Continuation/Research Progression Projects Form (7)

Required for projects that are a continuation/progression in the same field of study as a previous project.
This form must be accompanied by the previous year's abstract and Research Plan/Project Summary.

Student's Name(s) Ethan Chetkof

To be completed by Student Researcher: List all components of the current project that make it new and different from previous research. The information must be on the form; use an additional form for previous year and earlier projects.

Components	Current Research Project	Previous Research Project: Year: 18-19
1. Title	Mitochondrial Transfer From Mesenchymal Progenitor Cells to Macrophages	The Impact of IFNγ on Non-opsonized Phagocytosis
2. Change in goal/ purpose/objective	No longer focuses on the actual rate of phagocytosis in the progenitor-macrophage culture. Instead examines the movement of mitochondria from progenitor cells towards macrophages. Examined prominence of VCAM 1 protein in accordance to microtubule processes.	Focused on confirming the suppression of non-opsonized phagocytosis when the IFNγ protein is produced within macrophage cells.
3. Changes in methodology	Included co-cultures of aortic progenitors and macrophages. Did not include PCR or phagocytosis assay of any kind. Fluorescence microscopy was primary focus for results	Cultured only macrophages. Inoculation of various pathogens to said macrophages. Inoculation of IFNγ into experimental groups Phagocytosis assays and PCR analysis
4. Variable studied	Ind: Progenitor cell pure culture vs progenitor cell and macrophage co-culture Dep: Observable mitochondrial transfer	Ind: IFN-gamma expression Dep: Rate of phagocytosis
5. Additional changes		

Attached are:

☑ Abstract and Research Plan/Project Summary, Year 18-19

I hereby certify that the above information is correct and that the current year Abstract & Certification and project display board properly reflect work done only in the current year.

Ethan Chetkof	Ethan Chetkof	01/29/20
Student's Printed Name(s)	Signature	Date of Signature (mm/dd/yy)

Abstract 2018-2019

The Impact of IFNγ on Non-opsonized Phagocytosis

Individuals who have already been infected by viral pathogens are generally more susceptible to infection from bacterial pathogens than those who are not already ill at the time of bacterial infection. Numerous studies have hypothesized that this is due to inhibition of non-opsonized phagocytosis caused by IFNγ being produced as a response to the initial infection. Non-opsonized phagocytosis as a whole is understudied, and its supposed relation to IFNγ has been seldom examined by an entire investigation. In this study, IFNγ's effects on the process of non-opsonized phagocytosis was analyzed, specifically concerning *E. Coli*, *S. Aureus*, and zymosan. Additionally, the identification of the specific reason for this suppression was deemed a goal for the study. Ultimately, it was recognized that IFNγ did indeed have an inhibitory effect on the non-opsonized phagocytosis of *E. Coli* and *S. Aureus*, while the effect on zymosan was unclear. PCR assays determined minor influence of IFNγ on initial gene pathways of interest, however suggested specific genes concerning the phagocytic process which had their expressions significantly changed as a result of IFNγ induction. This study manages to confirm the notion that IFNγ is suppressive for the non-opsonized phagocytosis of several notable pathogens, as well as confirming IFNγ's specialization with the opsonized form of phagocytosis based on enhanced gene expression of genes associated with the process. Additionally, it also linked said genes with possible reasons for the phagocytic suppression and opened them up to further analysis.

Ethan Chetkof

Research plan 2018-2019

Research Plan - The Impact of IFN-gamma on Non-opsonized Phagocytosis

Introduction

A. RATIONALE: The effect of the cytokine Interferon-ɣ (IFNɣ) on the phagocytic activity of macrophage cells has already been well researched and become well understood. IFNɣ is already known to be effective in acting as a signal for the phagocytosis of opsonized foreign pathogens, however the role which IFNɣ plays in non-opsonized phagocytosis is not as well researched, and thus there is less of an understanding of its effect on this particular process throughout the scientific community. It is recognized that an individual is more likely to contract a bacterial disease when already infected with a viral disease due to the suppression of non-opsonized phagocytosis and prior literature has suggested that IFNɣ may be significant in the causation of this suppression. This results in the host being more susceptible to said pathogens, as the host's immune system is unable to conjure an adequate response. Ultimately, the success of this study would enhance the current understanding of the interface between adaptive and innate immune cells, allowing for possible advancements in the treatment of infectious diseases and the prevention of the aforementioned reality. Determining the reason for this suppression would result in possible advances in treatment for people who have been afflicted with some sort of medical condition, and ensure that they are not as susceptible to other invasive foreign bodies.

B. HYPOTHESIS/EXPECTED OUTCOMES: The specific aim of this study is to confirm the effect of IFNɣ on non-opsonized phagocytosis and determine how IFNɣ affects the regulation of specific genes which pertain to non-opsonized phagocytosis. Pathways of interest as indicated by prior research include the Rac1 and RhoA pathways, both of which will be examined in this study, along with a general plate of genes which pertain to the phagocytic process. Prior to execution of the study, it was hypothesized that IFNɣ would act as a suppressor of phagocytosis for all examined pathogens, being *Escherichia coli*, *Staphylococcus aureus*, and zymosan. Additionally, it is believed that observed changes in gene regulation within the Rac1 and RhoA pathways would lead to a determination of the reason for such suppression.

C. RESEARCH METHODS:

Procedures: To determine IFNɣ's regulation of the IFNɣ, Rac1 and phagocytic signaling pathways, mouse splenic macrophage cells acquired from the American Type Culture Collection will be used for analysis. After cell culturing within Dulbecco's Modified Eagle's Medium with with 4 mM glutamine containing 1.5 g/L sodium bicarbonate and 4.5 g/L glucose, IFNɣ will be induced into the macrophage cells, the pathogen being used will be introduced into the cell system, and a phagocytosis assay measured through fluorescence elicited by the pathogens while phagocytized will be run and recorded by a SpectraMax i3x multi-mode plate reader from Molecular Devices. Additionally, RNA extractions will be performed on cells, as well as reverse transcription PCR in order to determine the gene expression in pathways of interest. The machine used to conduct the PCR will be the CFX Connect™ Real-Time PCR Detection System

from Bio-Rad. The observed expressions will be utilized to identify of both positive and negative regulation of specific genes by IFNɣ.

Data analysis: Following this, data will be further analyzed through the creation of figures using the software Prism, as well as through an Excel spreadsheet.

Risk and Safety: There is little risk to the researcher associated with the execution of this study, however typical lab procedures (use of gloves, long sleeves, etc.) are of course in effect in order to protect any possible contamination.

3. POTENTIALLY HAZARDOUS BIOLOGICAL AGENTS RESEARCH: Considering the pathogens being used within the study can possibly be dangerous to humans if not carefully dealt with, some precaution does have to be taken. Consumption of *E. Coli*, *S. Aureus*, or zymosan can theoretically cause health issues, however do not require any extra precautions to be safely worked with. The biosafety level is determined based upon risk assessment, as well as former analysis by other scientists. As the cells are considered to be rather low-risk, they are assigned a biosafety level of BSL1, the lowest possible score when measuring the risk of using such cells. That said, when disposed of, all cells still are discarded in a special biohazard deposit bin.

4. HAZARDOUS CHEMICALS:

Culture mediums:

The medium used for cell culture was Dulbecco's modified Eagle's medium (DMEM) with 4 mM glutamine containing 1.5 g/L sodium bicarbonate and 4.5 g/L glucose, also supplemented with 10% fetal bovine serum, and 20% LADMAC. These are considered to be rather low-risk, so no extra precautions have to be taken in order to ensure safety.

IFNγ:

Inoculated into the cells at 250 ng/mL, with another experimental group both IFNγ and 1,000ng/mL lipopolysaccharide. This is considered rather low-risk, so no extra precautions have to be taken in order to ensure safety.

Chloroform:

200µl of chloroform will be used in preparation for RNA extraction. Chloroform gives off toxic fumes which can be dangerous if inhaled, so it will be applied under a specialized fume hood which provided sufficient ventilation. All other typical safety precautions also will be applied.

SYBR Green:

SYBR Green was used in preparation for reverse transcription PCR. This is considered rather low-risk, so no extra precautions have to be taken in order to ensure safety.

Ethanol:

Ethanol was used in preparation for reverse transcription PCR. This is considered rather low-risk, so no extra precautions have to be taken in order to ensure safety.

Bibliography

- Abbas, A.K., A. H. Lichtman and S. Pillai (2014). Basic Immunology: Functions and Disorders of the immune system, Elsevier.
- Garcia-Garcia, E. and C. Rosales (2013). Adding Complexity to Phagocytic Signaling: Phagocytosis-Associated Cell Responses and Phagocytic Efficiency. Austin (TX), Landes Bioscience.
- Gilberti, R. M. and D. A. Knecht (2015). "Macrophages phagocytose nonopsonized silica particles using a unique microtubule-dependent pathway." Mol Biol Cell **26**(3): 518-529.
- Janeway, C. A. J., P. Travers and M. Walport (2001). The Immune System in Health and Disease, New York: Garland Science.
- Wang, Z., S. Zhou, C. Sun, T. Lei, J. Peng, W. Li, P. Ding, J. Lu and Y. Zhao (2015). "Interferon-gamma inhibits nonopsonized phagocytosis of macrophages via an mTORC-c/EBPbeta pathway." J Innate Immun **7**(2): 165-176.