SOAR Proposal for Summer Research 2017

Project Title: Do antioxidants interfere with bacterial killing in a macrophage infection model?
Faculty Mentor: Dr. Kara Mosovsky, Assistant Professor, Department of Biological Sciences
Student: Michelle Pomposello, Biology Major, Class of 2018
Project Duration: May 22nd – August 4th (11 weeks). Please read "Rationale for Altered Hours" below for more information about our expected timeline and rationale for a 20 hr/week SOAR project instead of a 40 hr/week project.

Project Description

General Background: *Burkholderia pseudomallei* is a bacterial pathogen and the causative agent of the disease melioidosis. While melioidosis is rare in the United States, it causes deadly disease in south-east Asia and northern Australia where the pathogen is normally found in soil and water. *B. pseudomallei* is inherently antibiotic resistant and treatments to cure the disease are long and expensive. There is no preventative vaccine, and even with antibiotics the mortality rate for melioidosis is still 25-50%. New therapies are sorely needed to tackle the burden of this disease around the world.

One of the unique aspects of the bacterium that allows it to cause such devastating disease is the fact that it can live inside white blood cells of the immune system. In doing so, the bacteria are protected from antibiotics as well as antibodies from the host's immune response. It is quite an interesting model by which to study the interplay between the host's attack and the bacteria's defenses. In the laboratory, we study this interplay using a specific type of white blood cell called a macrophage. Using a purchased cell line, we grow and manipulate the macrophages in plastic cell culture flasks, outside of a living animal. We infect the macrophages, apply treatments, and then track surviving numbers of bacteria. Since *Burkholderia pseudomallei* is capable of causing such devastating disease, we instead work with the different, but related model organism, *Burkholderia thailandensis*. *B. thailandensis* is entirely safe to work with in an undergraduate laboratory because it doesn't normally cause infections in humans.

Specific Project Background: In previous studies we discovered an interaction between two drug treatments that led to enhanced killing of the bacteria inside the macrophage white blood cells. One drug is ceftazidime, a common antibiotic used to treat melioidosis, and the other drug is interferon-gamma, an immune system activator created by the human body to fight infections. However, the killing power of these two drugs was inhibited by addition of certain antioxidants. In other words, the drug combination of antibiotic and immune stimulant normally reduced the bacteria inside the infected macrophages, but there was less killing when this antioxidant was present. In those same studies, we determined that the reason for the decreased killing was due to the antioxidant eliminating the toxic free radicals that kill bacteria. This surprising result led us to hypothesize that other antioxidants (maybe even dietary antioxidants) may have a similar effect, and if that were true, it may suggest that antioxidants, while touted as the miracle compounds that they are, may not be beneficial in certain types of infection. What if, instead, antioxidants interfere with the ability of the body and/or antibiotics to eliminate infections? Would we continue to load-up on antioxidants when we are feeling sick?

Proposed Project: We will choose one new antioxidant to test in our macrophage infection model. We anticipate using one of the carotenoid compounds as our antioxidant, but will look into the best one to use prior to starting our project. We will research relevant concentrations to test and then titrate the antioxidant down to an appropriate concentration. An "appropriate concentration" will be one at which the antioxidant itself isn't killing bacteria nor promoting bacterial growth. Once we have a concentration to try, we will add the antioxidant to the infection model at the same time as the immune stimulant and antibiotic that have been shown to interact and promote bacterial killing. If we see that the killing power is reversed by the antioxidant addition, then we will have further evidence to suggest that 1) the mechanism of the drug interaction is based on the toxic free radicals, and 2) that high levels of antioxidants may be detrimental during certain infections where free radicals actually help kill bacteria.

Troubleshooting: Luckily there are as many things to learn from troubleshooting a particularly troublesome project as there are to learn from a smooth-running project. While I am confident that we *can* complete all of our project goals in this time-frame, and am hopeful for a smooth-running summer, I am equally confident that *should* we run into challenges and set backs, we will create an equally enriching experience through troubleshooting the issues at hand. I can foresee no situation arising that would permanently block progress on this project. And knowing that our time together is short and precious, we will be even more conscious of properly planning ahead, reading and discussing papers, and progressing steadily through our goals.

Roles and Responsibilities

Faculty vs. Student Engagement in Discipline-Appropriate Scholarly Research: In my interactions with student researchers, I'm always aware that I am training my future colleagues. My approach has always been to work side-by-side with students in the laboratory and it is in this fashion that Michelle will experience all aspects of her research project from start to finish. We will start by going over the background and theory and writing equations and calculations on the board. I will give Michelle the chance to explain things back to me to solidify her understanding. Then we will design and perform experiments together, collect and analyze data, and graph results. As much as possible I will nudge Michelle to draw her own conclusions from the data before I weigh-in. Data analysis is one of the most important quantitative skills of a researcher, but it takes practice to be able to do it reliably well. As everything about this project will be new to Michelle, I will work directly with her until she is comfortable working alone. Even once she is capable of performing experimental steps on her own, we will continue to act as teammates, discussing all aspects of the project, double-checking calculations, interpreting results, etc. Michelle will also keep and maintain a proper laboratory notebook. Finally, we will work together to draft a poster and presentation for Scholar's Day 2018 at Moravian College.

Qualifications of Student: I have never met a student more eager to participate in research than Michelle Pomposello. Her enthusiasm is obvious and her intentions are genuine. She knows that microbiology is her intended field of study for graduate school and she sought me out early to profess her desire to conduct research in my lab. Because of this, I know she will be an engaged and active learner in the laboratory this summer. Aside from her bubbling enthusiasm for this particular project, she is also

well qualified (academically speaking) to participate in research in my laboratory. She completed my microbiology class with good grades and has participated in research experiences before, albeit not in her intended discipline.

Benefits to Student: As with any laboratory-science research experience, Michelle will have the opportunity to put her coursework into practice with hands-on inquiry-based learning. She will experience some of the culture of science through the daily ups and downs, the reliance on reading primary literature, the trouble-shooting, etc. and will master mammalian cell culturing techniques that will be useful in a microbiology career. The questions she is asking for this project are relevant and important to the fields of microbiology and immunology and the time-intensive cell culture techniques that she will master this summer will enable her to continue research with me (on a separate but related project) during the next academic year−an otherwise time-prohibitive endeavor for me and my schedule. Michelle will also present her work at Moravian College's Scholar's Day in 2018 which will give her experience with presenting scholarly work.

Rationale for Altered Hours: Michelle has been lucky enough to find steady employment in a field directly related to her interests in the biomedical sciences field. Due to the necessity of Michelle's work, and her necessity to maintain her hours over the summer as a criterion for future employment, we have opted to submit this proposal for a half-SOAR project. We have talked extensively about our options for research, and this really is the best fit for us. I can assure the committee that if a full-length SOAR project were an option for her, she would jump at the opportunity. The shortened project does not come from a lack of ambition nor a lack of interest on her part. We have already arranged with her current employer that she would conduct research with me for 20 hours a week instead of the typical 40 hours, but we will spend 11 weeks together instead of the standard 10 weeks. She would attend the Wednesday student presentations and would certainly present her own work as well, since we both see these as crucial aspects of the SOAR program.

Expected Timeline of Project

Before Project Begins: Read the seminal papers that relate directly to our macrophage infection model.

Weeks 1-2: Discuss journal articles, review the background and theory pertaining to the project. Discuss procedure, calculations, and dilutions for macrophage infection model. Create reagents and antibiotics for summer research project and finalize the antioxidant we wish to test in our project.

Weeks 2-3: Work side-by-side on the typical macrophage infection. It is important to practice the basic cell culture infection model before advancing to the antioxidant additions we will try in this project.

Weeks 4-7: We will test different concentrations of our antioxidant to ensure that it does not kill nor promote growth of the bacteria by itself. We will also test different concentrations of antioxidant to ensure that the antioxidant does not harm the macrophages by itself.

Weeks 8-11: We will test the antioxidant with the combination of the antibiotic and immune stimulant in the macrophage infection model. We hope to perform the experiment at least twice to be more confident in our results.

2017 SOAR Student Proposal:
Do antioxidants interfere with bacterial killing in a macrophage infection model?
Michelle Pomposello: Biology Major, Class of 2018
Faculty Mentor: Dr. Kara Mosovsky
(I am requesting summer housing)

My name is Michelle Pomposello and I am a junior here at Moravian College. Throughout my academic journey as a Biology major, I have had the honor and privilege of taking various science classes and doing research with different professors to try and find my area of interest. This is important to me because I plan to go on to graduate school and become a professor, as well as continue on in the realm of scientific research. After taking microbiology with Dr. Mosovsky, I fell in love with the field and became very interested in various research topics related to it. Microbiology is in everything around us and the fact that we can study and manipulate microbes to yield resources such as food, medical care, and even cosmetics is quite extraordinary in my opinion. I find the subject incredibly fascinating and would love to make a career out of something that I enjoy so much.

Research experience is an important factor in being able to pursue my dreams of becoming a microbiologist. Fortunately, Dr. Mosovsky was kind enough to sit down with me and help map out what I need to do in order to achieve my goals. One of these activities includes microbiology research. Dr. Mosovsky has conducted intriguing research in the past with other students and I would love nothing more than to be the next student to join her in a SOAR project. The area that we hope to focus our attention on deals with studying the effects of antioxidants, immune stimulants, and antibiotics on a macrophage infection model. Antioxidants are believed to be beneficial in fighting off bacterial infections, however we have reason to believe that some antioxidants, when combined with immune stimulants that produces reactive oxygen, can actually harm the cells that are infected. This research relates to almost everyone at some point in his or her life, and would be nothing but beneficial for my academic and lifelong success. It would grant me the knowledge base I would need to continue research into graduate school, teach me the techniques and procedures that are important for microbiologists to know and practice on a daily basis, and give me hands on experience in real life research in the exact field I

plan to make a huge part of my life. SOAR would prepare me for the next step in my life that is fast approaching.

Therefore I am requesting permission to complete a half-SOAR project for this summer of 2017. Dr. Mosovsky and I will prepare a weekly work schedule that consists of research being done over the span of 20 hours per week instead of the full 40 hours per week. The reason that such a request is being made is due to some extenuating circumstances that I cannot control. Unfortunately, my parents were recently divorced and things have spiraled out of control and into an uncivil and expensive battle between both parties. While it is true my dad has helped me cosign for loans to attend Moravian, I am paying for everything myself, both now and when I graduate. I have to find the funds to pay for transportation, schooling, phone bills, and insurance costs and the only way for me to be able to do so is to work part time off campus. My position as a pharmacy technician directly relates to my biomedical interests because I am constantly handling and learning about medication used to fight off infections. In order to maintain my position throughout the upcoming academic year, I was told (upon being hired) that I will need to work through the summer. This along with the fact that I sometimes need to return home to help take care of my little brother who still lives at home makes a full 40 hour SOAR project seem unrealistic and overwhelming. A half-SOAR project at 20 hours per week would allow me to fully concentrate on and dedicate myself to my research while also being able to provide for myself and my family both during the summer and the upcoming year. This opportunity would be absolutely perfect for the project that Dr. Mosovsky and I have chosen as well because of the nature of the experiments themselves. I would be honored, privileged, and incredibly grateful for the opportunity to participate in the 2017 SOAR program.

Expense Proposal

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Expected Expenses:

1) Cell culture medium components

We already have several components required to support the culture of our macrophage cell line, so we will only need to purchase a few new components:

Two 500 mL bottles of minimum essential medium (base of cell culture medium)	2 @ $20.00

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. We will need to replace what we use during the project.	$50-100

Total = $90-140

****We understand that our project may not take priority above other full-length projects, so we would happily accept any funding, up to $140.** The Department of Biological Sciences will cover the remaining expected costs (antioxidant, antibiotics, etc.) as well as any unforeseen costs throughout the length of the project.