

SOAR Proposal for Summer Research 2016

Project Title: Recapitulating Immuno-Antimicrobial Synergy against *Escherichia coli* infections

Faculty Mentor: Dr. Kara Mosovsky, Assistant Professor, Department of Biological Sciences

Student: Paige Hart, Biology Major, Class of 2017

Project duration: May 31st – August 8th (10 weeks)

Project description:

Background:

Burkholderia pseudomallei is a bacterial pathogen and the causative agent of the disease melioidosis. Melioidosis is a frequent and deadly disease of South-east Asia and Northern Australia, with rapid onset of signs and symptoms as well as poor prognosis. There is no vaccine against *Burkholderia pseudomallei* and due to its inherent antibiotic resistance, infections are difficult to treat. Even with antibiotics, mortality rates are typically 20-50% and persistent infections frequently occur which can lead to relapse infections or recrudescence of the original infection. There is a great need for novel treatment methods to fight this deadly pathogen and the disease that it causes.

One major reason why the human host is so susceptible to this pathogen is because *Burkholderia* has the ability to live inside macrophages, a type of white blood cell of the immune system that is usually involved with killing bacterial pathogens. In this intracellular niche, it can avoid both antibiotics and detection by the immune system. Since macrophages are naturally infected by *Burkholderia* species, we have created a macrophage infection model to study potential treatment strategies outside of a living host. Using an immortalized macrophage cell line that can be grown in flasks and manipulated in the laboratory, we first subject the macrophages to bacteria to establish an intracellular infection, and then apply treatments in the hopes of decreasing the bacterial burden. Since *Burkholderia pseudomallei* is capable of causing deadly disease, we instead use the different, but related, *Burkholderia thailandensis*. *B. thailandensis* does not usually cause infections in humans and is therefore entirely safe to work with in an undergraduate laboratory.

Rationale:

We have previously found success in a treatment that combines activation of the infected macrophages and traditional antibiotic therapy to kill intracellular bacteria. To elicit this effect we combine IFN- γ , a potent chemical that increases the killing capability of macrophages, with ceftazidime, the current antibiotic of choice for treating human cases of melioidosis. When these two treatments are combined, their killing effects are greater than either drug treatment alone and greater than their additive effects. We call this *synergy*. We have discovered that the mechanism of this synergistic action is due to antibiotic-mediated killing of bacteria outside of the macrophages and IFN- γ -mediated killing within the macrophages. This combination of extracellular and intracellular killing by the two drugs creates a two-pronged attack of the

bacteria and reduces the bacterial burden of the entire macrophage system. Interestingly, we have also discovered the ability of some antioxidants to interfere with the killing of bacteria in this combination therapy. Our preliminary results suggest that although antioxidants are praised as the epitome of “healthy,” they may in fact hinder the immune system’s ability to naturally fight off intracellular bacterial infections. While these findings are significant on their own, we have only studied these mechanisms in *Burkholderia* species, and we still do not know if our findings apply to other bacterial infections. If we could prove immuno-antimicrobial synergy could be used to treat other intracellular bacterial infections caused by more common pathogens, such as *Escherichia coli*, we would not only suggest a novel treatment for these infections, but we may also reconsider our popularization of antioxidants.

Proposed project:

We will introduce a new organism, *E. coli*, into our macrophage infection model to test if our combined immuno-antimicrobial therapy is more broadly applicable as a treatment of intracellular infections. To begin we will need to optimize the infection dose, type of antibiotic used, concentration of antibiotic used, and timing of our infection model. After experimenting with these variables, we will then combine the antibiotic with IFN- γ stimulation and measure the resulting decrease in bacterial burden. Once we have found an antibiotic that combines with IFN- γ to synergistically kill *E. coli*, we can then use established methods to test if the mechanism of synergy is the same, and to evaluate whether or not antioxidants interfere with the killing.

Roles and responsibilities:

Qualifications of student: Paige is qualified to take ownership of this project. Paige has a rich background in clinical infection having worked for a veterinary clinic for two years, and she is currently enrolled in my immunology seminar on host-pathogen interactions. She has not taken microbiology yet, but she has already shown herself to be dedicated to this project and a very fast learner. I am confident that she would no doubt master the microbiology necessary for an in-depth understanding of her project, even before the May 31st start date. Her enthusiasm and background for this project will suit her well as she begins bench-level research under my guidance.

Student Roles and Engagement in Discipline-Appropriate Scholarly Research: Paige will have the opportunity to actively participate in all aspects of this project including reading and discussing the primary literature, developing an experimental design to test her research questions, and conducting the actual experiments. She will also collect and analyze data under my guidance. If Paige would like to continue a related line of research for an Honors project, she would certainly have the opportunity to compile her data into a publishable form. *Fine Focus*, a journal for undergraduate research in microbiology would be a suitable starting goal for Paige’s research.

Paige’s project will add to the growing body of research on therapies that can replace or add to traditional antibiotic therapy, and with increasing antibiotic resistance, this line of work could not be more pertinent. Her project will serve as outstanding hands-on experience, which will

introduce her to the process and culture of microbiology research. Additionally, she will develop skills in cell culture, an important tool for conducting research in host-pathogen interactions.

Roles of faculty mentor: I will guide and mentor Paige through all aspects of the project mentioned above. We will work side-by-side until she feels confident to perform calculations and carry out experiments on her own. Even after she has gained independence in carrying out experiments, I will continue to meet with her daily to discuss the primary literature, offer suggestions to improve her technique, and help her interpret data.

In addition to in-laboratory guidance, I will nurture her professional growth by helping her develop a poster and presentation of her results for Moravian College's Scholar Day and an abstract for the National Council for Undergraduate Research student conference. If our schedules allow, I would also like to introduce her to professional meetings of the Eastern Pennsylvanian branch of the American Society for Microbiology (ASM).

Expected Timeline of Project:

Weeks 1-2: Practice cell culture techniques required to perform the macrophage infection model and experiment with the initial infectious dose for *E. coli*. Ideally, intracellular *E. coli* numbers will reach 1×10^6 or 1×10^7 bacteria/mL within the macrophages.

Weeks 3-8: Optimize one or more antibiotics for use in the macrophage infection model. After finding a suitable antibiotic, titrate different concentrations of the antibiotic in the macrophage infection model. Optimum antibiotics will result in little killing of intracellular bacteria, but will result in good killing of extracellular bacteria. Test immuno-antimicrobial synergy with IFN- γ and optimum antibiotic concentration. Optimize timing of the synergistic effect.

Weeks 8-10 (or as time permits): Test if the mechanism of synergy with *E. coli* is the same as with *Burkholderia*, and evaluate whether or not antioxidants interfere with the synergistic mechanism.

Student Benefits: In addition to the scholarly benefits mentioned above (Student Roles and Engagement in Discipline-Appropriate Scholarly Research), it is our expectation that Paige will present her work at Moravian College's Annual Student Scholars Day in Spring 2017 as well as submit an abstract for acceptance to the National Council for Undergraduate Research student conference. Depending on the outcome of her project, we would like to publish her work in *Fine Focus*, a microbiology journal for undergraduate research. The writing process would engage Paige in yet another facet of scholarship in science. If she continues with a related project for Honors or independent study, there will be additional avenues for publication in peer-reviewed journals and presentation of her work at the American Society for Microbiology general meeting in Summer 2017.

Paige E. Hart Statement of Purpose

Biology Major

Moravian College Class of 2017

Recapitulating Immuno-Antimicrobial Synergy against *Escherichia coli* infections

Dr. Kara Mosovsky

On-Campus Housing Not Requested

Ever since elementary school when I first utilized a microscope and became acquainted with the basics of microbiology, I have been utterly fascinated with microscopic organisms and the diseases they cause. Throughout my youth, I did research papers on topics such as Diphtheria and the Bubonic Plague solely to indulge my need to investigate the microbes themselves, how they cause disease, and their effects on human history. In more recent years, I became employed at an emergency veterinary clinic to learn more about pathogenesis of veterinary ailments, zoonoses, veterinary parasitology, and the medications used to treat maladies that result from infection. Within roughly two years of working at the clinic, I have personally helped treat multitudes of patients suffering from ailments such as Leptospirosis, Methicillin-resistant *Staphylococcus aureus* (MRSA), Parvovirus infections, Feline Calicivirus infections, Giardiasis, and Haemobartonellosis (Feline Infectious Anemia) just to name a few. Due to this, I have increased interest in how microscopic organisms cause disease, their modes of transmittance, and how they can be effectively treated. As a result, I plan on pursuing a doctorate in microbiology so that I may one day be able to contribute to the field, and as a result help improve the treatments used in both human and veterinary medicine.

The original research project proposed by Dr. Mosovsky and myself will provide insight into the synergistic relationship between the cytokine interferon-gamma, which increases the cytotoxic activity of immune cells, and antibiotics in the treatment of intracellular pathogens. Previously, Dr. Mosovsky found that the intracellular pathogen *Burkholderia thailandensis* is more effectively treated with a combination of interferon-gamma and the antibiotic ceftazidime.

In this project, we hope to find the same relationship when treating *Escherichia coli* with a combination treatment of interferon-gamma and a suitable antibiotic. If the same relationship is present, a model will be developed that will elucidate the effectiveness of the combination of these two drugs as a treatment compared to that of each component separately. This model may very well result in better forms of treatment for diseases caused by resilient intracellular pathogens, and as such, will be an asset to the health and scientific community.

On a more personal level, this research project will provide me with the opportunity to learn in a kinesthetic style about the field of microbiology and immunology while also enabling me to gain important laboratory skills over the duration of the 10-week period. The knowledge I will gain throughout this experience will be invaluable in my pursuit of acceptance into a microbiology PhD program this coming fall as well as providing me with ideas for future research projects. Furthermore, this research project will be incredibly intellectually stimulating for me, thus unequivocally enabling me to grow as an aspiring scientist and individual. In summary, the proposed research project will provide me with an outstanding opportunity to obtain relevant hands-on experience in microbiology and immunology, cultivate my laboratory techniques, and aid in the advancement of knowledge in regards to more effective treatment of intracellular pathogens.

Expense Proposal

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Expected Expenses:

1) Cell culture medium components

Several components required to support the culture of mammalian cell lines in the lab:

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| 1) 4-500 mL bottles of minimum essential medium (base of cell culture medium) | 4 @ \$23.00 |
| 2) PenStrep solution (for maintenance of cell line sterility) | 1 @ \$20.00 |
| 3) Amino acid solutions (for added nutrients for cell line) | 1 @ \$60.00 |

2) Plastic consumable labware (petri dishes, bacteria medium, cell scrapers, tissue culture-treated plates, disposable tubes, dilution plates, pipette tips, serological pipettes)

Cell culture requires specific sterile, plasticware for growing large quantities of mammalian cells. ~\$150

3) *Escherichia coli* for intracellular infections

Research-grade organism, purchased from American Type Culture Collection ~\$300

4) GraphPad Prism Software for student computer

For the statistical analysis of microbiological data as well as graph formulation. An extra site license is available from CIT for the listed price (already received quote). \$450 per license

Total = \$1072

Funds requested from SOAR = \$500

**The Department of Biological Sciences will cover the remaining expected costs as well as any unforeseen costs throughout the length of the project.