Project Title

Mechanisms of Microvascular Endothelial Cell Apoptosis in Sepsis

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Project Description

Background. Sepsis is a common and serious clinical problem, with significant morbidity and mortality. A central role for injury/dysfunction of the microvasculature and specifically the microvascular endothelial cell (MVEC) has been proposed as central to the pathogenesis of septic multiple organ dysfunction and a high risk of death. For example, septic lung injury is characterized by injury and dysfunction of lung MVEC resulting in the key pathophysiologic feature, microvascular barrier dysfunction leading to high-permeability protein-rich edema. Our ongoing work in sepsis has led to our recent finding that lung microvascular MVEC dysfunction in septic mice in vivo is associated with significant MVEC apoptosis, which appears to be dependent on the presence and action of circulating blood neutrophils (PMN). Moreover, we have also recently reported on involvement of the calpain apoptotic pathway in apoptosis of isolated murine MVEC in vitro. We now propose to further define the relationship between septic MVEC dysfunction and MVEC apoptosis, and to dissect out the mechanisms of septic PMN-dependent MVEC apoptosis.

Hypothesis & Aims. The hypothesis of the current proposal is that septic microvascular dysfunction is due to PMN-dependent MVEC apoptosis. We will pursue this hypothesis in parallel murine models, including isolated murine MVEC in vitro and an in vivo mouse sepsis model, as well as subsequently explore the clinical relevance of important findings in isolated human MVEC in vitro. We will address 3 major aims:

1) To define the intracellular mechanisms of murine MVEC apoptosis under septic conditions in vitro and in vivo.

2) To define the mechanisms of PMN-dependent murine MVEC apoptosis under septic conditions in vitro and in vivo.

3) To define the mechanisms of human PMN-dependent human MVEC apoptosis under septic conditions in vitro.

Research Approach. The proposed experiments will focus on septic MVEC barrier dysfunction, and identifying the key mechanism controlling MVEC apoptosis.

Aim#1: In isolated murine MVEC under septic conditions in vitro, we will first characterize the time-course and relationship of septic MVEC barrier dysfunction and MVEC apoptosis, and then define activation of the major apoptotic pathways, focusing on the calpain-caspase effector pathway and oxidant stress/NADPH-dependent signaling. Similarly, in septic mice in vivo, MVEC dysfunction/apoptosis will be characterized and the key apoptosis signaling and effector pathways defined.

Aim#2: We will define the effects of the presence of PMN in murine PMN-MVEC co-culture in vitro and in vivo on septic MVEC dysfunction/apoptosis, and dissect out the specific mechanisms of PMN-dependent
septic MVEC apoptosis, focusing on PMN inducible nitric oxide synthase (iNOS/NOS2) and CXC chemokine receptor type 2-dependent signaling.

**Aim#3:** We will then assess the clinical relevance of key septic murine MVEC apoptosis mechanisms in human MVEC in vitro under septic conditions. We will use state-of-the-art techniques: (1) isolation and study specifically of MVEC from mouse lung, and in vitro co-culture of these MVEC with PMN under septic conditions, using various murine strains as sources of both cells (e.g. Wild-type, Nos2-/-) (2) our established in vivo clinically-relevant mouse model of cecal ligation/perforation-induced sepsis; (3) selective in vivo PMN depletion-reconstitution strategies to dissect out the molecular mechanisms of PMN-dependent MVEC dysfunction/apoptosis in the complex in vivo situation; (4) isolation of MVEC from septic vs. sham mice and ex vivo FACS assessment of MVEC signaling/apoptosis; (5) isolation and co-culture of human lung MVEC with human PMN under septic conditions.

**Feasibility / Future Directions.** Over the past 8 years of HSF-funded work and publications on septic MVEC injury in vivo and in vitro, we have demonstrated our extensive experience with in vivo murine sepsis models, in vivo selective cell (e.g. PMN) manipulation via depletion-reconstitution, as well as isolation and study of MVEC from mouse and human lung. As such, all required tools and expertise are available to successfully complete the proposed studies. Most exciting is that direct studies on mechanisms of human sepsis can be performed using human MVEC in vitro. Improved therapy for human sepsis will depend on a clear understanding of mechanisms regulating MVEC dysfunction/death, as well as the complex paracrine effects of other cellular influences.

**Research Environment**

My lab has a technician, 2 graduate students, 2 undergraduate honours thesis students, and 1 undergraduate volunteer. I have also joined labs with a well-established clinician scientist, Dr. Sanjay Mehta, who has a research scientist and technician. Together, we have all of the equipment and have established all of the techniques required to successfully complete the proposed project. Our senior staff has extensive experience with all proposed techniques and work with all undergraduate students in the lab to ensure they learn how to perform required techniques and understand why they utilize specific techniques.

**Expected Objectives/Accomplishments for Student within 16 weeks**

The student will be working on studies from Aim 2 of our overall project (Define mechanisms of neutrophil (PMN)-endothelial cell (EC) apoptosis under septic conditions in vitro). They will be using mouse PMN-EC co-cultures and fluorescent stains to identify the activation of apoptotic enzymes under basal and septic conditions. It is expected that they will be able to generate pilot data characterizing the time course of this apoptotic enzyme cascade.

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