SOAR Proposal for Summer Research 2016

Project Title: Antibiotic Tolerance: Distinguishing between Classical Resistance and Persistence in a Macrophage Infection Model
Faculty Mentor: Dr. Kara Mosovsky, Assistant Professor, Department of Biological Sciences
Student: Crystal Collins, 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 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 detection by the immune system and antibiotics. 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 cells 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 that activation of the infected macrophages, combined with traditional antibiotic therapy, can drastically reduce the number of intracellular bacteria in infected macrophages. 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. Although this novel combination can greatly decrease the bacterial load of infected macrophages, we have never achieved complete sterilization in the macrophage model. We suspect, that these resilient, remaining, bacteria may be tolerant to antibiotics. Just as any remaining bacteria after melioidosis treatment may cause relapse of the infection, the remaining bacteria in the macrophage infection model pose a significant threat to reactivation to a potential host. It is important to understand the basis of their antibiotic tolerance so as to be better

equipped to develop strategies that target the mechanism of their tolerance. There are only two possibilities to explain antibiotic tolerance in bacteria. Either the bacteria are classically resistant, which is a heritable trait in which genes have actually been altered to resist antibiotics, or the bacteria are persisters. Persister cells are slow-growing and temporarily tolerant to antibiotics, but their tolerance is not heritable like classical antibiotic resistance. When removed from the stressful environment, persister cells will regain sensitivity to antibiotics. Again, once we characterize the bacteria as resistant or persistent, we will be able to determine the best strategies to target their killing. Antibiotic resistant bacteria likely pose a greater threat to the host than persisters due to their permanent and heritable tolerance to antibiotics. Additionally, antibiotic therapy ceases to be effective after classical antibiotic resistance has developed.

Proposed project:
We will first prove that the remaining bacteria are capable of tolerating antibiotics. Using pre-established methods, we will then characterize the antibiotic tolerant bacteria as either antibiotic resistant cells or persister cells. We will determine the potential risk these bacteria play in infections by characterizing their ability to re-infect healthy macrophages, and time permitting, we will evaluate different strategies to enhance killing of these particularly stubborn remainders.

Roles and responsibilities:

Qualifications of student: Crystal is more than qualified to take ownership of this project. She excelled in my microbiology lecture and lab this past Fall semester, is currently enrolled in my immunology seminar, and is also serving as a teaching assistant for the laboratory component of microbiology. Her enthusiasm and relevant background for this project will enable her to immediately begin bench-level research under my guidance.

Student Roles and Engagement in Discipline-Appropriate Scholarly Research: Under my guidance, Crystal will actively participate in all aspects of this project including reading the primary literature, development of hypotheses and experimental design, conducting experiments, as well as collecting and analyzing data. As is inevitable in laboratory research, she will also participate in trouble-shooting technical issues as they arise. Pending the results of her project and the progress we make, Crystal may also have the opportunity to publish her work in an undergraduate journal.

Crystal'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. All aspects of this project serve as outstanding and relevant hands-on application and reinforcement of her classroom learning and will also deepen her understanding of the process and culture of science from the perspective of a laboratory scientist. Additionally, she will develop skills in cell culture, an important tool for conducting research in host-pathogen interactions.

Role of faculty mentor:
I will mentor Crystal through all aspects of the project mentioned above, ensuring that Crystal has completely mastered both the theory and technical aspects of each step. I will offer attentive, side-by-side guidance until she feels comfortable performing calculations and conducting experiments on her own. Research is never isolated, independent work, so even once she is capable of working more independently, I will continue to meet with her daily throughout the length of the project to assist with data interpretation, design of new experiments, and to discuss the primary literature.

I will further promote her professional growth by helping her develop a poster and presentation of her results for Moravian College's Scholar Day. In addition we will work together to develop an abstract for both the National Council for Undergraduate Research student conference as well as the student conference for Tri-Beta, a national biology honor society. 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) down in Philadelphia.

Expected Timeline of Project:

Weeks 1-2: Practice cell culture techniques required to perform the macrophage infection model and confirm the existence of a population of antibiotic tolerant bacteria following treatment with the combination of the macrophage activator, IFN-γ, and the antibiotic ceftazidime.

Weeks 3-7: Build on pre-established methods to characterize the remaining *Burkholderia thailandensis* bacteria as either antibiotic resistant cells or temporary persister cells.

Weeks 6-10: Evaluate the potential of the remaining bacteria to re-infect healthy macrophages. Hypothesize and test different methods to enhance killing of the remaining bacteria, thus eliminating the threat of future relapse.

Student Benefits: In addition to the scholarly benefits mentioned above (Student Roles and Engagement in Discipline-Appropriate Scholarly Research), it is our expectation that Crystal 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 and the annual undergraduate research conference hosted by Beta Beta Beta, a biology honor society. 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 Crystal in yet another facet of scholarship in science. She intends to continue research with a different but related project in my lab for her Honors project starting Fall 2016. An Honors project will provide additional opportunities for publication in peer-reviewed journals and presentation of her work at the American Society for Microbiology general meeting in Summer 2017.

SOAR Project Proposal
Summer 2016

Student Statement of Purpose

Project title: Antibiotic Tolerance: Distinguishing between Classical Resistance and Persistence in a Macrophage Infection Model
Student name: Crystal Collins
Major: Biology
Graduation: May 2017
Faculty Mentor: Dr. Kara Mosovsky, Assistant Professor, Department of Biological Sciences
Campus Housing: Yes

For a while now, I have been interested in completing my degree in biology and minoring in chemistry to pursue a career in the health field. I have concentrated merely upon completing my bachelors with the goal of medical school. However, after successfully completing a microbiology course this past fall and now assisting as a teaching lab assistant for microbiology, I find that I am captivated by research. My interests in the science field have evolved. I not only want to continue my education in the health field, but I am considering involvement with research as another possibility. Topics such as bacteria, antibiotic resistance, and infectious disease have piqued my interest after the various science classes that I have taken at Moravian. By completing a relevant research project in microbiology, I will gain the hands-on experience of studying intracellular pathogens that infect humans. I am intrigued by this project and excited to use different lab techniques, and to ask questions about how *Burkholderia* species interact with macrophages. The knowledge and experience that I will gain will provide me with important and fundamental microbiology skills used in the field.

Another reason for my interest in applying for this research experience is that it will lead to further research opportunities for me, such as an Honors project, which I intend to pursue after

this project ends. In addition to this research project, depending on the outcome, I would submit an abstract to the National Council for Undergraduate Research student conference as well as the student conference for Tri-Beta, which is a national biology honor society to which I currently serve as president at Moravian College. I am passionate and dedicated to the biological sciences, and learning the methods and techniques that are involved. This project will allow me to discover new material that would be beneficial for me in my future career.

The research that I aspire to embark on, with the guidance and encouragement of my faculty advisor, would be to characterize the *Burkholderia thailandensis* bacteria that remain after treatment of infected macrophages. We suspect that these organisms are tolerant to the antibiotics. This organism is a related species to *Burkholderia pseudomallei*, which is capable of causing a deadly disease and is difficult to treat. The reason why we will be using the related species, *Burkholderia thailandensis*, is because it is safer to work with in the laboratory. The next task is to characterize the remaining cells as resistant or persister cells. "Persister cells" are cells which are temporarily tolerant to the antibiotic that they are treated with. Finally, we will determine the risk posed by the antibiotic tolerant bacteria to re-infect healthy macrophages.

Participating in this SOAR project will be a unique opportunity for me to utilize the skills and techniques that I have developed through my laboratory work in microbiology as a teaching assistant and the past courses that I have taken. Involvement and proficiency in lab-based skills is essential to successfully obtain a position in that field. Engaging myself in research for a ten week period will ripen my appreciation for the sciences and will allow me to mature and develop as a scientist.

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:

   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) **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 = $772
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.