SOAR Proposal for Summer Research 2019

Project Title: Will an Antioxidant Decrease Bacterial Burden in an Infection Model?
Faculty Mentor: Dr. Kara Mosovsky, Assistant Professor, Department of Biological Sciences
Student: Kaitlyn Nemes, Biology Major, Class of 2020
Project Duration: May 28th – August 2nd, with 1 week off in-between, for a total of 9 weeks. Please read "Rationale for 9-week SOAR Project" below for more information about our expected timeline and rationale for a 9 week SOAR project instead of a 10 week SOAR project.

Project Description

General Background: Our lab studies the bacterial pathogen, *Burkholderia thailandensis* and it's ability to invade white blood cells in the immune system—the white blood cells that are typically involved in eliminating bacterial threats. *B. thailandensis*, which is easy to work with in a lab and does not cause severe infections, serves as a model organism for studying its very close relative, *Burkholderia pseudomallei*, which can cause severe, life-threatening disease. The two organisms are so similar in the way they invade the white blood cells and live inside, that studying the safe pathogen usually tells us something about the more dangerous one.

In the lab, we study the relationship between *B. thailandensis* and white blood cells through a validated approach—we culture and infect cells in plastic dishes, rather than infect live animals. Our previous work has identified a certain combination of antibiotics and immune system activators that work well to clear the bacterial infection in this cell culture model, and we continue to study and understand the mechanism behind that effect. In preliminary studies, we discovered an interesting finding that suggested that antioxidants, in certain circumstances, could inhibit killing of the bacteria in our white blood cell infection model. If this were the case, then dietary antioxidants might actually hurt a person's ability to fight and eliminate infections caused by *B. thailandensis*. People assume that antioxidants are always "healthy," and our early results showed otherwise. It was important to continue to look at the role of antioxidants to interfere with bacterial killing. During research in 2018, however, we were surprised to find that a particular dietary antioxidant actually helped clear bacterial infections—exactly the opposite of our original hypothesis. We spent a long time investigating that surprising result, and were quite confident that the effect was real.

Proposed Project: We now have extensive data showing that a particular dietary antioxidant (acquired through diet) can help clear bacterial infections, but we have very little data about the effects of other antioxidants, and other *cellular* antioxidants (naturally present in cells), in particular. Therefore, we need to study more antioxidants in our cell culture infection model. We need to return to our original, early and preliminary findings using cellular antioxidants and re-evaluate the role of one or more cellular antioxidants using the same series of studies that so confidently confirmed the benefits of the dietary antioxidant last year. We intend to go through the steps and stages of our infection model with N-acetylcysteine, a compound that acts as an

antioxidant and is present in our cells. We expect to determine whether our cellular antioxidants act the same way as the dietary antioxidant that we tested previously.

Student Engagement in Discipline-Appropriate Scholarly Research

We will build significantly off of the early studies on antioxidants, using protocols established only in the last few years. The proposed summer project is both entirely novel and incredibly significant. No one else has studied the role of this antioxidant to alter the bacteria in this infection model, and if our findings can confirm that a second antioxidant can help reduce the bacteria in our infection model, then we would have a good basis for trying to determine if antioxidants in general all have a similar broad effect. Alternatively, we might find that dietary and cellular antioxidants work in different ways. The questions we will address are directly related to Dr. Mosovsky's main scholarly research goals, and the results of this project will fit in nicely with previous and future research aims of the Mosovsky lab. In essence, Kaitlyn will work on this small piece of a larger project to understand how antioxidants play a role in the infection model. Kaitlyn's work will therefore directly contribute to discipline-appropriate scholarly research goals. She will disseminate our findings to the Moravian College community through a presentation at Scholar's Day (2020) and if circumstances allow, she will attend and present our work at the Annual Landmark Summer Research Conference this summer. A local microbiology summer undergraduate research conference (held annually) may also be an option, if timing works out. Finally, as Kaitlyn intends to conduct an Honors project related to antioxidants, parts of her work over the summer with SOAR might be presented through the opportunities available to her through her Honors work.

Roles and Responsibilities of Student and Faculty Mentor

Together as student and mentor, we will meet daily to perform laboratory experiments and collect/analyze data to determine the effects of N-acetylcysteine (NAC), a cellular antioxidant, on the bacterial infection model. We will work together, side-by-side until the point that Kaitlyn feels confident performing the assays on her own, at which point she will take more independence with the completion of the experiments, but we will still analyze the data and draw conclusions together. The goals of each week of the project can be seen in the expected timeline below. Basically, the outcome of each experiment will influence the next experiment to follow, so the order of steps and order of experiments we plant to conduct is fairly settled. If we come across unexpected results, we will need to repeat those experiments and reassess what comes next, but we are confident that we will be able trouble-shoot as needed. In terms of reading, Kaitlyn will necessarily read more papers to establish her own background for this project, but then we will both read relevant and recent peer-reviewed articles that help us make sense of the results we obtain.

Rationale for 9-week SOAR Project

Our schedules and availability for summer research are such that we are requesting to start SOAR one week early (starting on May 28th) and end SOAR one week early (ending on August

2nd), due to personal and professional obligations on either side of those dates (conference travel/presentation and already-planned family trip). Kaitlyn's only expected travel is to Paris, France, for a personal vacation during our SOAR timeline. Since she will be missing 1 week of research for that trip, and since it would be difficult to make up that time on either end of our proposed timeline, we will need to shorten the SOAR project to 9 weeks, instead of the normal 10 weeks. We would still plan to attend the last day of student presentations and end-of-SOAR picnic on Wednesday, August 7th. The good news is that Kaitlyn is already skilled at cell culture techniques (having taken my immunology course that taught these skills), as well as calculations of dilutions. We anticipate that she will be able to hit the ground running, and we also expect the same level of progress in our 9 weeks as would have been expected in 10 weeks with a student less prepared for this project. We understand that our summer pay will be adjusted appropriately for the slightly shorter project.

If the earlier start time and the 9-week project are unacceptable to the SOAR committee, we would appreciate the opportunity to try to amend our schedules to make something else work. It would take some rearranging for us both, but we would prefer not to miss out on SOAR completely because of either request.

Expected Timeline of Project

Weeks 1: Discuss the background and theory pertaining to her project. Discuss and draw out the procedures for the most used assays, including sample calculations. Prepare and sterilize reagents and media, begin culture of the macrophage cell line, make bacteria stocks. Complete biohazardous waste training and chemical safety training.

Week 2: Work side-by-side to perform the standard 3-day macrophage infection with *Burkholderia thailandensis,* followed by data analysis and generation of a graph. Kaitlyn will perform this assay or versions of this assay numerous times throughout the summer, so it will be important to build a strong foundation for these skills.

Weeks 3-4: We will test different concentrations of our cellular 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 determine the concentration that will cause harm to the macrophages. Multiple assays will be conducted each week. All experiments will be repeated at least 1 additional time.

Weeks 5-7: We will test different concentrations of the antioxidant with the combination of the antibiotic and immune stimulant in the macrophage infection model. Different sets of concentrations will be tested on different weeks. All experiments will be repeated at least 1x to show reproducibility of results. As needed we will use other assays and laboratory techniques to supplement or confirm our results.

Weeks 8-9: Wrap up experiments or repeat final experiments, finalize notebooks, results, conclusions, work on poster or presentation for Scholar's Day.

2019 SOAR Student Proposal:
Will an Antioxidant Decrease Bacterial Burden in an Infection Model?
Kaitlyn Nemes: Biology Major, Class of 2020
Faculty Mentor: Dr. Kara Mosovsky
(I am requesting summer housing)

My name is Kaitlyn Nemes and I am junior biology major here at Moravian College. I have had the tremendous privilege to explore the biology field with many different professors, in and out of biology. My academic journey has been nothing but amazing. I have gained valuable skills in and out of the laboratory that has helped me prepare for my future. I am also on the pre-medical track and I have just applied to medical school. My science courses here have given me an extraordinary advantage in my application and being involved in SOAR will only help improve my research knowledge and skills. The skills used and obtained in this in-depth research project will further the knowledge that I have already learned in the labs required with classes. I will be responsible for designing experiments and analyzing data in more depth. After taking immunology with Dr. Mosovsky, I fell in love with the field and how interesting the immune system is. Immunology and cell culturing will be a part of my future in medicine. I know I will be taking immunology in medical school and completing a research project in this field will provide me the ability to be advanced in this subject before I even begin medical school. Immunology is also a cross-discipline science and this project will also involve microbiology. I will be able to learn new material not only in immunology, but microbiology as well. This will help me be prepared for future classes by learning about host-pathogen interactions. I will apply my prior knowledge on immunology to learn new things about microbiology. Not only is this subject incredibly fascinating, it also extremely important for our survival against bacteria and viruses.

A research experience is extremely critical for my dreams of becoming a physician. I had worked on planning my career path with Dr. Mosovsky since my freshman year. As a freshman, I often dreamed about doing research alongside her one day and I would love nothing more than to be able to join her this summer in a SOAR research project. The area we plan to focus on is expanding the SOAR project she started during a previous summer on antioxidants, immune stimulants, and antibiotics on a macrophage infection model. We plan on testing an antioxidant, N-acetylcysteine which is an antioxidant naturally found in the body. We want to know if high concentrations of this antioxidant will harm or help the cells in fighting infection. This research is extremely relevant in my interest in medicine and would be beneficial for not only my academic success, but my personal success. I will gain the ability to troubleshoot issues when they arise, acquire the confidence needed to perform experiments on my own, and learn how to use different machinery in the laboratory. I will also be reading many research articles and this will improve my critical analysis skills and help me in a significant amount of ways. This project would grant me the ability to expand my knowledge to continue on into medical school or future research I would complete as a physician. SOAR will prepare my confidence for my next journey in life at medical school.

Expense Proposal

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Expected Expenses:

1) Cell culture medium components

We will need to purchase several components required to support the culture of our macrophage cell line, including the base medium as well as all of the individual supplements:

Four 500 mL bottles of minimum essential medium (base of cell culture medium)	$100.00
Two (100 ml) bottles of the following:	
antibiotic solution (Penicillin/Streptomycin)	$40.00
essential amino acids	$40.00
non-essential amino acids	$40.00
L-glutamine	$50.00

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 lots of specific, sterile, plasticware for growing and handling mammalian cells.	~$250-400

Total = $520-670

We are formally requesting up to $500 from SOAR to support our summer research project. The Department of Biological Sciences will cover the remaining costs as well as any unforeseen costs throughout the length of the project.