Summer Student Research Program
Project Description

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**PROJECT TITLE (200 Characters max):**

Interferon-dependent *M. tuberculosis* induction of antigen presentation via class I MHC

## HYPOTHESIS:

***We hypothesize that* M. tuberculosis *infection of macrophages up-regulates antigen processing and presentation that allows presentation of* M. tuberculosis *antigens on class I major histocompatibility complex, in part as an autocrine response to type I IFN induced by the infection.***

## PROJECT DESCRIPTION **(Include design, methodology, data collection, techniques, data analysis to be employed and evaluation and interpretation methodology)**

***Design: Macrophages have been infected by* M. tuberculosis *in the absence or presence of antibodies that neutralize type I IFN. (We have previously shown that M. tuberculosis infection of macrophages induces secretion of type I IFN). Controls have been performed using mock infection of macrophages cultured in the presence and absence of the neutralizing antibodies. Our unpublished data show that the secreted IFN is partially responsible for elevated cell-surface levels of class I MHC molecules induced by infection.***

***Methodology: We have isolated RNA and prepared protein extracts from several replicate experiments, which we will use to determine effects of infection and dependence on secreted type I IFN for the levels of proteins that mediate processing of antigens for presentation on class I MHC. We will assay for changes in TAP1 a transporter that moves peptides into the endoplasmic reticulum lumen so they can assemble on class I MHC complexes. Work performed on this project last summer demonstrated type I IFN-dependent induction of TAP-1 mRNA as a consequence of* M. tuberculosis *infection. The project will also involve designing primers and probes and measuring expression of the class I MHC molecule HLA-A2 and of the associated b2-microglobulin, which is the invariant chain of the class I MHC.***

***Techniques and Data Collection: Protein levels will be determined using Immunoblotting. Target gene expression will be assayed using quantitative RT-PCR, which involves real time measurement of amplicon production as each PCR cycle is completed. The increase in product above the detection limit defines a threshold cycle (Ct) for samples and standards. The abundance of a target transcript is determined by comparing the sample Ct to a standard curve produced with data from the assay standards.***

***Data Analysis and Evaluation: Expression levels from replicate experiments will be compared using a student's T test to determine whether statistically significant differences exist between uninfected and infected cells in expression of each target protein or gene, between cells infected in the presence and absence of anti-type I IFN neutralizing antibodies, and between mock-infected cells cultured with and without the neutralizing antibodies.***

***Interpretation: The determination of protein levels will establish the significance of changes in mRNA abundance. The mRNA assays will reveal whether regulation of gene expression is responsible for the increased cell surface level of MHC class I caused by* M. tuberculosis *infection and the extent to which secreted type I IFN is responsible for the induction that occurs. Any decrease in protein level or gene expression due to the presence of neutralizing antibodies during infection must fail to occur in the comparison of mock-infected cells cultured with and without the antibodies in order to conclude that secreted type I IFN contributed to induction by* M. tuberculosis.**

**SPONSOR'S MOST RECENT PUBLICATIONS RELEVANT TO THIS RESEARCH:**

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Weiden, M., Tanakia, N., Qiao, Y., Zhao, B.-Y., Honda, Y., Nakata, K., Canova, A., Levy, D. E., Rom,W. N., and Pine, R. (2000). "Differentiation of monocytes to macrophages switches the Mycobacterium tuberculosis effect on HIV-1 replication from stimulation to inhibition: modulation of interferon response and C/EBPβ expression." J. Immunol. 165, 2028-2039.

Prabhakar, S., Qiao, Y., Hoshino, Y., Weiden, M., Canova, A., Giacomini, E., Coccia, E. and Pine, R. (2003). "Inhibition of response to alpha interferon by *Mycobacterium tuberculosis*." Infect. Immun. 71, 487-2497.

Prabhakar, S., Qiao, Y., Canova, A., Tse, D., Pine, R. (2005). "IFNα/β secreted during infection is necessary but not sufficient for negative-feedback regulation of IFNα/β signaling by *Mycobacterium tuberculosis*" J. Immunol. 174, 1003-1012.

**IS THIS PROJECT SUPPORTED BY EXTRAMURAL FUNDS?**
**Yes ☐ or No ☒**
***(IF YES, PLEASE SUPPLY THE GRANTING AGENCY'S NAME)***

**THIS PROJECT IS:** ☐ **Clinical** ☒ **Laboratory** ☐ **Behavioral** ☐ **Other**

**THIS PROJECT IS CANCER-RELATED** ☐
**Please explain Cancer relevance**

**THIS PROJECT IS HEART, LUNG & BLOOD- RELATED** ☒
**Please explain Heart, Lung, Blood relevance**

**THIS PROJECT EMPLOYS RADIOISOTOPES** ☐

**THIS PROJECT INVOLVES THE USE OF ANIMALS** ☐
**PENDING** ☐ **APPROVED** ☐ **IACUC PROTOCOL #**

**THIS PROJECT INVOLVES THE USE OF HUMAN SUBJECTS** ☐
**PENDING** ☐ **APPROVED** ☐ **IRB PROTOCOL # M**

**THIS PROJECT IS SUITABLE FOR:**

| | | | |
|---|---|---|---|
| UNDERGRADUATE STUDENTS | ☐ | ENTERING FRESHMAN | ☐ |
| SOPHMORES | ☐ | ALL STUDENTS | ☒ |

**THIS PROJECT IS WORK-STUDY:** **Yes ☐ or No ☐**

**THIS PROJECT WILL BE POSTED DURING ACADEMIC YEAR**
**FOR INTERESTED VOLUNTEERS?:** **Yes ☒ or No ☐**

**WHAT WILL THE STUDENT LEARN FROM THIS EXPERIENCE?**
***Students will learn background on molecular immunology and molecular biology of the interferon system and of macrophage response to* M. tuberculosis *infection. The experimental work will provide hands-on experience with a major method used to measure gene expression. Interpretation of the data, particularly understanding the importance of control experiments, will teach students how to evaluate whether a difference in experimental protocol has caused a change in the results. The results will allow them to learn specifically about the effects of infection on antigen presentation.***