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14. ABSTRACT

During the first year of funding, we made substantial progress in characterizing the role of SCARF1 in hematopoietic vs non-hematopoietic cells in the development of lupus-like disease on our mouse model. This will help up identifying a group of cells that are essential in the development of lupus-like disease. We hypothesized that hematopoietic cells are essential in the development of disease and mice that lack SCARF1 in dendritic cells and macrophages will develop lupus like symptoms. We perform chimera experiments using the following groups: WT->WT, SCARF1->SCARF1 as our controls, and SCARF1->WT, WT->SCARF1 as experimental samples. While the experiment still on going, our initial findings show that WT mice that have SCARF1-/- cells developed dermatitis, the production of ANA and release of dsDNA. Presentation of symptoms was faster, with more pronounced symptoms in the SCARF1->WT group compared to the WT->SCARF1 group. However, WT->SCARF1 mice group show mild lupus-like disease suggesting that the non-hematopoietic cells also play a role disease development. Our data suggest that hematopoietic cells (DCs, macrophages, monocytes) are essential in the initiation of SLE; however, non-hematopoietic could play a role in the maintaining of the disease.

In addition, other accomplishments in the lab include: purchase and breeding of mouse lines to be use on Aim 3, initiation of new collaboration with the new chief of Rheumatology who will help to obtain the necessary samples for this proposal.

15. SUBJECT TERMS

None listed.

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1. Introduction

Efficient detection and clearance of apoptotic cells is essential in the maintenance of tolerance and tissue homeostasis. We recently identified the scavenger receptor expressed on endothelial cells-1 (SCARF1) as a receptor for apoptotic cells on dendritic cells via interactions with C1q/phosphatidylserine complexes on the dead cells (Ramirez-Ortiz *et.al.*; *Nature Immunology*). Loss of SCARF1 results in impaired uptake of apoptotic cells *in vitro* and *in vivo*, with accumulation of cell corpses in tissues and blood. Consequently, SCARF1-deficient mice develop lupus-like autoimmune disease. In this application, we propose to investigate the role of human SCARF1 in the onset and development of systemic lupus erythematosus (SLE).
We hypothesize that SCARF1 plays an important role in the physiological clearance of apoptotic debris, and that dysregulated or loss of SCARF1 expression on cells leads to impaired AC uptake, loss of self-tolerance, and development of SLE. To answer our hypothesis, we propose the following specific aims:

Aim 1: Determine whether dysregulated SCARF1 expression in SLE patients correlates with a defect in apoptotic cell recognition and severity of disease.
Aim 2: Define the contribution of SCARF1 in dendritic cell maturation, and antigen presentation.
Aim 3: Test the efficacy of soluble SCARF1 treatment in apoptotic cell removal and prevention of SLE.

2. Keywords

ACs- Apoptotic cells
ANA- Antinuclear antibodies
DCs- Dendritic cells
SLE- Systemic Lupus Erythematosus
SCARF1- Scavenger Receptor Class F #1

3. Accomplishments

During the funding period of 10/21-9/22 the laboratory answer the following aim:

A major contributing factor in autoimmune diseases, like lupus, is a defect in efferocytosis—the process in which phagocytes engulf and remove dying cells. Defective efferocytosis leads to an autoimmune response to self-antigens and a break in immune tolerance[1]. The molecular mechanisms of efferocytosis are unknown, and elucidating this process is critical to treat autoimmune diseases.

We demonstrated that phagocytes express Scavenger Receptor Class F member 1 (SCARF1) on their cell surface, which serves as a non-redundant receptor for ACs[2]. Mice with global *Scarf1* deficiency spontaneously develop autoimmune disease with clinical manifestations similar to human systemic lupus erythematosus (SLE). Our publications and preliminary data support the hypothesis that SCARF1-expressing phagocytes engulf and clear apoptotic cells (ACs) to prevent loss of tolerance, inflammation, and spontaneous development of autoimmunity[2]. In order to dissect the essential cells involved in this process, we decided to investigate the role of hematopoietic vs non-hematopoietic cells in the removal of cellular debris. To answer this question, we used a congenic bone marrow chimeric approach. Briefly, 4 week-

old mice, WT (45.2) and SCARF1-/- (45.1), were radiated and allowed to recover for 4 hours. After recovery, mice are injected intravenous with bone marrow from either WT (45.2) or SCARF1-/- (45.1) cells. After engraftment of bone marrow cells, mice are bled every other week to measure the levels of dsDNA using *Crithidia luciliae* and levels of antinuclear antibodies (ANA) using Hep2 immunofluoresence reporter system. We observed development of ANA and the presence of dsDNA at a higher percentage in the SCARF1->WT chimeric mice compare to the WT->SCARF1 or the experimental controls (Figure 1A-D).

Our preliminary data shows that global SCARF1-/- mice develop dermatitis in the head and back. We observed the mice for occurrence of skin disease. Both experimental groups acquire dermatitis as the lupus-like disease progresses (Figure 2). However, symptoms develop earlier in the experimental group SCARF1->WT.

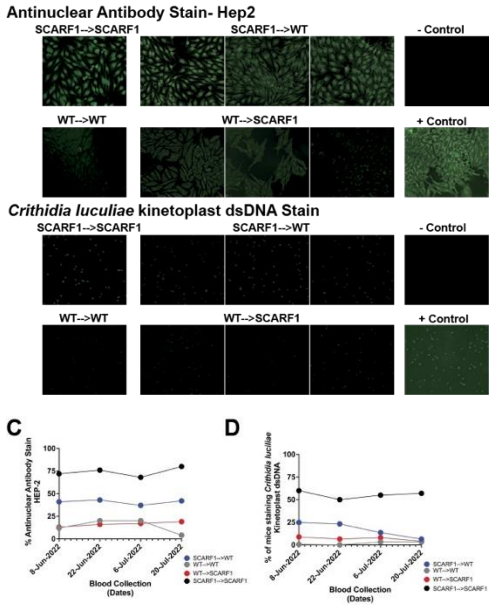

Figure 1. *Increase autoantibodies in SCARF1→WT chimeras.* (A-D) Serum was collected bi-weekly (n=12 mice). (A,C) Fluorescent HEp-2 cell was used to analyze the presence of anti-nuclear antibody (ANA) and *Crithidia luciliae* was used to measure the presence of dsDNA (B,D). Briefly, 1:100 serum dilution was added to HEp-2 (A) or *Crithidia luciliae* (B) slides. Anti-mouse IgG AF-488 was used as secondary antibody. Five-independent pictures were taken by fluorescence microcopy using equal exposure. (C, D) Quantification (percentage) of positive cells for ANA (C) or dsDNA (D).

Overall our data suggest that SCARF1 expressed on phagocytic cells is required in the development of autoimmunity. However, based on our observations we speculate that SCARF1 may play a role in non-hematopoietic cells in the advancement of lupus-like symptoms. In our model, SCARF1 expressed on phagocytic cells is necessary to maintain tissues homeostasis and immune tolerance.

Figure 2. *SCARF1→WT develop lupus-like skin symptoms faster than WT→SCARF1 chimeric mice.* Representative pictures of chimeric mice expressing alopecia and butterfly rash (red arrows) compared to control mice (n=12 mice).

4. Impact

Our initial findings fill several major gaps in our understanding of apoptotic cell clearance and regulation of autoimmunity; however, additional work is needed to identify the mechanisms necessary for SCARF1-mediated removal of apoptotic cells and prevention of spontaneous autoimmunity *in vivo*. ACs clearance requires numerous receptors and bridging molecules. For example, mice with a single deficiency in C1q, MFG-E8, or TIM4 accumulate apoptotic cells in tissues and develop spontaneous autoimmune disease[3,4]. A critically important question raised

from these studies and ours is, “What is the mechanism and relative contribution of these seemingly redundant pathways to the clearance of apoptotic cells and prevention of autoimmunity?”. This led us to hypothesize that the immune system has developed failsafe mechanisms involving multiple receptors and bridging proteins expressed by different cell types present in specific tissues for the removal of dying cells to maintain tolerance and prevent autoimmunity.

5. Changes/Problems

During the funding period of 10/01/2021-9/30/2022 the laboratory encounters certain challenges that require changes in the SOW. We ordered and initiated breeding for the mouse colonies to be used on Aim 3. The colonies are expanding at this time. However, there is lack of personnel in the Ramirez-Ortiz Lab. During the first year of funding, the laboratory hired two research associates. My first research associate transition into pharma/industry, and the second got accepted into Graduate School to complete a PhD in immunology. At this point, there is no personnel in the lab to perform the duties and experimental work. In addition, Umass Chan recently hired a new Chief of Rheumatology, delaying the collaborations between researchers and clinicians. We are expected to obtain SLE and control patients samples starting Spring of 2023. Even with all the problems encountered, the laboratory made significant progress in characterizing the role of hematopoietic vs non-hematopoietic in the presence or absence of SCARF1.

6. Products

None to report.

7. Participants & Other Collaborating Organizations

None to report.

8. Special Reporting Requirements

Nothing to report.

9. Appendices

References:

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