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PART I:

Name of Schulich faculty member who will supervise the project	Bryan Heit
Supervisor's Schulich, Western, Hospital or Lawson Email	bheit@uwo.ca
Schulich Department	Microbiology & Immunology

PART II - Project Description

Title of Project	Quantifying Phagocyte Defects in Pneumonia and Acute Respiratory Distress Syndrome

Background

We have known for nearly a century that pulmonary infections such as influenza can lead to long-term immunological sequelae including a persistent suppression of anti-microbial immune responses and aggravation of inflammatory disorders such as atherosclerosis – often leaving these patients susceptible to re-infection and myocardial infarct. Despite an extensive history of investigation, the molecular and cellular mechanisms that produce these immunological defects remain unknown.

We believe that these immunological defects arise from epigenetic "reprogramming" of lung-resident immune cells such as alveolar macrophages, resulting in a polarization of these cells to a poorly anti-microbial and anti-inflammatory phenotype. Consequently, these cells are unable to respond appropriately to new infectious challenges, or to chronic inflammatory conditions such as atherosclerosis.

To investigate this possibility, we have established a biobank of lung and blood tissues from patients with infection-associated acute respiratory distress syndrome (ARDS), from which we can isolate and analyze the function of macrophages and related immune cells. The goals of this project are:

Year 1: develop a tool for the multi-plex analysis of macrophage anti-microbial function, allowing for the simultaneous quantification of phagocytosis (engulfment of microbes), microbe killing (proteolysis & reactive oxygen species production), and acidification of the anti-microbial compartment.

Year 2: using the tool from year 1, assess the anti-microbial activity of lung (bronchoalveolar lavage-derived) macrophages and peripheral-blood derived macrophages from patients with ARDS, and to combine these functional readouts with RNA sequencing and other procedures to investigate the epigenetic reprogramming of these patients' immune cells. By comparing the functional and genetic characteristics of these cells, we hope to identify the processes which induce the immunological defects which leave these patients susceptible to new infections and inflammatory disorders.

Hypothesis

We hypothesize that exposure to the extreme inflammatory conditions present in the lungs of ARDS patients leads to the epigenetic reprogramming of resident alveolar macrophages to a poorly phagocytic and minimally antimicrobial phenotype, thus leaving patients susceptible to repeat pulmonary infections and altered inflammatory responses.

Aim 1: Establish a microparticle-based phagocytic system which incorporates fluorescent reporters of proteolysis, reactive oxygen species production, and phagosome pH into a single phagocytic target. Once developed, this model will be refined and validated using cultured macrophages, with defects in these antimicrobial pathways mimicked by RNA knockdown or pharmacological inhibitors.

Aim 2: Using the platform developed in Aim 1, characterize the immunological defects of alveolar and blood-derived macrophages from patients with ARDS, and then correlate these results with RNA sequencing data of the same cells to identify the genes which are responsible for creating the immunological defects in these patients, and to identify

epigenetic regulators which act as the “master regulator” of these immune-defect driving genes.
Through identifying the genes and epigenetic regulators, we may identify druggable targets to either reverse these immunological defects, or to prevent these defects from forming in patients with ARDS.

Proposed Methodology
Aim 1: Gram negative (E. coli), Gram positive (S. aureus), and fungal (yeast) microbes will be grown, chemically inactivated, and then reporters of proteolysis (DQ green BSA), pH (pHrodo), reactive oxygen species production (CellROX Deep-red), and phagocytosis (AlexaFluor 405) covalently linked to the microbes. The efficacy, dynamic range, and sensitivity of these microparticle phagocytic reporters will then be assessed using cultured macrophages (J774.1 macrophages), with inhibition or gene knockdown of key parts of these pathways used to confirm the specificity of each readout. This aim will produce a tool providing a multiplexed measure of the phagocytic and antimicrobial activity of macrophages and other phagocytic immune cells.

Aim 2: Macrophages will be isolated from banked ARDS patient bronchoalveolar lavage fluid and differentiated from banked ARDS peripheral blood mononuclear cells. Phagocytosis assays will be performed with these cells, using the microparticle phagocytic targets from Aim 1. One the phagocytosis experiments are complete, mRNA will be extracted and sequenced from these cells and analyzed for changes in phagocytosis, antimicrobial, and inflammation-associated genes, as well as for changes in the expression of epigenetic regulators (e.g. histone deacetylases, methyltransferases), in order to identify genetic and gene pathways which alter the function of macrophages in ARDS patients. This aim will characterize any defects in the phagocytic or antimicrobial activities of ARDS patient macrophages and identify the epigenetic pathways which induce these defects. This will serve as a foundation for future studies into therapeutic interventions to prevent, or reverse, these defects.

Expected Outcomes
Year 1: Generation, optimization, and characterization of a multiplex reporter of macrophage (and other phagocytic immune cells) phagocytic and antimicrobial activities, with this system implemented on microparticles derived from Gram negative, Gram positive, and fungal organisms in order to enable investigations into antimicrobial responses again a range of pathogen types. Ultimately, this will be incorporated into our current microscopy-based microimaging platform which allows for quantification of cellular behaviours using minute numbers of patient-derived cells.

Year 2: Using the microparticle-based assays developed in year 1, characterize the phagocytic capacity, activity of the antimicrobial proteolytic and reactive oxygen species systems, and the acidification of the antimicrobial compartment formed after phagocytosis, in the alveolar and monocyte-derived macrophages of patients with infection-associated ARDS. These functional readouts will be correlated with gene expression data derived from RNAseq analysis of the same cells, with the goal of identifying genes and genetic regulators which mediate defects in the macrophages’ microbicidal systems. We expect to observe a loss of both pathogen engulfment (phagocytosis) and of the antimicrobial reactive oxygen species (ROS) pathway, with inactivation of these pathways driven by an epigenetic regulator such as HDAC2.

Research Environment – Description of the number of research personnel, primary location of research, size of lab, etc
The Heit lab is located in the Department of Microbiology and Immunology, on the 3rd floor of the Health Sciences Addition building. Our lab is currently comprised of Dr. Bryan Heit (PhD), a lab manager Angela Vrieze, five graduate students, and between 3 and 6 undergraduate students. Our lab contains the equipment for the collection, manipulation, and assessment of immune cells derived from patient/donor samples, as well as for the use of cultured macrophage cell lines. This includes analyses of macrophage function, gene expression, subcellular protein localization, tissue processing, and live-cell assays. Dr. Heit also leads the MNI Microscopy Facility, which contains microscopes dedicated to 3-dimensional live-cell imaging (including live-cell analyses of macrophage function), single-molecule imaging, and tissue imaging, all of which are central to this project.

This project is in collaboration with the laboratory of Dr. Aleksandra Leligdowicz (MD/PhD). Dr. Leligdowicz oversees the Early Severe illness TrAnslation BioLogy InformaticS in Humans (ESTABLISH) and MacrOphages and carDiopULmonAry criTical illnEss (MODULATE) studies under which the ARDS patient bronchealveolar lavage and blood samples are collected, with Drs. Heit and Leligdowicz collaborating on these studies with the goal of better understanding how processes such as macrophage polarization and epigenetics alter patient immune responses to

severe infectious challenges. Dr. Leligdowicz's lab is located in the Robarts Research Instiute and contains the resources necessary for processing and banking clinical samples, and performing a range of immunological and gene expression analysis of cells derived from these samples.
Note that all biosafety and human ethics approvals required for this project are already in place, or will be in place by February 2024.

Names and titles of other individuals who will be involved with the research project?
Dr. Bryan Heit received his PhD in immunology from the University of Calgary, and subsequently specialized in the cell biology of phagocytes during a post-doctoral fellowship at the Hospital for Sick Children in Toronto. Dr. Heit leads the phagocyte biology laboratory at Western University (www.phagocytes.ca) where he leads investigations into the cellular and molecular processes which mediate pathological macrophage function in inflammatory diseases such as atherosclerosis, and in infectious diseases including influenza and SARS-CoV-2. Dr. Heit is also co-director of the Western Infection, Immunity & Inflammation Center, which promotes and coordinates joint clinical/basic sciences research programs.

Dr. Aleksandra Leligdowicz received her PhD from Oxford University, completed post doctoral fellowships at University of California San Francisco and the University of Toronto, completed her training as a MD at McGill University, and completed fellowships in internal medicine and critical care at the University of British Columbia and the University of Toronto. Dr. Leligdowicz's lab is focused on understanding how immune cells and the vascular endothelium become dysregulated during acute and severe inflammatory challenges such as sepsis and ARDS, with the goal of understanding how changes in these cells during the early stages of disease mediate patient outcomes. Dr. Leligdowicz leads the ESTABLISH and MODULATE programs which collect, bank, and analyze clinical samples from sepsis and ARDS patients.

Angela Vrieze is the lab manager and technician in the Heit lab. Angela has extensive expertise in microscopy, assay development, and the handling of patient-derived immune cells. Angela will oversee much of the training of the SRTP student, and will assist in performing some of the proposed experiments.

Yasamin Minazadeh and Matthew Arbolino. Yasi and Matt are graduate students who are jointly supervised by Drs. Heit and Leligdowicz. Their research projects respectively focus on the polarization phenotype of ARDS patient macrophages and the mechanisms by which influenza infection polarize heart macrophages into a pro-atherosclerosis phenotype. While the proposed SRTP project is independent of these students' work, the methods and knowledge generated by these students is foundational to the SRTP project, and it is expected that the SRTP student will collaborate closely with Matt and Yasi.

Can this project be done remotely?	No
Duration of Project	Two Summers

Expected Objectives/Accomplishments for Student for Year 1?
Generation, optimization, and characterization of a multiplex reporter of macrophage (and other phagocytic immune cells) phagocytic and antimicrobial activities, with this system implemented on microparticles derived from Gram negative, Gram positive, and fungal organisms in order to enable investigations into antimicrobial responses again a range of pathogen types. Ultimately, this will be incorporated into our current microscopy-based microimaging platform which allows for quantification of cellular behaviours using minute numbers of patient-derived cells.

Expected Objectives/Accomplishments for Student for Year 2?
Using the microparticle-based assays developed in year 1, characterize the phagocytic capacity, activity of the antimicrobial proteolytic and reactive oxygen species systems, and the acidification of the antimicrobial compartment formed after phagocytosis, in the alveolar and monocyte-derived macrophages of patients with infection-associated ARDS. These functional readouts will be correlated with gene expression data derived from RNAseq analysis of the same cells, with the goal of identifying genes and genetic regulators which mediate defects in the macrophages' microbicidal systems. We expect to observe a loss of both pathogen engulfment (phagocytosis) and of the antimicrobial reactive oxygen species (ROS) pathway, with inactivation of these pathways driven by an epigenetic

regulator such as HDAC2.

PART III - Certifications

If the project will require any certification approvals from one or more of the following offices, please check the appropriate box below.	- Human Ethics - Biohazard
Human Ethics: If you have the protocol information, please enter it below (or enter the status of the approval).	Human Ethics Protocol 104010 (Dr. Heit), ESTSBLISH and MODULATE protocols (Dr. Leligdowicz)
Biohazard: If you have the protocol information, please enter it below (or enter the status of the approval).	BIO-UWO-0262

Note: certification approval should be obtained prior to the start of the summer. Projects without this approval will not be a priority for funding.