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

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14. ABSTRACT

The long term goal of our research program is to therapeutically target critical marrow microenvironmental defects that contribute to hematopoietic dysfunction in myelodysplastic syndromes (MDS). MDS are group of acquired bone marrow failure disorders that are not adequately cured in part because they preferentially affect elderly patients, who are less likely to be eligible for the only curative treatment, stem cell transplantation. Therefore, novel treatment approaches are needed. Data in humans and in murine models have shown that, in addition to cell-autonomous defects in hematopoietic cells, the bone marrow microenvironment (BMME) is dysfunctional in MDS. In this proposal, we aimed to determine if functional defects in bone marrow resident macrophages contribute to age-dependent changes in the MDS BMME may contribute to severity of hematopoietic dysfunction and rate of transformation to leukemia. We found that defects in phagocytosis of apoptotic cells recapitulate hematopoietic stem cell skewing and is also found in the MDS BMME.

15. SUBJECT TERMS
Aging, hematopoietic stem cell, bone marrow failure, bone marrow microenvironment, efferocytosis, myelodysplastic syndrome, macrophages, phagocytosis.

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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 cell, bone marrow failure, bone marrow microenvironment, efferocytosis, myelodysplastic syndrome, macrophages, phagocytosis.

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 4 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 efferocytic 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.

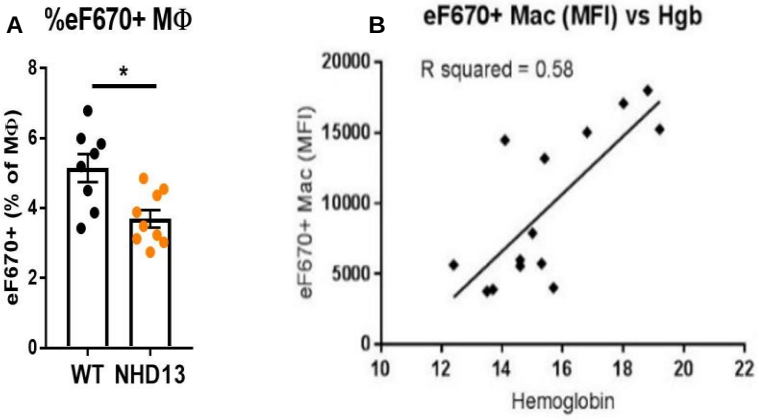

Figure 1*:* eF670 labelled neutrophils were injected in mice expressing the Nup98/HoxD13 translocation under the control of the Vav promoter (NHD13) and in mice transplanted with cells carrying the Asxl1 truncation (MT2). 18 hours later macrophages were obtained from the bone marrow and tested for the presence of eF670 as an index of efferocytosis. In A, significant loss of efferocytic activity is seen. In B, lower hemoglobin level (evidence of anemia) is associated with loss of efferocytic activity in macrophages. Each dot represents an experimental mouse. *:p<0.05

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 (**Figure 1A**). We developed of a second model of MDS, with expression of a mutant ASXL1 by viral vector (**Figure 1B**). We identified defects in efferocytosis in bone marrow macrophages from both murine models. These data are currently being written up as a manuscript.

Subtask 2 included conditional deletion or overexpression of signals that mediate efferocytosis. A genetic murine model in which there was global loss of the efferocytic receptor Axl (Axl ko) induced early skewing of hematopoietic stem cells to the megakaryocytic lineage (identified by increased phenotypic marker CD41 in long term hematopoietic stem cells). These data were published (Frisch

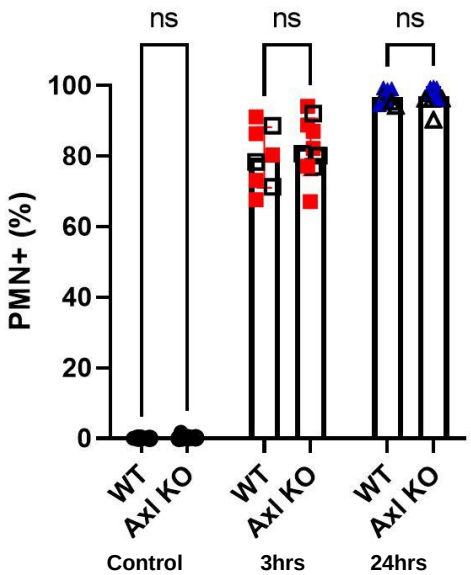

Figure 2*:* Bone marrow derived macrophages and stromal cells were grown in vitro and when at 80% confluence eF670 labelled neutrophils were added in 10:1 excess to the plated cells. Rate of efferocytosis was quantified by flow cytometry in macrophages (PMN+ =eF670+CD45+F4/80+ cells) 3 and 24 hours later. Macrophages with genetic loss of Axl (Axl ko) had no evidence of defective efferocytosis. Each dot represents an experimental mouse. NS:p>0.05

BJ et al, listed in 3d). Surprisingly, in vitro testing of efferocytosis of bone marrow-derived macrophages demonstrated no defect in efferocytosis when tested with labelled end stage neutrophils (**Figure 2**). Surprisingly, we found that bone marrow derived stromal cells (BMSCs) were participating in efferocytosis, and that loss of Axl was causing in reduction of efferocytic capacity (**Figure 3**). Based on these data we decided to perform targeted overerexpression of efferocytosis in BMSCs by mating Bai1tg mice (which we obtained from the University of Virginia from Dr. Ravichandran) with PrxCre mice.

Subtask 3 included mating mice with MDS with mice with modulation of efferocytosis. Prx-Crex BaiTg mice are viable. The initial data on cells obtained from these mice demonstrate increased efferocytosis in BMSCs. We also find that increased efferocytosis has a deleterious effect on BMSCs, increasing their rate of senescence (**Figure 4**). We have also found evidence of increased efferocytosis in BMSCs from the two murine models of MDS (NHD13 and Asxl1, data not shown). These data suggest that increased efferocytosis in BMSC may be a mechanism leading to increased senescence in the bone marrow microenvironment in the setting of MDS.

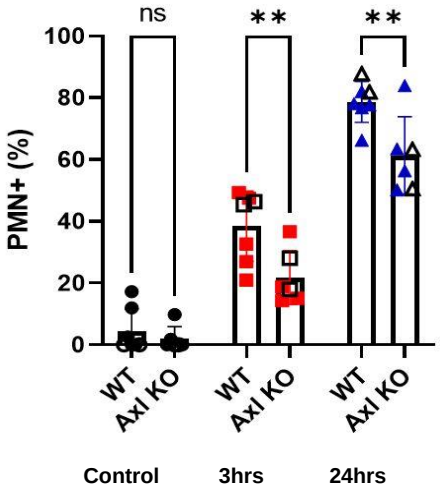

Figure 3: Bone marrow derived macrophages and stromal cells were grown in vitro and when at 80% confluence eF670 labelled neutrophils were added in 10:1 excess to the plated cells. Rate of efferocytosis was quantified by flow cytometry in BMSCs (PMN+ =eF670+CD45-CD31-F4/80- cells) 3 and 24 hours later. BMSC with genetic loss of Axl (Axl ko) had decreased efferocytosis compared to wt control. Each dot represents an experimental mouse. **:p>0.01

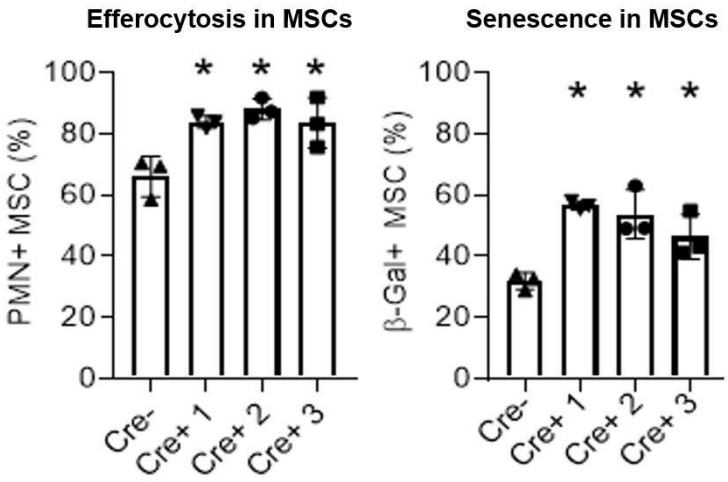

Figure 4: Bone marrow derived stromal cells were grown in vitro from mice with targeted overexpression (via the Prx-Cre) of the efferocytic receptor Bai1 (Cre+) and normal littermates (Cre-)when at 80% confluence eF670 labelled neutrophils were added in 10:1 excess to the plated cells. Both Cre+ and Cre- have the Bai1Tg, but Bai1Tg expression is activated only where the Cre recombinase is expressed. Rate of efferocytosis was quantified by flow cytometry in BMSCs (PMN+ =eF670+CD45-CD31-F4/80- cells) 24 hours later. BMSC of Cre+ mice had increased efferocytosis compared to wt control. Rate of senescence was measured in PMN+ cells (using beta galactosidase activity) and was increased in BMSCs from PrxCrexBai1tg mice. Each dot represents an experimental mouse. *:p>0.05 1

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. Consistent with the data in mice, we have found transcriptional evidence of increased efferocytosis in BMSCs from patients with MDS, regardless of mutation.

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

4. Impact

This work is defining a novel defect in the bone marrow microenvironment of MDS, which is similar to changes that are induced by aging. We have identified a novel mechanism of increased senescence in BMSCs that is associated with increased efferocytic activity.by uncovering the receptors and signaling pathways involved in these interactions, we may be able to mitigate the disruption in the bone marrow microenvironment of MDS, with the goal of improving normal bone marrow function while decreasing the rate of transformation to leukemia.

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

7. Participants & Other Collaborating Organizations

Laura Calvi Project Role: No change

Michael Becker Project Role: No change

Michael Elliott Project Role: No change

Benjamin Frisch Project Role: No change

Daniel Byun Project Role: No change

8. Special Reporting Requirements

N/A

9. Appendices N/A