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Role of the Aged Bone Marrow Microenvironment in Modulation of Hematopoietic Failure and Transformation in Myelodysplastic Syndrome

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CONTRACTING ORGANIZATION: University of Rochester

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Our research focus is on ways in which the cells and tissues supporting blood forming stem and progenitor cell can be rallied to improve blood count, prevent marrow failure and transformation to acute leukemia. For several years, the core of our research team has been studying the microenvironment in the context of normal marrow function, aging and blood cancers. Recently, we have discovered that the professional scavenger cells in the bone marrow of aged mice play a critical role in instructing all other microenvironmental populations, and together they can change the function of stem cells that produce blood. To study this process, and whether it plays a role in marrow failure, we recruited an expert in these scavenger populations. Together, we were able to show that aged and MDS scavenger cells have a significant defect in engulfing dying cells in the marrow. This is very important, because dying cells that are not taken up by scavenger cells release inflammatory signals in their vicinity that can disrupt normal production of blood cells and even accelerate transformation to leukemia. In fact, it is known that in marrow failure due to myelodysplastic syndromes, the disease we are studying, there is a great increase in programmed cell death in the marrow. In this proposal, we seek to use mouse models where the scavenger function is increased or decreased, and induce marrow failure to see if pharmacologic modulation of scavenger function could be a way to treat marrow failure. This is critical because patients with myelodysplastic syndrome are primarily elderly and often are not candidates for bone marrow transplantation, the curative treatment for this disease. Our results will add a complete new class of treatments that focus on the contribution of the cells and tissues supporting the blood forming cells, and it is therefore very innovative. Since there are pharmacologic ways to modulate scavenger function, this idea, if supported by data generated by this proposal, has a strong potential to be translated to the clinic.

15. SUBJECT TERMS

Aging, hematopoietic stem cells macrophages, phagocytosis, apoptosis, efferocytosis, pancytopenia, stem cell niche

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PROGRESS REPORT -

1. INTRODUCTION

The aim of this proposal was to test if aging is associated with defects in macrophage function (specifically loss of efferocytosis), if these changes impact hematopoietic stem cells, and whether macrophages changes are involvement in development of cytopenia, including in models of myelodysplasia and in patients with MDS. Since the inception of this funding, we have made significant progress, outlined below.

2. KEYWORDS

Aging, hematopoietic stem cells, macrophages, phagocytosis, efferocytosis, apoptosis, pancytopenia, stem cell niche.

3. ACCOMPLISHMENTS

a. What were the major goals of the project?

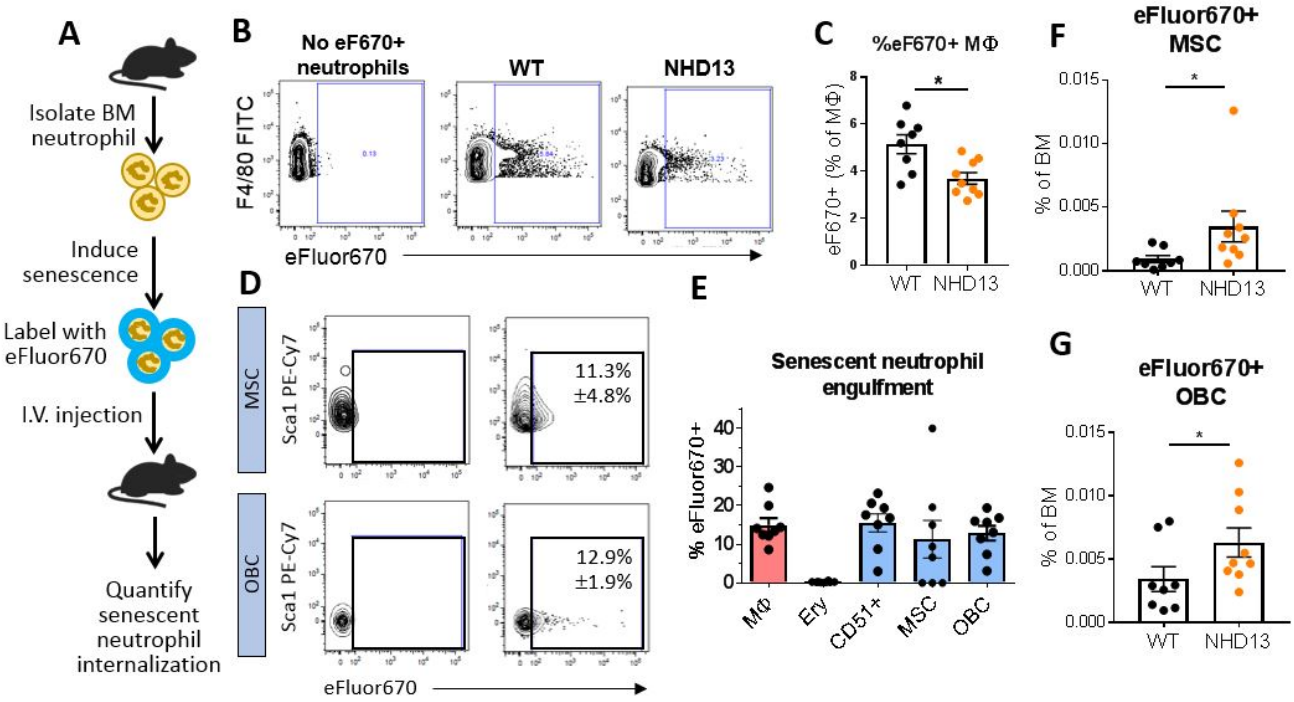
Major Task 1
To seek DoD ACURO approval for the animal use, and DoD HRPO approval for the human subject use.
Major Task 2
Subtask 1 Test in vivo whether NHD13 and ASXL1 MDS models have defects in efferocytosis. Efferocytosis will be confirmed in vivo and in vitro as shown in figure 6. 8 wt and 8 NHD13 littermates will be tested at 18-20 weeks of age. The mutant ASXL1 viral vector or control normals will be infected in sorted LSK cells (5 wt donor mice), positive cells will be transplanted in 8 6-9 wks old conditioned recipient mice per experimental group. Recipients will be initially tested at 6-9 weeks after transplantation.
Subtask 2 Csf1r-Cre mice will be crossed with Axlfl/fl mice (LOF) and BAI1tg (GOF) to achieve targeted modulation of efferocytosis in macrophages. Modulation of efferocytosis will be confirmed in vivo and in vitro as shown in figure 6. 6-8 mice per experimental group will be studied.
Subtask 3 MDS will be induced in mice with modulation of efferocytosis (subtask2) by crossing with NHD13 mice or transplantation. We will then Determine in vivo and ex vivo whether modulation of phagocytosis causes changes in hematopoietic changes, transformation to leukemia, defects in the

phenotype and function of non-MDS HSCs, in BMME populations and in inflammatory mediators in the bone marrow, as we previously published.[1] 6-8 mice per experimental group will be studied.
Milestone(s) Achieved Determine if 1) loss of efferocytosis worsens marrow failure (including rate of transformation to leukemia), and 2) improvement of efferocytosis ameliorates marrow failure in 2 murine models of MDS.
Local IRB/IACUC Approval Already obtained
Major Task/ Specific aim 2
Subtask 1 Characterize the efferocytotic capacity of BMME Mφs of young and aged normal volunteers as well as MDS patients in vitro.
Subtask 2 Knock down AXL in Mφs from young volunteers and quantify their efferocytic function as well as ability to support HSCs, on MSCs and inflammatory signals.
Subtask 3 BAI1 will be overexpressed in Mφs from aged or MDS patients and their efferocytic function as well as ability to support HSCs and change MSC in cocultures will be quantified.
Milestone(s) Achieved: 1) Mφs from marrow of MDS will be similar to Mφs from marrow of aged normal volunteers and will be defective in their ability to engulf apoptotic cells compared to Mφs of young volunteer. 2) Knock down of AXL in young Mφs will cause efferocytic defects and HSC dysfunction, expansion in dysfunctional BMME MSCs, and increases in marrow inflammatory signals. 3) BAI1 overexpression in Mφs from aged or MDS patients will improve their efferocytic function and their ability to support HSCs and change MSC in cocultures.

b. What was accomplished under these goals?

Major tasks are listed below from the SOW (verbatim in italics, with accomplishment listed right next to each task/subtask):

Major task 1 was: *To seek DoD ACURO approval for the animal use, and DoD HRPO approval for the human subject use.* These tasks were completed



DoD ACURO approval for the animal use on 10/18/2018
DoD HRPO approval for the human subject use on 8/19/2019

Major task 2 included the proposed animal studies, under 3 subtasks.

Subtask 1 included:

Part 1: *Test in vivo whether NHD13 and ASXL1 MDS models have defects in efferocytosis*. Analysis of the NHD13 model for efferocytosis is completed and shows defective efferocytosis in macrophages as well as enhancement in efferocytosis in mesenchymal populations (Figure 1). This work is now in a manuscript draft. Analysis of the ASXL1 model is in progress (see part 2).

Part 2: *The mutant ASXL1 viral vector or control normals will be infected in sorted LSK cells (5 wt donor mice), positive cells will be transplanted in 8 6-9 wks old conditioned recipient mice per experimental group. Recipients will be initially tested at 6-9 weeks after transplantation.*

We developed of a second model of MDS, with expression of a mutant ASXL1 by viral vector, as well as the analysis of efferocytosis by MDS mice (NHD13) compared to normal mice. This task is partially completed, as analysis at 6-9 weeks, showed low levels of engraftment. Analysis of this model is continuing.

Figure 1. Changes in efferocytic cell populations in MDS A. *Schematic of in vivo assay of eFluor670-labeled senescent neutrophil phagocytosis.* ***B.*** *Engulfment of eFluor670-labeled senescent neutrophils by marrow macrophages (Ly6C- Ly6G- CD45+ F4/80+). eFluor670+ gate was established based on negative population from control not treated with eF670+ senescent neutrophils. Representative plots shown from WT and NHD13 mice.* ***C.*** *Quantification of eFluor670+ senescent neutrophil engulfment by marrow macrophages. 2 independent experiments with 3-6 mice per group for each experiment.*p<0.05, **p<0.01, Mann-Whitney test.* ***D.*** *Engulfment of eFluor670-*

labeled senescent neutrophils by MSCs (lineage-CD45-CD31-CD51+Sca1+), and osteoblastic lineage cells (OBC, lineage-CD45-CD31-CD51+Sca1-). Lineage markers include Ter119, B220, CD3e, and Gr1. Mean frequency ± SEM shown for each population. ***E.*** *Quantification of eFluor670+ senescent neutrophil engulfment by marrow macrophages (MΦ, Ly6C- Ly6G- CD45+ F4/80+), erythroid-enriched cells (lineage+ CD45-), CD51+ mesenchymal-osteolineage, multipotent stromal cells (MSC), and osteoblastic lineage cells (OBC). 2 independent experiments, n=4/experiment. Error bars indicate SEM.* ***(F,G)*** *Expansion of efferocytic MSCs (G), and OBCs (H) in MDS marrow of NHD13 mice compared to WT. Results from 2 experiments, n=3-6/group for each experiment*.

Subtask 2 included conditional deletion or overexpression of signals that mediate efferocytosis.

We are currently conducting these matings.

Subtask 3 included mating mice with MDS with mice with modulation of efferocytosis. These matings have been established and analysis of development of pancytopenia and transformation are ongoing.

Major task 3/ specific aim 2 included the proposed human samples studies.

Analysis of human samples from young, aged and MDS patients for macrophage ability to engulf apoptotic changes began after approval in 8/2019 and is ongoing.

c. What opportunities for training had the project provided?

Nothing to report

d. How were the results disseminated to communities of interest?

Work showing the impact of aging on defects in phagocytosis was recently published.

Frisch BJ, Hoffman CM, Latchney SE, LaMere MW, Myers J, Ashton J, Li AJ, Saunders J 2nd, Palis J, Perkins AS, McCabe A, Smith JN, McGrath KE, Rivera-Escalera F, McDavid A, Liesveld JL, Korshunov VA, Elliott MR, MacNamara KC, Becker MW, **Calvi LM**. Aged marrow macrophages expand platelet-biased hematopoietic stem cells via Interleukin1B. JCI Insight. 2019 Apr 18;5. pii: 124213. doi: 10.1172/jci.insight.124213. PMID: 30998506

e. What do you plan to do during the next reporting period

During the next reporting period, we are continuing analysis of the established murine models, submitting the initial report of defects in efferocytosis in MDS models and continuing the analysis of human samples.8

4. IMPACT

What was the impact on development of the principal disciplines of the project?

This work is defining a novel defect in the bone marrow microenvironment of MDS, which is similar to changes that are induced by aging. The experiments ongoing are defining a novel cause for the inflammatory changes in MDS that could represent a novel therapeutic target.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS

Nothing to report.

6. PRODUCTS

One manuscript has been published including work supported by this award. In it, DOD support was acknowledged:

Frisch BJ, Hoffman CM, Latchney SE, LaMere MW, Myers J, Ashton J, Li AJ, Saunders J 2nd, Palis J, Perkins AS, McCabe A, Smith JN, McGrath KE, Rivera-Escalera F, McDavid A, Liesveld JL, Korshunov VA, Elliott MR, MacNamara KC, Becker MW, **Calvi LM**. Aged marrow macrophages expand platelet-biased hematopoietic stem cells via Interleukin1B. JCI Insight. 2019 Apr 18;5. pii: 124213. doi: 10.1172/jci.insight.124213. PMID: 30998506

PARTICIPANTS

Individuals that have worked on the project:

Name:	Laura Calvi
Project Role:	No change
Researcher Identifier:	
Nearest Person Month Worked:	
Contribution to Project:	

Name:	Michael Becker
Project Role:	No change
Researcher Identifier:	
Nearest Person Month Worked:	
Contribution to Project:	

Name:	Michael Elliott
Project Role:	No change
Researcher Identifier:	
Nearest Person Month Worked:	
Contribution to Project:	

Name:	Benjamin Frisch
Project Role:	No change
Researcher Identifier:	
Nearest Person Month Worked:	
Contribution to Project:	

Name:	Daniel Byun
Project Role:	No change
Researcher Identifier:	
Nearest Person Month Worked:	
Contribution to Project:	

OTHER SUPPORT

CALVI, LAURA M.

ACTIVE

W81XWH1810485: PI: Laura M. Calvi, M.D.
Title: Role of the Aged Bone Marrow Microenvironment in Modulation of Hematopoietic Failure and Transformation in Myelodysplastic Syndrome 07/01/2018-06/30/2020 1.8 calendar months
Funding Agency: Department of Defense $207,500
Goal: This project studies disruption of the normal hematopoietic stem cell niche by aging and myelodysplastic syndrome and its mechanism.

1P30AR069655: PI: Edward M. Schwarz, Ph.D.,
Title: University of Rochester Resource-Based Center for Musculoskeletal Biology and Medicine.
Funding Agency: NIH 07/01/2016-06/30/2021 1.2 calendar months
$499,998
Goals: to provide shared facilities and services to NIH-funded investigators who are addressing scientific problems in musculoskeletal biology and medicine, in order to improve efficiency, accelerate the pace of research, and facilitate clinical translation. It will also facilitate the development and promotion of Research Assistant Professors (RAP) and unfunded physician-scientists (UPS) to become national leaders.
Role on Project: Associate Director

Harry T. Mangurian, Jr. Foundation: MPI: Laura M. Calvi, Michael W. Becker
Title: Myelodysplastic Syndrome (MDS) Microenvironment Study
Performance Period: 07/01/2018-06/30/2022 0.6 calendar months
$125,000
Goal: The primary goal of this research proposal is to perform the first in human clinical trial that combines Abaloparatide with Bevacizumab in patients with MDS to improve bone marrow function and decrease the risk for transformation to AML.
Overlap: None

Dresner Foundation: PI: Michael W. Becker
Title: Role for bone marrow mesenchymal populations in modulating Interleukin 1 signaling in Myelodysplastic Syndrome
Performance Period: 10/01/2018-09/30/2020 0.6 calendar months
$250,000
Goal: The primary goal of this research proposal is to confirm the role of IL-1 signaling in development and progression of MDS via its impact on BMME MSCs and demonstrate the value of targeting IL-1 β to improve the care of patients with MDS.
Role on Project: Co-Investigator
Overlap: None

Taub Foundation: PI: Laura M. Calvi
Title: Role of myeloid populations in microenvironmental regulation of Myelodysplastic Syndromes
Performance Period: 07/01/2019-06/30/2022 0.6 calendar months
$181,818
Goal: The primary goals of this research proposal is to (1) To define MDS-related changes in marrow mesenchymal stem and progenitor cells and their dependence on macrophage defects and inflammation; and (2) To determine defects in the myeloid components of the specialized erythroid BMME that direct progression of anemia in MDS.
Overlap: None

OTHER SUPPORT

BECKER, MICHAEL
ACTIVE

W81XWH1810485 (Calvi) 07/01/2018-06/30/2020 1.8 calendar months
Department of Defense $207,500
Role of the Aged Bone Marrow Microenvironment in Modulation of Hematopoietic Failure and Transformation in Myelodysplastic Syndrome
The major goals of this project is to study disruption of the normal hematopoietic stem cell niche by aging and myelodysplastic syndrome and its mechanism.
Overlap: None

(Calvi, Becker) 07/01/2018-06/30/2022 0.6 calendar months
Harry T. Mangurian, Jr. Foundation $125,000
Myelodysplastic Syndrome (MDS) Microenvironment Study
The major goal of this research proposal is to perform the first in human clinical trial that combines Abaloparatide with Bevacizumab in patients with MDS to improve bone marrow function and decrease the risk for transformation to AML.
Overlap: None

(Becker) 10/01/2018-09/30/2020 0.6 calendar months
Dresner Foundation $250,000
Role for bone marrow mesenchymal populations in modulating Interleukin 1 signaling in Myelodysplastic Syndrome
The major goal of this research proposal is to confirm the role of IL-1 signaling in development and progression of MDS via its impact on BMME MSCs and demonstrate the value of targeting IL-1 β to improve the care of patients with MDS.
Overlap: None

7020-19: (Jordan) (Subcontract PI Becker) 10/01/2018-09/30/2023 0.6 calendar months
Leukemia and Lymphoma Society $80,039
Therapeutic targeting of AML stem cells
The major hypothesis of this project is that different therapies should have different impacts on LSCs and their microenvironment, altering selection for adaptive phenotypes. Using samples from patients we will perform a detailed analysis of LSC extrinsic factors that may influence LSC drug sensitivity and/or drive evolution towards more drug resistant phenotypes. We will ask how therapy-induced changes in extrinsic factors like inflammation affect LSC progression, responses to therapy and drug-resistant relapse.
Overlap: None

Taub Foundation: PI: Laura M. Calvi
Title: Role of myeloid populations in microenvironmental regulation of Myelodysplastic Syndromes
Performance Period: 07/01/2019-06/30/2022 .12 calendar months
$181,818
Goal: The primary goals of this research proposal is to (1) To define MDS-related changes in marrow mesenchymal stem and progenitor cells and their dependence on macrophage defects and inflammation; and (2) To determine defects in the myeloid components of the specialized erythroid BMME that direct progression of anemia in MDS.
Overlap: None