Biology Proposal 2015 Randolph College Summer Research Program

Applicants

Tetiana Poliakova, Undeclared, 2018, Olivia Reed, B.S. Biology, 2016, Amanda Rumore, Assistant Professor of Biology

Project Title

Entry and Localization of Allergen Alt a 1 in Human Airway Epithelial Cells

Abstract

Alternaria alternata is a ubiquitous airborne fungus associated with an increased risk of allergy, asthma, and chronic obstructive pulmonary disorder (COPD). When inhaled, the fungus secretes the allergen Alt a 1 into the lung tissue. The interaction between the allergen and the epithelial cells lining the airway can cause an inflammatory response, increased mucous production, and irritation due to cell death. Our project aims to determine the timing and localization of the Alt a 1 allergen in airway epithelial cells using fluorescent microscopy. We will also examine if entry increases the release of pro-inflammatory cytokines from the airway cells and/or if cell death is induced by allergen entry.

Project Description

Allergies and asthma affect millions of people worldwide and a better understanding of these conditions or how they are induced could lead to new treatments. Asthma is a chronic disease that is characterized by the inflammation of the airway. This inflammation may cause coughing, difficulty breathing, and in extreme cases it may even lead to death. Though it can be triggered in various manners, some of which are still not understood, irritants in the environment are a known cause for many people (1, 2). One such irritant, the allergen Alt a 1, has been implicated in the development of asthma and its chronic obstructive pulmonary disorder (COPD) (3, 4). The Alt a 1 allergen can also trigger an immune response in humans, causing a condition known as hypersensitivity, or allergies. This condition can range from minor to life threatening, and varies highly from case to case.

Allergen Alt a 1 is produced by the fungus *Alternaria alternata*, and has been suggested as one of the primary causes of fungal allergies (5). Alt a 1 is the protein secreted by *A.alternata* in the lung and it is extremely important to understand its mechanism of its entry into human cells. This project seeks to investigate the entry and localization of Alt a 1, into human airway epithelial cells. The understanding of how this allergen enters human airway epithelial cells could have many benefits in both the research and clinical setting. In addition, it is known that production of cytokines (inflammatory proteins) and cell death are exacerbated by the presence of allergens such as Alt a 1. Understanding if cytokine production and cell death are initiated by Alt a 1 entry may help develop better therapeutic strategies.

Significance:

It is hypothesized that allergens must cross the epithelial barrier in order to activate the immune cells that reside in the lung tissue. However, the visualization and localization of Alt a 1 inside these cells has never been observed. Since the ability of Alt a 1 to enter airway cells is likely essential to initiating an antagonistic response in the lung, understanding if the Alt a 1 allergen enters the target airway epithelial cell and where it localizes would allow researchers to better understand the allergen as a whole. If our research is successful it could be used to propose a much larger research project such as identifying the cell receptor for Alt a 1 or how it can be degraded inside the cell before signaling a response. When the entry of the allergen into the cell is visualized and understood it may be possible for future research to target a block of the pathway of entry. This may even lead to research that could create a novel drug pathway to help treat asthma and allergies.

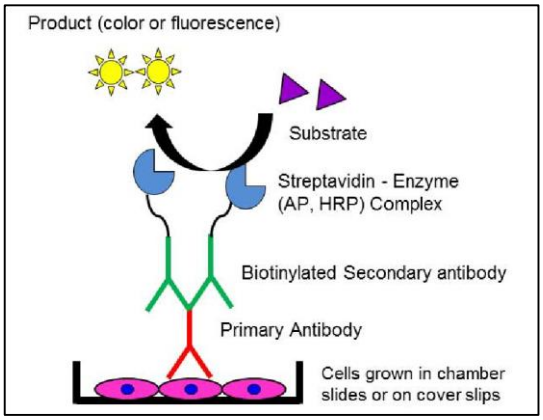
Goals of this Research

The primary goal is to visualize the entry and localization of the Alt a 1 allergen in human airway epithelial cells using fluorescent microscopy. In addition, changes in cellular biochemistry, such as inflammatory cytokine production and cell death will also be examined. We will attempt to find a correlation between Alt a 1 entry and cytokine production and cell death.

Methods:

Cell Culture:

The research will begin by learning cell culture techniques by the student researchers. This includes preparation of culture media, understanding culture conditions (timing, temperature, cell density), and aseptic (sterile) technique. This will allow the researchers to work somewhat independently and gain a mastery of basic cell culture.



Fluorescent Cell Staining/ Localization:

Once cell culture techniques are established, time course studies will be set up. Human airway epithelial cells will be incubated for various times (0 – 24hrs) with the Alt a 1 allergen. Cells will then be “fixed” (permanently frozen) in paraformaldehyde, allowing us to see the state of the cell at that exact time. Cells will be stained with anti-Alt a 1 antibody which will detect where, if any, Alt a 1 is inside the cell. A second antibody with a

fluorescent tag will then be added. This will bind to the first antibody and allow us to use fluorescent microscopy to image the localization of Alt a 1 inside the cell. A schematic of this method is shown in Figure1. In order to determine if the allergen is localized in the cytoplasm or a specific organelle, fluorescent stains will be used. DAPI (nucleus stain), Rhodaminephalloidin (cytoskeleton), DiO (cell membrane), Lysotracker Red (lysosomes) will be employed and are readily available from the Biology Department. These will allow us to pinpoint exact location of Alt a 1 based on if we see co-localization of the fluorescent stain and the antibody tag.

The time course investigation will allow us to see how long it takes the allergen to enter the cell, and possibly be released from it. This entry and release timeline would mimic what occurs inside the lung tissue as the allergen crosses the epithelial barrier.

Cytokine Production:

An ELISA assay will be used to quantify pro-inflammatory cytokines (IL6, IL8) produced by the epithelial cells. Supernatants (liquid) from the cell cultures will be collected and tested. The ELISA will determine is the cells secreted any inflammatory cytokines when the allergens interacted or entered the cell. We will determine amount of cytokines produced at the various time points.

Cell Death:

Cells will also be evaluated as to whether or not Alt a 1 entry causes apoptosis (programmed cell death) or necrosis (spontaneous cell death). Levels of lactase dehydrogenase (LDH) will be measured to determine if cell death is occurring. If this is confirmed, then cells will be again treated and stained with SYTOX green and LysoTracker Red. Positive cell staining with these dyes will indicate apoptosis. If no staining is observed, it will be concluded that necrosis is the form of cell death.

The Randolph College Biology department is already in possession of many of the supplies that will be needed during this research.

Project Timeline

Week 1 Start cell cultures from frozen stocks and training on basic cell culture techniques; conduct literature review

Week 2 Practice cell culture technique (prepare media, practice aseptic technique); complete literature review

Week 3 Continue cell cultures in preparation for experimentation. Set up time course (phase 1) and sample collection schedule

Week 4 Conduct time course experiment of treating cells with Alt a 1; collect samples at appropriate times

Week 5 Fluorescent microscopy, ELISA, and cell death assays; set-up time course (phase 2) and sample collection schedule

Week 6 Repeat time course experiment of treating cells with Alt a 1; collect samples at appropriate times

Week 7 Fluorescent microscopy, ELISA, and cell death assays of second time course experiment

Week 8 Compilation and final analysis of results and SRP Symposium presentation; submit SRP Travel Grant Application

This project can be completed in the eight-week duration of the Summer Research Program.

Project Continuation

This project is not a continuation of any prior research project. If the student researchers decide to continue this research after the eight-week summer research program, it will be conducted as an Independent Study during the fall 2015 semester. If more funding is needed to continue the project at that time, then the students will apply for a RISE Grant.

Course Release

No course release is being requested.

Works Cited

"Allergic Diseases." *Allergic Diseases*. National Institute of Allergy and Infectious Disease, 29 Dec. 2014. Web. 05 Feb. 2015.

"What Causes Asthma?" - *NHLBI, NIH*. National Heart, Lung, and Blood Institute, 14 Aug. 2014. Web. 05 Feb. 2015.

Andersson M, Downs S, Mitakakis T, Leuppi J, Marks G. (2003). Natural exposure to Alternaria spores induces allergic rhinitis symptoms in sensitized children. *Pediatr Allergy Immunol.* 14:100–105.

O'Hollaren MT, Yunginger JW, Offord KP, Somers MJ, O'Connell EJ, Ballard DJ, Sachs MI. (1991). Exposure to an aeroallergen as a possible precipitating factor in respiratory arrest in young patients with asthma. *N Engl J Med.* 324:359–363.

Song HG, Cramer RA, Lawrence CB, Pryor BM. Christopher B. Lawrence, and Barry M. Pryor. (2004) Alt a 1 Allergen Homologs from Alternaria and Related Taxa: Analysis of Phylogenetic Content and Secondary Structure. *Fungal Genetics and Biology.* 42(2):119-29.

Dissemination Goals

Upon completion of this project it will be presented at the MARCUS Conference at Sweet Briar College in fall 2015 and the Randolph College Symposium of Artists and Scholars in spring 2016. If the results of the project are significant, we also intend to submit an abstract to a national conference, such as the National Council on Undergraduate Research (NCUR). We intend to apply for a Summer Research Project Travel Grant in order cover costs associated with traveling and presenting at NCUR.

Past Outcomes

Summer 2013:

Sydney Henson ('14, Education and Spanish, '15 Special Education) and Katherine Lesnak ('15, Political Science) participated as student researchers on the SCHEVfunded grant to Peter Sheldon, Peggy Schimmoeller, and Amanda Rumore entitled "Science and Math Links: Research-Based Teaching Institute". Sydney and Katherine assisted us with a weeklong Teaching Institute for local K-6 teachers while learning how to develop problem based learning activities. They also received hands on teaching experience by leading a Science Camp at the Jubilee Child Development Center. Both students spent time completing extensive literature reviews in preparation for their senior papers. They

also compiled, input, and analyzed data collected from the teachers and students in the previous (2012) study. They presented the outcomes of the 2012 study at the Virginia Educational Research Foundation (VERA) conference, MARCUS, and the Symposium of Artists and Scholars. In addition, Peter Sheldon presented the results at the American Association of Physics Teachers (AAPT) conference in January 2014. *The grant was again submitted and funded by SCHEV for 2013-14.*

Summer 2014:

Hart Gillespie ('15, Math and Physics), Thao Nguyen ('17, Undeclared), and Shaun Chopp ('15, Biology) participated as student researchers on the SCHEV-funded grant to Peter Sheldon, Peggy Schimmoeller, and Amanda Rumore entitled "Science and Math Links: Research-Based Teaching Institute". Although the students were from different disciplines, they each showed enthusiasm towards learning about educational practices. They also received hands on teaching experience by leading a Science Camp at the Jubilee Child Development Center. The students presented at MARCUS in fall 2014 and have been asked to apply to present at 2015 Symposium of Artists and Scholars. In addition, Thao Nguyen and Hart Gillespie presented the results at the American Association of Physics Teachers (AAPT) conference in January 2015 through a SRP Travel Grant Award. *The SCHEV grant was not funded for 2014-15 however we intend to re-submit for the 2015-16 grant cycle.*

External Funding

No external funding has been sought for this project. Supplies remaining from previous RISE funded projects will be used throughout the summer as their expiration dates are near. Dr. Rumore will use start-up finds to cover any additional supply and shipping costs.

Academic Credit

No academic credit is being sought.

Project Budget

Cell culture supplies from Atlanta Biologicals
RPMI w/ L-glutamine (6 bottles) $102
DPBS (w/o Ca^{++}/Mg^{++}) (4 bottles) $52.88
Penicillin/Streptomycin (1 bottle) $18.26
Trypsin/EDTA (1 bottle) $9.02
Allergens and Antibodies from Indoor Biotech/Biologend
Alt a 1 (purified protein) $70
anti-Alt a 1 antibody $525
FITC anti-mouse IgG2a Antibody $395
Consumables from Fisher Scientific $150 (est)
250 mL sterile filter units; 500 mL sterile filter units; 5 mL serological pipettes; 10 mL serological pipettes; 25 mL serological pipettes; 75 cm2 TC-flasks
Other from VWR $126
Nunc® Lab-Tek™ II Chamber Slide™
System (4-well/ set of 16)
Total: $1,298

Justification for request over $1000

The purified Alt a 1 protein and Alt a 1 antibody are essential to conducting this research project but cost a combined $920. These two items are only available from a single manufacturer in the United States. The other supplies are necessary for culturing the airway cells. We are requesting a budget above the $1000 limit to accommodate the high costs of the supplies necessary for this project to occur. Any additional costs, such as shipping, will be covered by the Biology Department or Dr. Rumore's start-up funds.

Moreover, many other supplies that are needed for this research are already available from the Randolph Biology Department including: DiO stain, SYTOX Green, LysoTracker, DAPI, Rhodamine Phalloidin, A549 and BEAS2b epithelial cells, LDH Cytotoxicity Kit, and IL6 and IL8 ELISA kit. It is estimated these supplies are worth a combined $2000 and available free of charge.

IRB or Animal Research Approval

This project does not need IRB or Animal Research Committee Approval.

Faculty Statement

As project supervisor, I will train both student researchers on all experimental techniques described above. I anticipate spending 15 – 30 hours per week mentoring this project in addition to my duties as Director of SRP; however, I have also indicated to both students that biological research alternates between independent and collaborative states. Once appropriately trained, I will expect them to design and conduct their experiments with my consultation rather than me giving them explicit and step-by-step instructions each day (note: detailed protocols for each technique will be available). This will maximize their experience and prepare them for a normal research laboratory atmosphere. I will support their productivity throughout the summer so that high-quality and significant data is obtained. I will be available to meet and train both students throughout the summer.

Olivia Reed is a qualified and studious third-year Biology major. She is academically ready for this project since she has already completed my BIOL 336/336L (Cell Biology) courses and all 200 level BIOL courses. Olivia has also worked with me as a Work Study student and I have seen first hand her ability to analyze and follow detailed protocols. She is a reliable and productive student that will greatly benefit from this experience in her pursuit of graduate education. I anticipate she will also serve a mentoring role to other students in the program.

Tetiana Poliakova is an ambitious first year student. She intends to major in Biology and I have spoken extensively with her regarding her career goals. She is a wonderful scholar, earning the highest marks in my BIOL 103/ 100L courses in the Fall 2014 semester. She asks engaging questions and shows innate talent for laboratory research. I anticipate this summer experience will further fuel her desire to attend medical school and enhance her vitae in that pursuit.

The nature of time-course experiments requires two students. Since samples will need to be collected nearly every 2 hrs over a 24-hour period, it is necessary to split the collection times between two students. In addition, extensive microscopy, such as what is proposed in this project, is strenuous on the vision of the researcher. It is ideal to have two "sets of eyes" with the large number of samples we intend to generate.

Student Statements

Tetiana Poliakova:

My name is Tetiana Poliakova, and I am a first year student at Randolph College. Biology is my intended major and that is why I am pursing a summer research project in the Biology Department. It will be a great opportunity for me to increase my knowledge in the field and improve my skills in the laboratory setting.

I am extremely interested in our proposed project, not only because of experience I will gain in culturing human cells, but also because it will give me a chance to investigate the entry and localization of a specific allergen into a cell. This will use techniques such as fluorescent microscopy and cell-based assays that I have not yet experienced. My main career goal is to obtain an MD degree and investigation of allergens is a critical area of research in the medical and pharmaceutical fields. Participating in this summer research program will make me more competitive when applying to medical schools since laboratory research is becoming an essential part of medical school curricula. I also intend to pursue other summer research programs at medical schools over the next two summers in preparation of the MCATs and medical school application process.

I am a very hard working and enthusiastic student. I believe I can greatly add to this project by my eagerness to learn more advanced laboratory skills. Moreover, although I am just a first year student and this would be my first time doing a Summer Research Program at Randolph College, I have already gained some experience while working in the Introduction to Biology Lab and Introduction to Genetics and Molecular Laboratory courses. This summer project will allow me to practice and perfect the skills I have already learned in those two classes and prepare for future laboratory courses.

In conclusion, I would be honored if I was accepted into the 2015 Summer Research Program. It would be an amazing addition to my academic career, and I assure you it will have a positive and profound impact on my future professional goals.

Olivia Reed:

My name is Olivia Reed, and I will be graduating in 2016 with a B.S. in Biology. The field of Biology has always interested me, but that passion truly thrived when I began to take biology courses at Randolph College and was able to learn more about the different topics in the discipline. Through this exploration, I found a specific part of biology that I fell in love with: cell biology and microbiology research. Our proposed summer research project focuses in the field of cell biology and piqued my interest because it involves examination of multiple cell processes and figuring out how these basic components affect cell physiology. I have already completed courses such as Microbiology and Cell Biology, and their accompanying lab courses, and have worked for the Randolph College Biology Department for over a year and a half. I believe that this prior experience will allow me to contribute a great amount to the project and that the knowledge gained in my many biology courses will allow me to work with independence and skill.

During the Randolph College Summer Research Project, I hope to practice and perfect my laboratory techniques, as well as gain new ones that will aid me later in life. Eventually,

I intend to go to graduate school for either a Masters or Doctorate degree in Microbiology or Cell Biology. This research would improve not only my chances of being accepted into one of these highly competitive programs, but allow me to excel in them once I am accepted. Eventually, I hope to work for the Centers for Disease Control or the World Health Organization doing research on human diseases. This summer research project is very similar to the research done in major organizations and the experience would give me an advantage in the field that I seek to work in. Though I have not participated in summer research in the past, I hope that this experience will be educational and allow me to excel in my field later in life. If accepted, I am sure that my prior knowledge, eagerness to learn, and motivation as a science student will aid in making this research a success.