CRITICAL CARE AWARD 2018

DIVISION OF DERMATOLOGY

DAVID GEFFEN SCHOOL OF MEDICINE AT UCLA

PROGRESS REPORT, MARCH 2019

David Geffen School of Medicine | UCLA Health

PROJECT PROGRESS REPORT

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Research Study: MOLECULAR CHARACTERIZATION OF NEUTROPHIL DYSFUNCTION IN VASOPLEGIC AND SEPTIC SHOCK

Primary Investigator: Philip Scumpia, MD PhD

Co-Investigators: Anaar Siletz MD, Kindra Kelly-Scumpia PhD, Yas Sanaiha MD, Peyman Benharash MD, Henry Cryer MD, Stephen Smale PhD.

Primary Objective: Use next generation RNA sequencing, lipidomics, and NETosis assays to characterize neutrophil programs following heart surgery, including a subset of patients who developed vasoplegic shock.

Secondary Objective: Compare neutrophil function in those patients to those who present with sepsis.

Progress Report (Through March 2019)

We thank the UCLA Critical Care Center for funding this project, and on behalf of the co-investigators, I am pleased to provid3e this progress report.

While much has been learned about the molecular underpinnings of sepsis and septic shock, no new therapies to treat sepsis have been developed since Drotrecogin alfa (Xigris), which has been since pulled off the market. Septic shock remains the leading cause of morbidity and mortality in patients in non-cardiac intensive care units. The variability of the timing when patients present with sepsis limits the ability to truly understand the early, critical events that lead to the development of sepsis.

We wished to better understand the timing of transcriptional reprogramming that occurs during the systemic inflammatory response and shock by studying patients undergoing cardiac surgery, including a subset that develop sterile vasoplegic syndrome, a distrutive type of shock. This allowed us to examine the development of shock over a defined time course following the insult. Then we wished to compare this to patients who present with septic shock.

We gathered peripheral blood neutrophils and plasma from 8 healthy control patients, 12 septic shock patients, 8 cardiopulmonary bypass patients who did not develop vasoplegic shock and 8 cardiopulmonary bypass patients who did develop vasoplegic shock. Among these latter 8 patients, 4 had very severe disease.

We performed RNA Sequencing on neutrophils, examined neutrophil extracellular trap (NET) formation, and cytokine analysis from patient plasma (only on fasted patients undergoing cardiac surgery).

Septic patients and patients after cardiac surgery displayed increased plasma IL-6 expression, confirming a systemic inflammatory response. Neutrophils from patients after cardiopulmonary bypass and those with septic shock displayed increased NET formation, displaying increased neutrophil activation. There was a trend to increased neutrophil NET formation in patients with vasoplegic shock over those with uncomplicated cardiac surgery.

RNA sequencing analysis of neutrophils demonstrated that cardiac surgery dramatically reprograms the neutrophil transcriptome, with many changes occurring immediately after bypass is turned off and 24 hours later. The neutrophil program following cardiopulmonary bypass was similar to that in patients with septic shock. Interestingly, metabolic reprogramming, including lipid biosynthesis pathways, was among the most strongly induced programs in septic shock and cardiopulmonary bypass patients. We also identified a gene program in patients with refractory vasoplegia that had a complicated clinical course.

We performed shotgun lipidomics on patients' plasma following surgery (as they were fasting), and found a dramatic decrease in many lipids, including precursors of vasoactive lipids, which suggests that the metabolic/lipidomic transcriptome

reprogramming was a consequence of depletion of lipid stores during the systemic inflammatory response.

We are currently performing time course experiments to determine the transcriptomic reprogramming of neutrophils that occurs in vitro following treatment with a bacterial (lipopolysaccharide) or a non-bacterial stimulator (phorbol myrestate acetate). We already determined the degree of net formation caused by these stimuli, and know that within 24 hours most neutrophils have degranulated. We are now performing RNA-Seq on these ex-vivo activated neutrophils to determine if transcriptional state of the blood neutrophils from the septic and cardiac surgery patients are more similar to activated neutrophils, and at which point of activation their neutrophils are in.

This award has allowed us to gather a tremendous amount of data that can be used to study the response of neutrophils to systemic inflammation caused by infectious and non-infectious immune activation and will be used in future grant applications. Our plan is to continue to analyze the data to write a manuscript detailing our findings. Anaar Siletz has presented some of this research at the Society of Critical Care Medicine Meeting in 2019 and received the 2019 Star Research Achievement Award. We again thank the Critical Care Center for providing the support for this project.