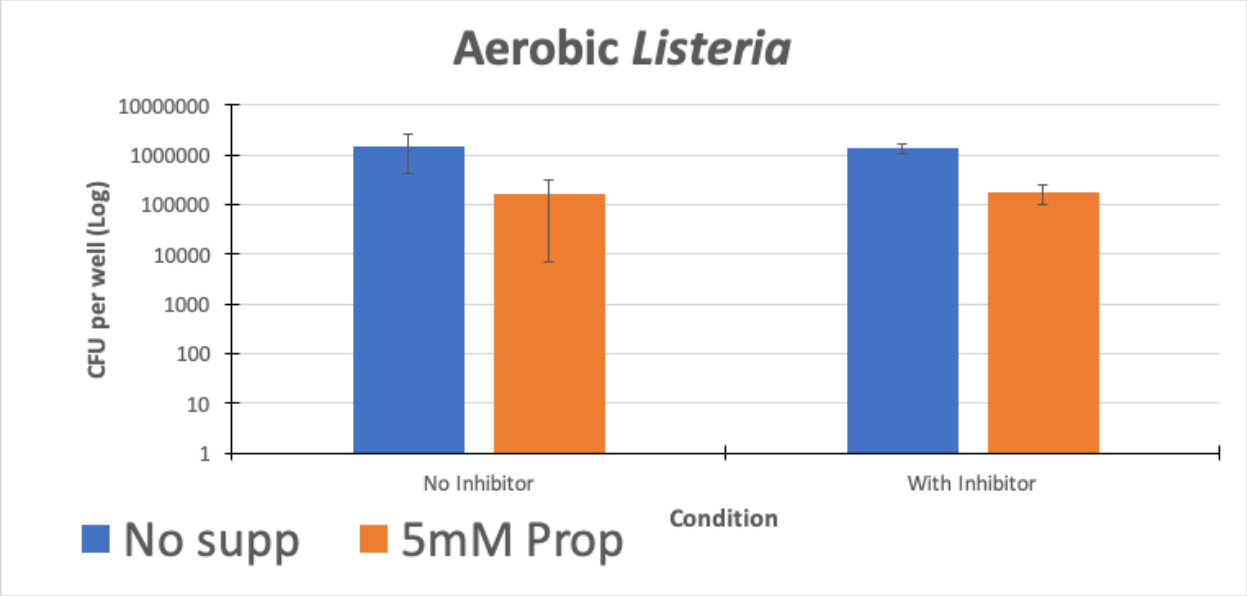

Figure 6

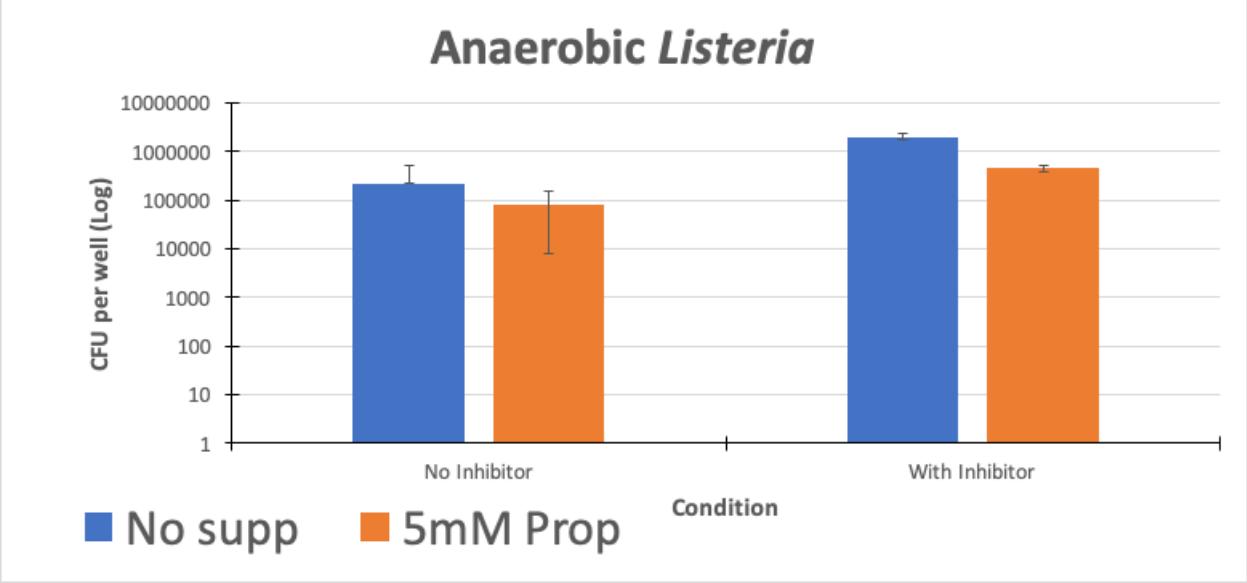

Figure 7

Macrophages were activated with interferon gamma (IFN-gamma) and supplemented with propionate to investigate if propionate is acting as an activator of macrophages. Propionate supplementation enhanced macrophage's bactericidal effects during the early hours of the infection. However, after 24 hours of infection, there was no statistically significant differences between the activated macrophages with propionate supplementation and the activated macrophages without propionate supplementation. Only Aerobic *Listeria* is shown below as the anaerobic *Listeria* gave me too confounded and inconsistent data to draw a conclusion.

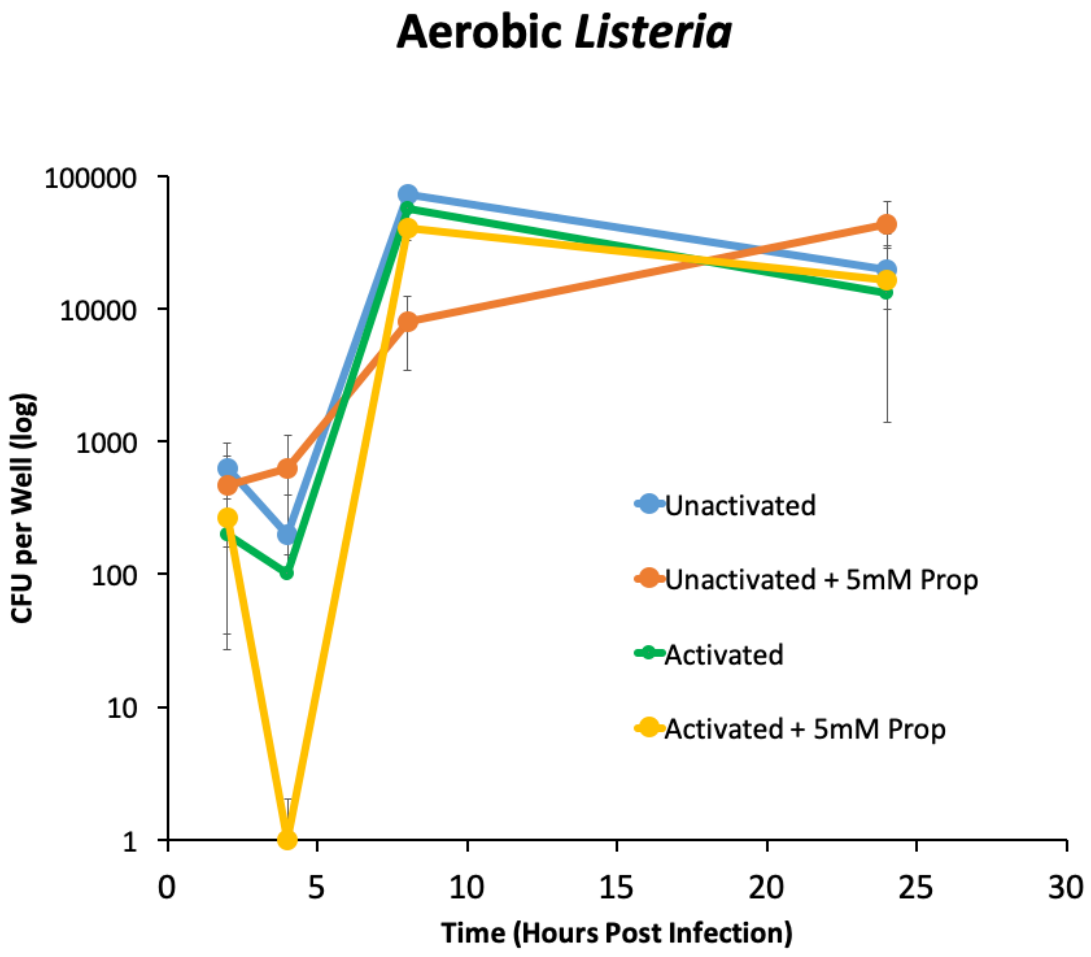

Figure 8

With the RAW 264.7 macrophage cell being an immortalized cell line, we wanted to test the efficacy of propionate on an un-immortalized cell line. We obtained Bone Marrow-derived macrophages and repeated the original cell culture infection that I had designed. Again, at 2 hpi I saw a statistically significant decrease for both anaerobically and aerobically grown *L. monocytogenes* inside bone marrow-derived macrophages (**Figure 9, 10**). Interestingly, at 1 hour hpi anaerobically grown *Listeria* had a higher intracellular input in the bone marrow-derived macrophages that were treated with 5mM propionate (**Figure 10**).

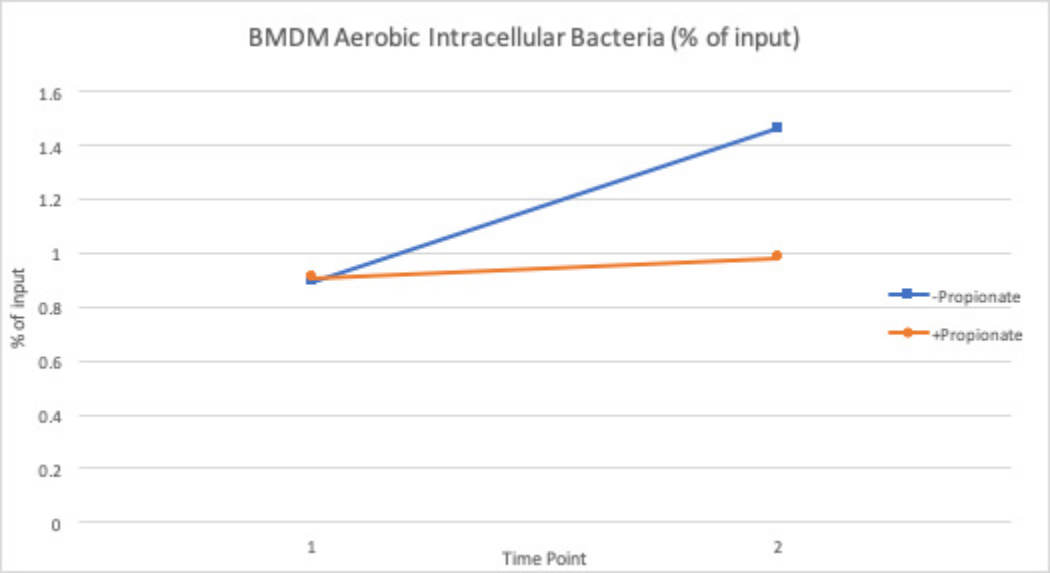

Figure 9

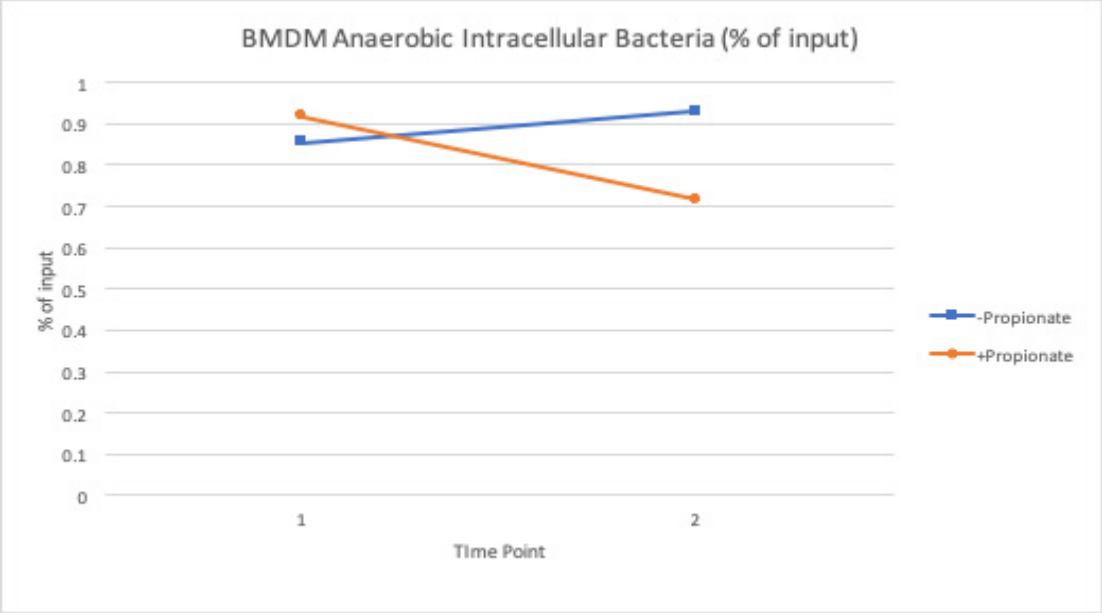

Figure 10

Discussion

Elizabeth Abram's data showed that the addition of propionate to *Listeria* overnight cultures did not lead to different infection outcomes in macrophages. This prompted me to investigate if propionate alters infection outcomes when supplemented in macrophage's culture media. Because of Elizabeth's finding, It was clear that propionate did not inhibit *Listeria's* ability to infect macrophages. From this, I was confident that the results seen in **Figures 2** and **3** (increased clearing of *L. monocytogenes*) were because propionate was in some way altering macrophage physiology and not directly inhibiting *Listeria's*. Our next goal was to isolate what propionate is actually doing to enhance the bactericidal features of macrophages. We wanted to locate where the killing was taking place. The majority of killing done by macrophages occurs in the phagolysosome almost immediately when a foreign invader is engulfed. *Listeria's* virulence factor LLO enables it to escape the phagolysosome and continue to survive and replicate. To solve this problem and isolate if the killing was in fact taking place in the phagosome we used a mutant of *Listeria* that does not have the gene to produce LLO (hly-). Results from **Figures 4** and **5** show that *L. monocytogenes* that is trapped inside the phagosomes of macrophages supplemented with propionate are killed off more efficiently. This finding suggests that the anti-microbial enhancement of propionate is credited to enhancing some type of macrophage killing mechanism inside phagosomes.

Macrophages have many different mechanisms of eliminating invaders from their phagosomes. This include Reactive Oxygen Species, Nitric Oxide Species, pH change, among others. One specific way that Macrophages eliminate Invaders from their phagosomes is through the poisonous nitric oxide (NO). I performed another cell culture infection supplementing a Nitric Oxide inhibitor, L-NMMA. L-NMMA takes away the ability of macrophages to use NO as a defense mechanism. Figures 6 identifies once again that propionate supplementation to macrophages that are infected with aerobically grown *Listeria* enhanced antimicrobial activity of macrophages.

Figure 7 shows that inhibiting nitric oxide production resulted in increased intracellular growth for only the anaerobically grown *L. monocytogenes*. From this experiment, it was not clear how exactly propionate effects NO production in macrophages. Because during this infection there is still a significant decrease in intracellular *L. monocytogenes*, it is most likely not one of the anti-microbial mechanisms that is seen being enhanced when propionate is added to earlier infections.

In **Figure 8** there is an extreme drop in intracellular *L. monocytogenes* to macrophages that have been activated and treated with 5mM propionate supplementation. However, this drop levels out and by the end of the experiment there are no significant differences between the four sets of conditions. It is most likely that this instance is an outlier and will have to be repeated to see if the result is consistent and can be repeated. Experiment 6 (**Figures 9** and **10**) is very similar to experiment 2. The experiment is being used to test the efficacy of using RAW 264.7 macrophages to compare to the macrophages found in our body. Bone marrow derived-macrophages (BMDM) were taken from mice ,and are not an immortalized cell line. These cells more so mimic the physiologically conditioned of cells found in the human body. When propionate was added to the BMM, very similar results occurred as when propionate was added to the RAW264.7 immortalized cell line of macrophages. This experiment proved the efficacy of using RAW264.7 macrophages and that they are in fact a good model cell line and can be compared to what would happen in vivo.

Conclusion

Through my research, there is a relationship between propionate and *Listeria*'s ability to intracellularly infect macrophages. There is still much more to learn about this relationship, but we have discovered some findings that are very intriguing and show the importance of investigating short chain fatty acids antimicrobial effects. It is clear that propionate is in some way enhancing macrophage's ability to eliminate *Listeria monocytogenes* during intracellular infection. More research must be conducted to find how exactly propionate is altering the anti-microbial mechanism that is allowing macrophages to more efficiently kill *L. monocytogenes*. With the increasing fear of antibiotic resistance to eliminate microbial threats such as *L. monocytogenes*, looking at other ways to combat pathogens is very important. Research in Short Chain Fatty acids has erupted over the recent years and findings like this show strong promise, as well as how important short chain fatty acids may be in human physiology.

Chapter 4 Funding and Appendix

Funding & Acknowledgement

This research was funded by American Heart Association (16GRNT27260219). E.R. and N.W. were supported by Graduate Student Summer Fellowship. I was supported by the Stephen E. and June H. Szabo Grant from the Premedical Program, the Dr. Robert Kearns Fellowship Travel Award, the Deans Summer Fellowship, and The Honors Program. Y.S. was supported by the University of Dayton Research Council, Hanley Sustainability Institute, and Department of Biology. I would like to acknowledge Dr. Nancy Freitag for sharing the *hly* reporter strain.

I would like to thank Dr. Sun for her resources and time, all members of Dr. Sun's Research lab, the University of Dayton Biology department, and all those that orchestrated the University of Dayton 2019 Brother Joseph W Stander Symposium. I would like to thank Erica Rinehart, Dr. Nathan Wallace, Elizabeth Abrams, and the rest of Dr. Sun's lab. I would especially like to thank Ryan Restrepo, Megan Bias, Megan Marasco, Andrea, Sam, and Ashton. I would also like to thank the rest of The University of Dayton Faculty & Staff and The University of Dayton Honors Program.

Appendix

References

1. Aoyama, M., Kotani, J. & Usami, M. Butyrate and propionate induced activated or non-activated neutrophil apoptosis via HDAC inhibitor activity but without activating GPR-41/GPR-43 pathways. *Nutrition* 26, 653–661 (2010).
2. Boets, E. *et al.* Systemic availability and metabolism of colonic-derived short-chain fatty acids in healthy subjects: a stable isotope study. *The Journal of Physiology* 595, 541–555 (2017).
3. Brunkhorst, B. A., Kraus, E., Coppi, M., Budnick, M. & Niederman, R. Propionate induces polymorphonuclear leukocyte activation and inhibits formylmethionyl-leucyl-phenylalanine-stimulated activation. *Infect. Immun.* 60, 2957–2968 (1992).
4. Cook & Sellin. Review article: short chain fatty acids in health and disease. *Alimentary Pharmacology & Therapeutics* 12, 499–507 (1998).
5. Dussault, D., Vu, K. D. & Lacroix, M. Development of a model describing the inhibitory effect of selected preservatives on the growth of Listeria monocytogenes in a meat model system. *Food Microbiol* 53, 115–121 (2016).
6. Glass, K. A., McDonnell, L. M., Rassel, R. C. & Zierke, K. L. Controlling Listeria monocytogenes on sliced ham and turkey products using benzoate, propionate, and sorbate. *J. Food Prot.* 70, 2306–2312 (2007).
7. Glass, K. A., McDonnell, L. M., Von Tayson, R., Wanless, B. & Badvela, M. Inhibition of Listeria monocytogenes by propionic acid-based ingredients in cured deli-style Turkey. *J. Food Prot.* 76, 2074–2078 (2013).

8. Kawamura, T. *et al.*Inhibitory effects of short-chain fatty acids on matrix metalloproteinase secretion from human colonic subepithelial myofibroblasts. *Dig. Dis. Sci.*54, 238–245 (2009).
9. McFarland, A. P. *et al.* RECON-Dependent Inflammation in Hepatocytes Enhances Listeria monocytogenes Cell-to-Cell Spread. *MBio* 9, (2018).
10. Morrison, D. J. & Preston, T. Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. *Gut Microbes* 7, 189–200 (2016).
11. Naccache, P. H., Faucher, N., Caon, A. C. & McColl, S. R. Propionic acid-induced calcium mobilization in human neutrophils. *J. Cell. Physiol.*136, 118–124 (1988).
12. Nastasi, C. *et al.*The effect of short-chain fatty acids on human monocyte-derived dendritic cells. *Sci Rep*5, 16148 (2015).
13. Niederman, R., Buyle-Bodin, Y., Lu, B. Y., Robinson, P. & Naleway, C. Short-chain carboxylic acid concentration in human gingival crevicular fluid. *J. Dent. Res.*76, 575–579 (1997).
14. Pitts, M. G., Combs, T. A. & D'Orazio, S. E. F. Neutrophils from Both Susceptible and Resistant Mice Efficiently Kill Opsonized Listeria monocytogenes. *Infect Immun* 86, (2018).
15. Rath, M., Müller, I., Kropf, P., Closs, E. I. & Munder, M. Metabolism via Arginase or Nitric Oxide Synthase: Two Competing Arginine Pathways in Macrophages. *Front Immunol* 5, (2014).
16. Rinehart, E. *et al.* Listeria monocytogenes Response to Propionate Is Differentially Modulated by Anaerobicity. *Pathogens* 7, (2018).

17. Rotstein, O. D. Interactions between leukocytes and anaerobic bacteria in polymicrobial surgical infections. *Clin. Infect. Dis.*16 Suppl 4, S190-194 (1993).
18. Stempelj, M., Kedinger, M., Augenlicht, L. & Klampfer, L. Essential Role of the JAK/STAT1 Signaling Pathway in the Expression of Inducible Nitric-oxide Synthase in Intestinal Epithelial Cells and Its Regulation by Butyrate. *J. Biol. Chem.*282, 9797–9804 (2007).
19. Vonk, R. J. & Reckman, G. Progress in the biology and analysis of short chain fatty acids. *The Journal of Physiology* 595, 419–420 (2017).
20. Wallace, N., Newton, E., Abrams, E., Zani, A. & Sun, Y. Metabolic determinants in Listeria monocytogenes anaerobic listeriolysin O production. *Arch Microbiol* 199, 827–837 (2017).
21. Wallace, N., Rinehart, E. & Sun, Y. Stimulating Respiratory Activity Primes Anaerobically Grown Listeria monocytogenes for Subsequent Intracellular Infections. *Pathogens* 7, 96 (2018)
22. Weigert, A., von Knethen, A., Fuhrmann, D., Dehne, N. & Brüne, B. Redox-signals and macrophage biology (for the upcoming issue of molecular aspects of medicine on signaling by reactive oxygen species). *Mol. Aspects Med.*(2018).
23. Whiteley, A. T. *et al.* c-di-AMP modulates Listeria monocytogenes central metabolism to regulate growth, antibiotic resistance and osmoregulation. *Molecular Microbiology* 104, 212–233 (2017).
24. Rinehart, E.; Newton, E.; Marasco, M.A.; Beemiller, K.; Zani, A.; Muratore, M.K.; Weis, J.; Steinbicker, N.; Wallace, N.; Sun, Y. *Listeria monocytogenes* Response to Propionate Is Differentially Modulated by Anaerobicity. *Pathogens* 2018, *7*, 60.

25. Tan, J. *et al.* The role of short-chain fatty acids in health and disease. *Adv. Immunol.*121, 91–119 (2014).
26. Feng, W., Ao, H. & Peng, C. Gut Microbiota, Short-Chain Fatty Acids, and Herbal Medicines. *Front Pharmacol* 9, (2018).
27. Elhelu, M. A. The Role of Macrophages in Immunology. *J Natl Med Assoc*75, 314–317 (1983).
28. Weiss, G. & Schaible, U. E. Macrophage defense mechanisms against intracellular bacteria. *Immunol Rev*264, 182–203 (2015).
29. Burden of Foodborne Illness: Findings | Estimates of Foodborne Illness | CDC. (2018). Available at: https://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html. (Accessed: 17th April 2019)
30. CDC. People at Risk for Listeria Infection. *Centers for Disease Control and Prevention* (2017). Available at: https://www.cdc.gov/listeria/risk.html. (Accessed: 17th April 2019)
31. Outbreak of Listeria Infections Linked to Pork Products | Outbreak of Listeria Infections Linked to Pork Products | November 2018 | Listeria | CDC. (2019). Available at: https://www.cdc.gov/listeria/outbreaks/porkproducts-11-18/index.html. (Accessed: 17th April 2019)
32. Meningitis | About Bacterial Meningitis Infection | CDC. (2019). Available at: https://www.cdc.gov/meningitis/bacterial.html. (Accessed: 17th April 2019)
33. Dramsi, S. & Cossart, P. Listeriolysin O. *J Cell Biol*156, 943–946 (2002).
34. Doyle, M. E. Virulence Characteristics of Listeria monocytogenes. 13 (2001).

35. Marler Clark, L. L. P. The Economic Impact of Listeria Infections. *About Listeria*(2019). Available at: http://about-listeria.com/the-economic-impact-of-listeria-infections. (Accessed: 17th April 2019)

Poster Presentations

John Weis, Yvonne Sun. 2017. Propionate enhances the antimicrobial response of macrophages against *Listeria*. University of Dayton Summer Symposium. July 28th, 2017. Dayton, OH.

E. Rinehart, K. Beemiller, E. Newton, M. Muratore, J. Weis, M. Marasco, N. Steinbicker, A. Zani, N. Wallace, Y. Sun. 2018. Propionate Perturbation of Listeria Monocytogenes Growth and Listeriolysin O Production is Modulated by Anaerobicity. American Society of Microbiology Microbe Conference 2018. June 9th. 2018. Atlanta, GA.

John Weis, Erica Rinehart, and Yvonne Sun. 2018. Understanding the role of propionate in the enhancement of antimicrobial responses in macrophages against *Listeria*. University of Dayton Summer Science Research Symposium. July 25th, 2018. Dayton, OH.

Robert Busch, John Weis, Farzia Karim, Chenglong Zhao, Yvonne Sun, Erick S. Vasquez. Mechanistic study of *Listeria* antibodies binding onto Au Nanoparticles at different pH conditions. Dayton American Chemical Society Poster Session. April 11th, 2019. Dayton, OH.

Publications

Erica Rinehart, Eric Newton, Megan Marasco Kaitlin Beemiller, Ashley Zani, Melanie Muratore, John Weis, Nicole Steinbicker, Nathan Wallace, Yvonne Sun. 2018. Listeria monocytogenes response to propionate is differentially modulated by anaerobicity. *Pathogens*, 7(3).