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**ANNOUNCEMENT OF FINAL ORAL EXAMINATION**

**FOR THE DEGREE OF DOCTOR OF PHILOSOPHY**

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| **CANDIDATE**: | **Evan L. Chiswick** |
| **DEPARTMENT OR PROGRAM:** | **Pathology, Program in Immunology** |
| **TITLE OF DISSERTATION:** | **"Altered Phagocyte Function Precedes Death in Polymicrobial Sepsis"** |
| **DATE, TIME, AND PLACE:** | **Friday, March 28, 2014 at 10:30a.m.**<br>**Boston University School of Medicine**<br>**72 E. Concord Street - Room R-115**<br>**Boston, MA 02118** |

**EXAMINING COMMITTEE**

| | |
|---|---|
| **FIRST READER:** | **Dr. Daniel Remick** |
| **SECOND READER:** | **Dr. Lee Quinton** |
| **THIRD READER:** | |
| **CHAIRMAN OF THE EXAMINING COMMITTEE:** | **Dr. Jan Krzysztof Blusztajn** **Phone: (617) 638-4829**<br>**Email:jbluszta@bu.edu** |
| **ADDITIONAL COMMITTEE MEMBERS:** | **Dr. Susan Winandy** |
| | **Dr. John Bernardo** |

**Members of the committee are asked to confirm attendance by replying directly to the Chairman of the Examining Committee.**

**ALL MEMBERS OF THE SCHOOL OF MEDICINE FACULTY ARE INVITED TO ATTEND.**

# ALTERED PHAGOCYTE FUNCTION PRECEDES DEATH IN POLYMICROBIAL SEPSIS

**EVAN L. CHISWICK**

Boston University School of Medicine, 2014

Major Professor: Daniel Remick, M.D., Chairman, Department of Pathology and Laboratory Medicine

ABSTRACT

Sepsis is an immunological condition defined by a pathogen inducing the Systemic Inflammatory Response Syndrome (SIRS), which itself is a clinical diagnosis involving temperature, heart rate, respiration, and white blood cell (WBC) count.

Our lab uses Cecal Ligation and Puncture (CLP) to induce polymicrobial sepsis in mice, with a mortality rate of 50 percent. Previous research in our lab has demonstrated that the plasma levels of IL-6 collected six hours after the start of sepsis can be used to predict which mice will live (Live-P) and which mice will die (Die-P) during the acute phase (<5 days post CLP). This predictive tool enables stratification of mice prior to mortality to determine immunological differences between groups. With this approach it was found that both Live-P and Die-P mice have equivalent bacterial burden and phagocyte recruitment within 6 hours of CLP. Yet by 24 hours, Die-P mice have increased bacterial burden while recruiting *more* phagocytes than Live-P. This suggested a phagocytic impairment.

This study reproduced the aforementioned findings and subsequently determined that Die-P peritoneal phagocytes kill fewer bacteria than Live-P. This bactericidal deficit was associated with multiple cellular defects. The reduced cellular function included:

decreased phagocytosis, decreased phagosomal acidification, and decreased generation of reactive oxygen species (ROS). All of these are integral components of the bacterial killing process. Furthermore, it was found that this deficit was due to cellular suppression and not to cellular exhaustion. The study of phagocytic function was then extended to the bone marrow, a source of phagocytes, and to the peripheral blood. Die-P bone marrow phagocytes showed increased phagocytic activity despite a similar capacity to respond to bacteria as Live-P. Additionally, Die-P bone marrow phagocytes were found to express higher levels CD11b, a marker of activation. Conversely, Die-P peripheral blood phagocytes expressed higher levels of activation markers while exhibiting decreased phagocytic functions.

This study then recapitulated the phagocytic dysfunction of septic cells with naïve healthy cells. A surge in pro and anti-inflammatory mediators is a hallmark of sepsis, with Die-P mice producing a significantly larger surge. Naïve phagocytes were incubated with plasma or peritoneal fluid from Live-P and Die-P mice and it was found that Die-P fluids significantly compromised the phagocytic activity of naïve phagocytes.

These studies collectively suggest that mortality from CLP induced sepsis is due to failure to kill bacteria and that differential production of inflammatory mediators contributes to the differences in phagocytic function.