

# **Allegheny-Singer Research Institute**

## **Annual Progress Report: 2010 Formula Grant**

### **Reporting Period**

January 1, 2011 – June 30, 2011

### **Formula Grant Overview**

The Allegheny-Singer Research Institute received \$120,384 in formula funds for the grant award period January 1, 2011 through June 30, 2012. Accomplishments for the reporting period are described below.

### **Research Project 1: Project Title and Purpose**

*Complement Activation Product C4d Binding to Platelets in Systemic Lupus Erythematosus* - We have previously observed that in 18% of patients with systemic lupus erythematosus (SLE) the protein C4d, a product of complement activation, is deposited on the surface of platelets. This project will explore the impact of this phenomenon on platelet function, based upon the hypothesis that these platelet C4d+ patients are a distinct subset of lupus patients. Given the established role of platelets in some of the pathological sequelae of lupus, we will explore differences in the expression of platelet proteins between C4d+ and C4d- platelets, in order to further characterize the mechanisms of platelet dysfunction in lupus.

### **Anticipated Duration of Project**

1/1/2011 – 6/30/2012

### **Project Overview**

This project is designed as a comparative study looking at all platelet proteins simultaneously, a technique known as whole-proteome scanning, and comparing differences in the proteome of C4d+ and C4d- negative platelets. These studies are performed by collecting platelets from 10 platelet C4d+ lupus patients and 10 platelet C4d- lupus patients and disrupting the platelets to release all of the intracellular proteins. The comparative analysis is then performed using Difference Gel Electrophoresis (DiGE), a technique which allows an overlay of two different proteomes on the same gel, and allowing identification of differences between the two images, with each dot corresponding to a single protein. 10 proteins showing the maximum difference between C4d+ and C4d- patients will be identified initially using statistical analysis software, and will subsequently be identified using mass spectrometry. The end result of these studies will be to identify 10 proteins that are expressed differently in the platelets of C4d+ vs. C4d- patients, potentially implicating signaling mechanisms in platelet dysfunction.

## **Principal Investigator**

Michael J. Passineau, PhD  
Assistant Professor  
Allegheny-Singer Research Institute  
Allegheny General Hospital  
Room 841, South Tower  
320 East North Avenue  
Pittsburgh, PA 15212-4772

## **Other Participating Researchers**

Chau-Ching Liu, MD – employed by Allegheny-Singer Research Institute  
H.M. Kingston, PhD – employed by Duquesne University

## **Expected Research Outcomes and Benefits**

This research will explore a proposed mechanism for platelet dysfunction in systemic lupus erythematosus (SLE), a disease that afflicts approximately 1.5 million patients, predominantly women of child-bearing age, in the United States. Owing to its complex etiopathogenesis and heterogeneous clinical features, SLE has remained a formidable challenge to both treatment and research. Amid the myriad manifestations of SLE, vascular involvement is a hallmark. Our discovery that a component of the complement system, C4d, is deposited on the surface of platelets in a subset of lupus patients suggests one mechanism by which these vascular symptoms arise during the natural history of the disease.

As the result of these studies, we will develop a fuller understanding of the alterations in physiology and function of C4d-bound platelets. By using an unbiased, full-proteome scanning technique, we will be able to focus on the pathways that are most affected by this platelet-complement interaction. Identification of perturbed signaling and function may identify directly druggable targets and will provide a clear direction for future research intended to inhibit the platelet pathology involved in lupus.

## **Summary of Research Completed**

Purchase of robotic spot picker. As proposed in the research plan, we have purchased the robotic spot picker that will extract the individual proteins from polyacrylamide gels after DiGE analysis. This instrument has been installed in the laboratory and integrated with the existing DiGE system.

Establishment of methods for full proteome scanning of human platelets. As the first step toward accomplishing our main aim, we have sought to establish the full-proteome scanning technique in platelet samples. Since Lupus patient samples are extremely precious, this first series of experiments used platelets from healthy controls.

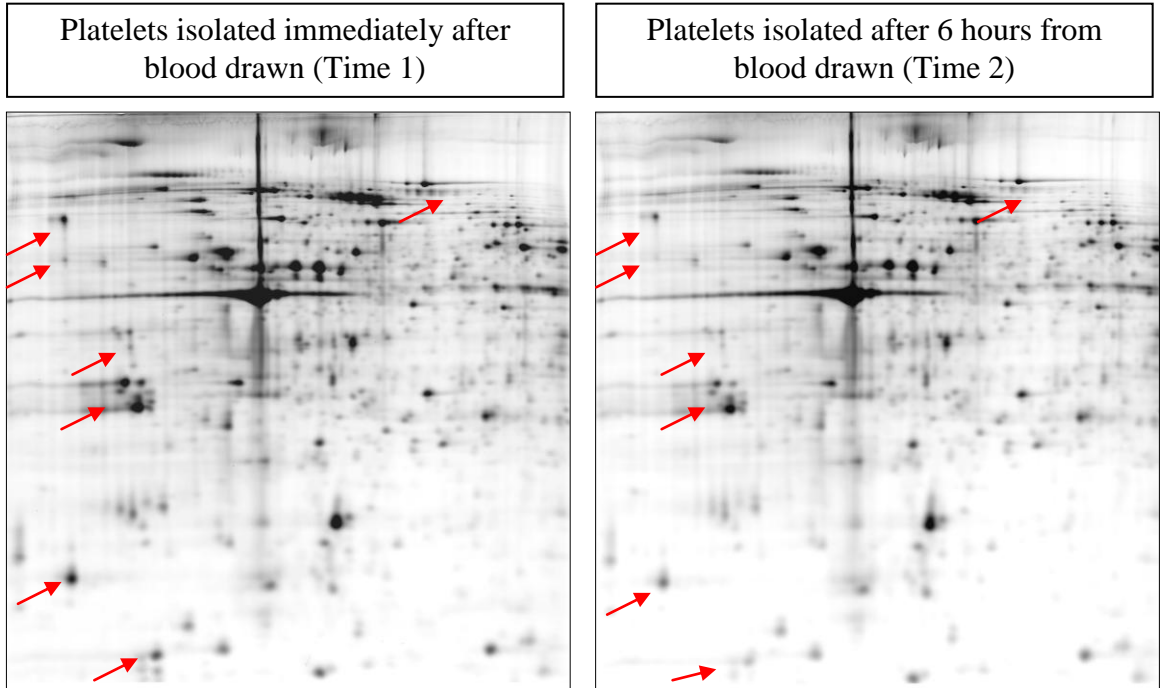
Within the platelet research community, it is unclear how soon after collection platelets need to be processed for proteomic analysis. Since our sample collection occurs at a different location from the laboratory analysis, this issue substantially impacts our research. Therefore, in this first series of experiments, we collected blood from three different healthy volunteers, with 2 samples taken from each volunteer. One sample from each volunteer was processed and platelets isolated within 1 hour of collection. The other sample was kept at ambient temperature for 6 hours before platelets were isolated and processed. These samples were then analyzed using DiGE, with comparisons made between the 1 hour and 6 hour timepoints on an *intra*-volunteer basis.

The results of these experiments are shown in Figures 1A, 1B and 1C. Red arrows indicate proteins for which the expression pattern changed between the two timepoints.

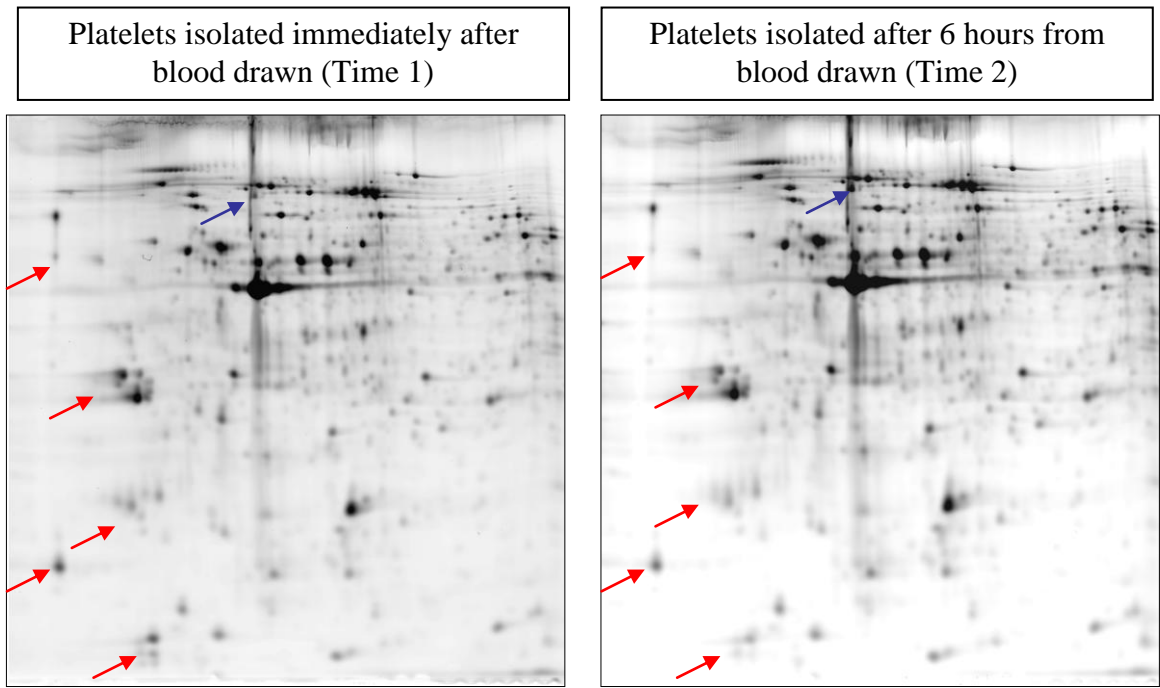
Findings from these experiments were striking in their reproducibility from one volunteer to the next, as well as for the relatively small number of proteins that changed between the two timepoints (see below).

Application of DeCyder analysis to full proteome scans of human platelets. Using the difference gels shown in Figures 1A, 1B and 1C, we applied the DeCyder software to determine intra-volunteer differences between the two timepoints. The results of this analysis are shown in Table 1.

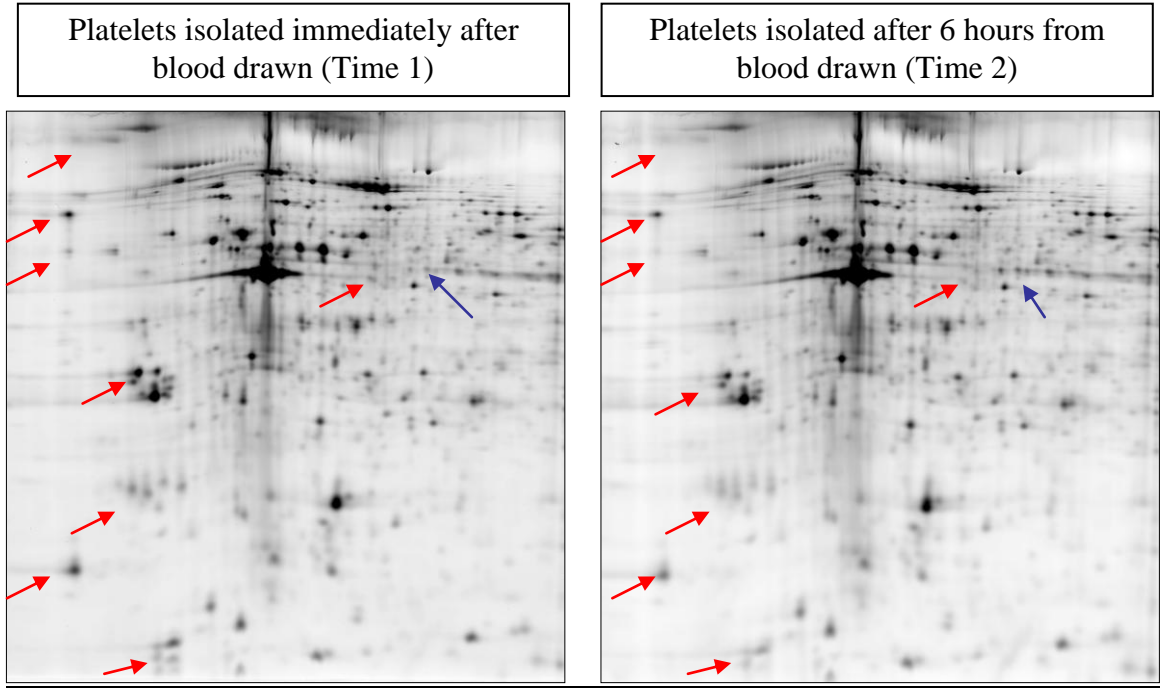
**Figure 1A - Volunteer A**



**Figure 1B - Volunteer B**



**Figure 1C - Volunteer C**



**Table 1: 1700 spots analyzed**

	Volunteer A	Volunteer B	Volunteer C
Similar spots	99.1 %	99.2 %	98.6 %
Spots Increasing	0 %	0.3 %	0.4 %
Spots Decreasing	0.9 %	0.5 %	1.1 %