SRTP - Project Description Form #238

PART I:

Name of Schulich faculty member who will supervise the project	Aleks Leligdowicz
Supervisor's Schulich, Western, Hospital or Lawson Email	aleligdo@uwo.ca
Schulich Department	Medicine

PART II - Project Description

Title of Project	Immune control in critical illness (ICICI)

Background

Sepsis is the most common diagnosis in patients admitted to the Intensive Care Unit (ICU). Sepsis is a syndrome caused by a dysregulated immune response to infection that leads to life-threatening organ injury which has no specific treatments. The syndrome is associated with variability in immune responses, with some patients experiencing excessive inflammation ("cytokine storm") while other patients experience a diminished immune response. Immune responses can be studied outside the body by exposing blood cells to exogenous bacterial products. The magnitude of the response to bacterial products outside the body (ex vivo) is a valuable correlate of the in vivo host immune state. Cellular reprogramming that occurs during sepsis can render some patients "endotoxin tolerant", a blunted immune state that prevents immune cells from responding to secondary stimuli. Understanding ex vivo immune cell responses and their association with the host in vivo inflammatory state could provide a novel way to identify patients most likely to respond to immune-based treatments for sepsis.

Hypothesis

1. The magnitude of the ex vivo immune responses in early sepsis is specific to bacterial products used to elicit them
2. The in vivo host immune state will influence immune responses to ex vivo stimulation with different bacterial products

Proposed Methodology

Fresh whole blood will be obtained from 20 patients with sepsis on the day of ICU admission who are enrolled in the Early Sepsis TrAnslational BioLogy InformaticS in Humans (ESTABLISH) cohort at the London Health Sciences Centre (LHSC). Blood will be centrifuged and separated into cellular and non-cellular fractions (plasma). The cellular fraction will be diluted 1:5 with cell culture media and stimulated for 4 hours at 37°C with three bacterial products: Lipopolysaccharide (LPS), Lipoteichoic acid (LTA), and Staphylococcal enterotoxin B (SEB). After stimulation, cell culture supernatants will be separated from the stimulated cell fraction. Multiplex cytokine bead arrays (ELLA) will be used to quantify cytokines in cell culture supernatants in response to different bacterial products (ex vivo immune response) and matched patient plasma (in vivo host immune state). Paired ANOVA will compare cytokine production after stimulation with the three bacterial products and in unstimulated cell supernatant samples. Linear regression will be used to correlate ex vivo immune responses (cell culture supernatants) to the in vivo host immune state (plasma). The analysis will be stratified by ICU diagnosis, and relevant confounders (age, sex, comorbidities) will be incorporated into the modelling.

Expected Outcomes

Determining the impact of bacterial products on ex vivo immune responses will provide information about whether a blunted immune state is specific to an antigen or whether it is a broader concept of cross-tolerance due to reprogramming involving key immune pathways. The impact of the in vivo host state on ex vivo immune responses will

provide important information that could inform future clinical decisions. If the ex vivo response can be predicted with in vivo markers of inflammation, this would provide a point-of-care measure to enable the seamless translation of our findings to the bedside, allowing the delivery of immune-modulating therpies to the patients most likely to benefit.

The ESTABLISH research infrastructure comprehensively characterizes immune responses during early critical illness. The output of this research will provide important information about the breadth of the immune response in the early phase of critical illness and inform many future translational projects. Upon completing this project, an appreciation of translational biology studies will be gained. Specifically, the ability to work in a category 2 laboratory and to perform cell culture, processing of blood samples, performing standard immunological assays, conducting independent statistical analysis, presenting data, and preparing manuscripts.

Research Environment – Description of the number of research personnel, primary location of research, size of lab, etc

The project will be carried out in the Cardiology and Critical Care Research Program (C3RP) laboratory (www.c3rp.org) at Robarts Research Institute under the supervision of Dr. Aleks Leligdowicz and the co-supervision of Dr. Mark Chandy. There will be opportunities to interact with lab managers, lab technicians, graduate students, postdoctoral fellows, and other scientists at Robarts, as well as in the Department of Microbiology and Immunology and the Department of Physiology and Pharmacology. The Robarts Research Institute is a rich academic environment and is located next to the University Hospital, providing an ideal location for translation biology studies. The experiments will be carried out in the biosafety level-2 laboratory, and all required training will be provided by the C3RP laboratory team (https://c3rp.org/team).

Names and titles of other individuals who will be involved with the research project?

Dr. Aleks Leligdowicz, MD PhD, Critical Care Medicine, LHSC University Hospital
Dr. Mark Chandy, MD PhD, Cardiology, LHSC University Hospital
Dr. Kerry-Ann Nakrieko, Robarts Research Institute, C3RP laboratory manager
Michelle Si, Robarts Research Institute, C3RP laboratory technician
Tracey Bentall, Research Coordinator at University Hospital, Critical Care Unit

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

Expected Objectives/Accomplishments for Student for Year 1?

1) Recruit 40 patients with early critical illness
2) Perform ex vivo biological cellular assays
3) Store samples for biological marker quantification

Expected Objectives/Accomplishments for Student for Year 2?

1) Quantify biological markers in cell culture supernatants and in plasma
2) Perform statistical analyses
3) Prepare and submit a manuscript of the results

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).	HSREB: 122418 (ESTABLISH); 123599 (ICICI)
Biohazard: If you have the protocol information, please enter it below (or	

enter the status of the approval).	BIO-RRI-0086

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