Aging-Related Upregulation of Pro-Inflammatory Signaling Pathways in Platelets and Monocytes

Introduction

Aging is associated with dysregulated immune and inflammatory responses and an increased susceptibility to infections, thrombosis, and systemic inflammatory disorders\textsuperscript{1,2}. Platelet-monocyte interactions are emerging as key mediators of these injurious responses. Platelets become hyperreactive with age, leading to increased adhesion and heterotypic binding with target monocytes. Amplified platelet-monocyte interactions in the elderly result in enhanced expression of pro-inflammatory cytokines that contribute to events such as sepsis, thrombosis, and death\textsuperscript{3}. Nevertheless, the underlying signaling pathways and mechanisms regulating enhanced platelet adhesion to monocytes and subsequent synthesis of pro-inflammatory cytokines in older adults remains inadequately understood. This is an especially important issue as exaggerated inflammation in older adults is a risk factor for cardiovascular disease, stroke, and venous thromboembolism. As such, this proposal is focused on a research area directly relevant to the mission of the National Heart, Lung, and Blood Institute.

Hypothesis

We hypothesize that aging alters the platelet molecular signature, leading to increased adhesion of platelets to monocytes and exaggerated pro-inflammatory cytokine synthesis by target monocytes. We further hypothesis that platelets from older adults will have significant upregulation of the expression of specific molecules that control exaggerated cytokine synthesis in target monocytes. These include Perforin 1 (PRF1), Chemokine Receptor 1 (CX3CR1), Tumor Necrosis Factor Receptor Superfamily Member 9 (TNFRSF9), Receptor Tyrosine-Protein Kinase erbB-2 (ERBB2), and Matrix Metalloproteinase-9 (MMP9).

Experimental Methods

Subjects

The studies proposed below will utilize stored biospecimens and existing patients currently participating in the Utah Study of Translational Research in Aging Registry (U-STAR). This registry, developed by Dr. Rondina and colleagues, consists of >100 male and female subjects aged 21 - 90 meeting inclusion/exclusion criteria and willing to donate blood for this research. This study is active and approved by the University of Utah Institutional Review Board (IRB # 51506). For the experiments proposed in Aim 1 (where fresh cellular isolates are required), after each participant provides informed consent, approximately 50mLs of whole blood will be drawn through sterile techniques from each subject. All blood will be collected in our laboratory where physicians are on-site to ensure the patient’s safety. The Mentor Laboratory has drawn this quantity of blood in over 500 donors during the last 7 years without any adverse effects to study participants. For Aim 2 (see below), we will use existing biospecimens banked in the U-STAR registry. Importantly, the current IRB allows for all experimental assays proposed in Aims 1 and 2.

Aim 1

From the whole blood, platelets and monocytes will be purified from healthy younger (age<40, n=15) and older (age≥65, n=15) subjects using protocols that the Mentor laboratory has established and optimized\textsuperscript{1,4-7}. All patients will be free of cardiovascular disease, heart failure, cancer, diabetes, or other
chronic conditions which could confound experimental results. Platelets and monocytes will be incubated together (initially at a 1:100 ratio to approximate physiological conditions) in the presence and absence of activating signals. The synthesis of the pro-inflammatory cytokines interleukin 6 (IL-6) and monocyte chemotactic protein-1 (MCP-1) (both secreted and intracellular levels) will be measured at the protein level using commercially-available ELISA kits, as we have done before. In addition, switch experiments will be used to determine whether platelets from elderly subjects can induce similar responses in monocytes from younger subjects.

**Aim 2**

Based on robust preliminary data, we expect the synthesis of IL-6 and MCP-1 to be significantly greater in older subjects compared to younger subjects. Next, we will identify the signaling molecules and pathways regulating increased IL-6 and MCP-1 synthesis in older adults. We have performed preliminary pathway analyses using deep sequencing data on isolated platelets from younger (age<40, n=3) and older (age≥65, n=3) healthy adults. The results of these discovery analyses demonstrate that in platelets isolated from older adults, transcripts involved in the leukocyte activation pathway are significantly upregulated (p=1.16x10^{-12}, activation z-score=2.34). Examination of the top, differentially expressed candidates identified several molecules in platelets that are significantly increased in older adults, induce monocyte activation, and lead to increased IL-6 and MCP-1 synthesis. These top candidates are PRF1, CX3CR1, TNFRSF9, ERBB2, and MMP9. We will validate the increased expression of these molecules in platelets from older adults (n=15) compared to younger adults (n=15), by quantitative real-time PCR and western blotting. In addition, we will leverage our existing deep sequencing data to examine other potential candidates identified by RNA-seq. For these experiments, we will use platelet lysates from older and younger healthy adults currently available in the Rondina U-STAR biospecimen repository. Validation will incorporate housekeeping genes, for normalization, and expression of each of the candidates will be compared in older versus younger adults.

**How our study will improve upon previously published work in this field**

Limitations in our understanding of how aging leads to dysregulated platelet-monocyte interactions and associated inflammatory syndromes hinders the development of safe and effective treatments and preventative strategies for inflammatory diseases in older adults. Moreover, no studies to date have specifically examined inflammatory signaling pathways at the molecular level in platelets from older adults. Finally, much of the past research has been done in older subjects with chronic diseases, thus confounding conclusions. This project will fill current knowledge gaps and the discoveries made during the course of this work may help improve the development of more effective therapeutic interventions in the elderly. In addition, as our studies will be performed using primary human cells from older and younger subjects, the results we generate will have immediate clinical relevance.

**What I hope to learn from this summer experience**

My interests in medicine stem from a deep desire to understand signaling pathways. Since my first biology class, I have always been interested in how molecules interact with one another and how dysregulation of these signaling cascades may result in injurious cellular responses, potentially influencing adverse clinical outcomes in patients. Nevertheless, while I have extensive experience in the basic science aspects of these signaling pathways, I wish to garner a broader understanding of how changes at the molecular level translate to human diseases. A passion for bridging the basic and clinical research fields is what drew me to work with Dr. Mentor, who I believe will help me connect these two
areas. By the end of the summer, I will develop a better understanding of how dysregulated and injurious platelet-monocyte interactions contribute to inflammatory and infectious syndromes in older adults.

Throughout the course of this program, I hope to learn much from Dr. Rondina. He is not only an excellent physician, but also an enthusiastic research mentor. Moreover, he has extensive experience in the field of aging research and my project builds on robust preliminary data developed in his laboratory. Thus, this project allows me to pursue my passion for molecular biology and apply it to practical problems. I hope to not only achieve the goals of this research project, but also learn from Dr. Mentor how to balance clinical and research interests. It is exciting to be a part of a project that is at the forefront of its field, but it is more exciting to have the support of a passionate mentor.

References