AWARD NUMBER: W81XWH-21-1-0803

TITLE: Role of SCARF1 in Efferocytosis and Prevention of Lupus

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CONTRACTING ORGANIZATION: University of Massachusetts Chan Medical School

REPORT DATE: October 2023

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2023	Annual	30SEPT2022 - 29SEPT2023

4. TITLE AND SUBTITLE

Role of SCARF1 in Efferocytosis and Prevention of Lupus

5a. CONTRACT NUMBER
W81XWH-21-1-0803

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

We demonstrated that the scavenger receptor SCARF1 (Scavenger Receptor Class F member 1) is a non-redundant AC receptor[1]. Mice with global *Scarf1* deficiency spontaneously develop autoimmune disease with clinical manifestations that are strikingly similar to human SLE. Our publication and supporting data provide ample support for the hypothesis that SCARF1 engulfs and clears ACs to prevent loss of tolerance, inflammation, and spontaneous development of autoimmunity. Therefore, 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. During the 10/22-9/23 cycle we identify an increased in soluble SCARF1 on SLE patients when compared to controls. Suggesting that part of the dysregulation could be mediated by cleavage of SCARF1 from cells. Furthermore, in our mouse models we performed some chimeras to characterize the role of SCARF1 in hematopoietic vs non-hematopoietic cells in the development of autoimmunity. Our data shows that both groups of cells are essential; however, hematopoietic cells are necessary in the initiation of disease. Non-hematopoietic cells are needed for the maintenance. While out data suggest a non-redundant role for SCARF1 in the initiation and maintenance of autoimmunity, additional work is needed to dissect the specific role of the receptor. For 10/23-9/24 our lab efforts will investigate the relation on soluble SCARF1 and autoantibodies, identify if SCARF1 autoantibodies are pathogenic and how N-modifications affect their role.

15. SUBJECT TERMS

NONE LISTED

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
U	U	U	UU	7	19b. TELEPHONE NUMBER *(include area code)*

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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 10/22 to 9/23 the laboratory focus on answering the following two aims:

Aim 1: Determine whether dysregulated SCARF1 expression in SLE patients correlates with a defect in apoptotic cell recognition and severity of disease.

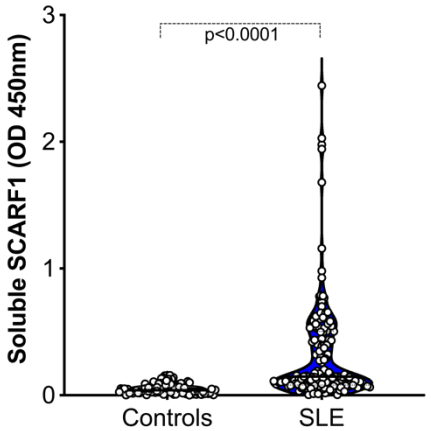

Figure 1. Increased soluble SCARF1 in SLE patients. Levels of soluble SCARF1 in serum from healthy (n=99) and SLE (n=169) patients determined by ELISA. P<0.0001 by t-test with Welch correction.

Five different splice isoforms have been documented for SCARF1, with two of them being soluble. Recently, it was demonstrated a 60KDa truncated soluble form of SCARF1 in human serum generated by exofacial cleavage. Recently, it was demonstrated a 60KDa truncated soluble form of SCARF1 in human serum generated by exofacial cleavage. This work also showed that sSCARF1 was present in chronically inflammatory liver disease, but sSCARF1 was absent in healthy controls[2, 3]. To date, the function of soluble scavenger receptors remains elusive; however, soluble scavenger receptors like CD36 and CD163 are regulated by the extent of disease progression and can be potential biomarkers[3]. The levels of sSCARF1 in SLE patients remains to be elucidated.

The goal of this experiment is to determine the levels of soluble SCARF1 in SLE patients compared to control patients, and to correlate with disease level. In order to answer this question, we will use sandwich ELISA assay. Briefly, we utilized SLE and healthy serum samples from the Partners Biobank that were previously collected. We will used human anti-SCARF1 antibody (R&D Biosciences) as primary antibody. Then added the serum 1:100 dilution, finally biotin-SCARF1 antibody (R&D Biosciences) as secondary antibody. We used recombinant SCARF1 to identify the concentration of the protein being express on the serum. We can observe an increase in soluble SCARF1 in SLE patients when compared to controls (Figure 1).

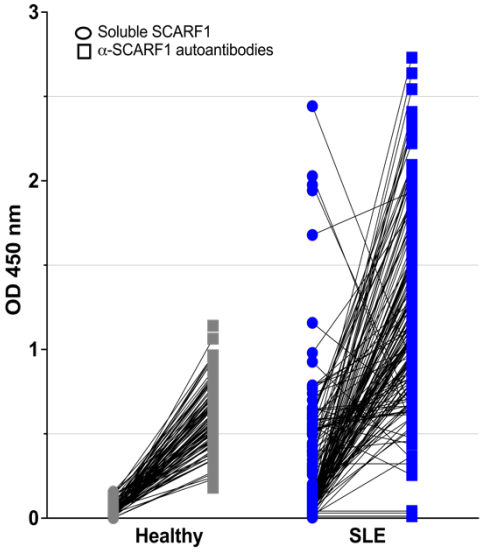

Figure 2. Soluble SCARF1 correlates with levels of autoantibodies to SCARF1. Levels of soluble SCARF1 (circles) and anti-SCARF1 autoantibodies (squares) in serum from healthy (n=99) and SLE (n=169) patients determined by ELISA. Lines match the same patients connection between the two measurements.

We recently discovered the presence of autoantibodies in SLE patients[4]. Therefore, we question whether there is correlation between the presence of SCARF1 autoantibodies and the presence of soluble SCARF1. Using the data from SLE and healthy serum samples from the Partners Biobank, we compared the presence of autoantibodies to soluble SCARF1 from the serum of the patients. Our data shows most of the patients exhibit a positive correlation for sSCARF1 and SCARF1-autoantibody (Figure 2). However, interestingly we observed that the patients with the most soluble SCARF1 have low levels of SCARF1 autoantibodies.

Our data implies a correlation of SCARF1 autoantibodies and soluble SCARF1, posting the hypothesis that SCARF1 gets cleaved from the cells resulting in the production of autoantibodies therefore affecting efferocytosis. In order to characterized the interaction between soluble SCARF1 and the presence of autoantibodies by mass spectrometry and ssRNAseq.

Aim 2: Define the contribution of SCARF1 in dendritic cell maturation, and antigen presentation.

Global mouse model demonstrates that SCARF1 is non-redundant efferocytosis receptor, as absence leads to an accumulation of apoptotic debris and autoimmunity[1]. Our lab demonstrated that phagocytes express Scavenger Receptor Class F member 1 (SCARF1) on their cell surface, which serves as a non-redundant receptor for ACs[1]. 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[1]. 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 antinuclear antibodies (ANA) using Hep2 immunofluorescence reporter system. We observed the

presence of ANA in SCARF1→WT mice as early as 2 months post radiation, suggesting that absence of SCARF1 on hematopoietic cells drive lupus-like disease. However, we noted that WT→SCARF1 develop ANA, albeit, at a delayed state suggesting that SCARF1 expression on non-hematopoietic cells play a small role in the autoimmune development.

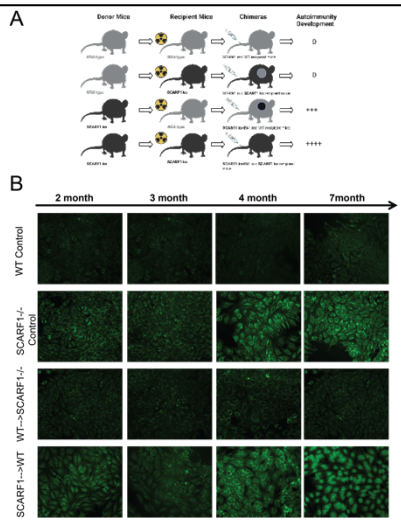

Figure 3. SCARF1 expression on hematopoietic cells is critical in lupus-like disease development. (A) Experimental design to generate bone marrow chimeras. Female mice (8-12 weeks of age) are lethally irradiated and reconstituted by intravenous injection of 10^7 of total bone marrow from female donor mice (8-10 weeks old) for 8-12 weeks. (B) Representative images of ANA stains of chimeras over time. ANA immunofluorescence was performed using HEp-2 cells, with sera from chimera mice every 2 weeks by cheek blead. Serum was collected for 28 weeks post radiation recovery (5 WT>SCARF1 and 5 SCARF1>WT). N=5 female mice

To determine the immune activation state in mice, we will analyzed the numbers of immune cells in the spleen of the chimeric mice. Seven months post-radiation, mice were euthanized and spleens were harvested and stained for flow cytometry. Data shows increase immune cell activation of SCARF1→WT chimeras with increased $CD4^+CD44^+CD25+$ T cells and $CD19^+GL7^+$ B cells (data not shown). In addition, we perform histology of kidney, spleen and liver where we can observed increased glomeruli in the kidney and inflammation in the spleen (data not shown). Therefore, our data suggest that SCARF1 expressed on hematopoietic cells is important in immune homeostasis and self-tolerance.

4. IMPACT

Characterizing the molecular mechanisms of AC removal will reveal novel biomarkers and identify new potential therapeutic strategies. Furthermore, the studies proposed in this application will provide insight into the general biology of AC removal, the consequences to the phagocyte, its residing tissue, and the organism. A better understanding of all the processes required for effective AC removal will most likely present new targets not only for lupus but for many autoimmune, metabolic, and infectious diseases.

5. CHANGES/CHALLENGES/PROBLEMS

During the funding period of 10/01/2022-9/30/2013 the laboratory encounters small challenges, however our group managed to make progress on a modified SOW. Changes in personnel, that required training in working with mouse models and additional training for the techniques routinely performed in the lab caused some delay. However, once the person got comfortable working in the bench and working with mice, new and exciting data was produced. Furthermore, currently the department of rheumatology is the beginning stages of generating a SLE biobank. Therefore, a new cohort from UMass Chan was not available for the assays performed. We used our frozen

samples to the data produced for Aim 1. Even with the challenges encounter during this founding year, the Ramirez-Ortiz lab make significant progress for Aim 1 and Aim 2.

6. PRODUCTS

Nothing to report.

7. PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

Nothing to report.

9. APPENDICES

Reference:

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4. Jorge, A.M., et al., *SCARF1-Induced Efferocytosis Plays an Immunomodulatory Role in Humans, and Autoantibodies Targeting SCARF1 Are Produced in Patients with Systemic Lupus Erythematosus.* J Immunol, 2022.