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TITLE: Photosensitivity and Lymph Node Immune Responses in Lupus

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Patients with systemic lupus erythematosus (SLE) are photosensitive, demonstrating an increased skin sensitivity to ultraviolet radiation (UVR) whereby even ambient exposure to sunlight can result in the development of inflammatory skin lesions. Advances have provided insight into both skin-intrinsic and immune cell-stromal contributions to photosensitivity, but, beyond the skin, photosensitive skin responses can trigger systemic disease flares, resulting in increased circulating autoantibodies which can deposit in and further injure end organs (1-3).

The mechanisms by which photosensitive responses can lead to systemic disease flares are not well understood. Our **long-term goal** is to delineate the mechanisms that connect photosensitivity with autoimmunity; as such, **our goal with this proposal** is to examine how SLE skin influences draining lymph node function. Interstitial fluid from skin drains via lymphatic vessels into sentinel lymph nodes where lymph fluid is channeled into a conduit system that provides the structural framework of the lymph node and is a distinct compartment from the parenchyma where T and B cells are located.

15. SUBJECT TERMS

Lupus
Photosensitivity

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1. INTRODUCTION:

Patients with systemic lupus erythematosus (SLE) are photosensitive, demonstrating an increased skin sensitivity to ultraviolet radiation (UVR) whereby even ambient exposure to sunlight can result in the development of inflammatory skin lesions. Advances have provided insight into both skin-intrinsic and immune cell-stromal contributions to photosensitivity, but, beyond the skin, photosensitive skin responses can trigger systemic disease flares, resulting in increased circulating autoantibodies which can deposit in and further injure end organs (1-3). The mechanisms by which photosensitive responses can lead to systemic disease flares are not well understood. Our **long-term goal** is to delineate the mechanisms that connect photosensitivity with autoimmunity; as such, **our goal with this proposal** is to examine how SLE skin influences draining lymph node function. Interstitial fluid from skin drains via lymphatic vessels into sentinel lymph nodes where lymph fluid is channeled into a conduit system that provides the structural framework of the lymph node and is a distinct compartment from the parenchyma where T and B cells are located. The conduit system is ensheathed by fibroblastic reticular cells (FRCs) that are among the first lymph node cells to directly sense lymph fluid signals and then, in turn, regulate lymphocytes function accordingly. Going into this project, we had data that, in SLE murine models, a subset of FRCs that normally limit plasmablast survival are activated and are prone to dying with UVR exposure of the skin. Our project thus tests the **hypothesis** that photosensitivity is associated with systemic autoimmunity at least in part because excess interferons (IFNs) and other signals from SLE skin activate FRCs and sensitize them for cell death upon UVR exposure of skin, resulting in loss of FRC-mediated plasmablast regulation. We are approaching this project by **1)** Understanding the signals from SLE skin that contribute to activation of lymph node FRCs, **2)** Understanding the role of UVR-induced lymph node stromal modulation in promoting plasmablast accumulation and map FRC state alterations in SLE models.

2. KEYWORDS:

Lupus
Photosensitivity
Skin
Lymph node
Fibroblastic reticular cells
Lymphatics
Autoimmunity
Ultraviolet radiation
Interferon

3. ACCOMPLISHMENTS:

A. What were the major goals of the project:

Aim 1) Understand the signals from SLE skin that contribute to activation of lymph node FRCs.

Aim 2) Understand the role of UVR-induced lymph node stromal modulation in promoting plasmablast accumulation and map FRC state alterations in SLE models.

B. What was accomplished under these goals

Aim 1: Understand the signals from SLE skin that contribute to activation of lymph node FRCs.

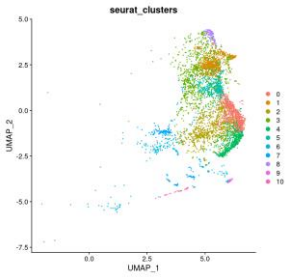
1. Major Task 1. Identify the IFN(s) that contribute to lymph node FRC activation and UVR-induced FRC loss in SLE models
    a. Subtask 1. Treat SLE model mice with IFN blocking antibodies and examine FRC activation state (timeline 6-30 months; status: on track). We have begun these experiments, blocking with anti-IFNAR. Preliminary results suggest that anti-IFNAR reduces the UVR-induced FRC loss in SLE model mice.
2. Major Task 2. Asses the sufficiency of IFN from the skin in activating lymph node FRCs by injecting IFN into skin and assess lymph node FRCs
    a. Subtask 1. Inject IFN into skin and assess lymph node FRC phenotype (timeline 6-9 months; status: on track with slight delay). We have performed these experiments and will finish replicate experiments in the coming weeks. Results suggest that IFN-I is sufficient to activate lymph node FRCs.
3. Major Task 3. Assess the extent to which interstitial fluid from human SLE vs healthy control skin can sensitize FRCs in vitro
    a. Subtask 1. Obtain interstitial fluid from SLE patients and healthy controls (timeline 6-30 months; status: delay in initiation). We have changed the site where we obtain the human skin interstitial fluid. The original plan was to collect at my home institution at HSS and we changed it to obtaining de-identified samples from co-investigator Dr. Rashighi at University of Massachusetts (as recommended for approval per Ms. Kimberly Carter, Grants Management Specialist, of 11/14/22). This is because Dr. Rashighi has a center for collecting fluid more efficiently. We have completed the Material Transfer Agreement (MTA) with the University of Massachusetts, and, with the MTA complete, my IRB has just approved the protocol for this exempt study. We will be submitting the OHRO forms within the next week. Collection will start shortly after OHRO review and approval.
    b. Subtask 2. Culture FRCs in preparation to set up vitro experiments assessing the role of IFNs and other mediators in SLE interstitial fluid that activates FRCs (timeline 1-30 months; status: on track). We have successfully set up the system using murine cells and observe that we can indeed assess differential effects of murine SLE interstitial fluid on murine FRC activation.
4. Major Task 4. Analyze the molecular composition of SLE vs healthy control skin interstitial fluid by mass spectrometry to better understand the nature of the signals that are transmitted from skin to the immune system in SLE
    a. Subtask 1. Mass spectrometry of interstitial fluid from SLE patients and healthy controls (timeline 6-24 months; status: delay in initiation because of delay in Major Task 3). We will perform the assay once we collect the interstitial fluid samples.

Aim 2: Understand the role of UVR-induced lymph node stromal modulation in promoting plasmablast accumulation and map FRC state alterations in SLE models.

1. Major Task 1. Assess the role of stromal CCL2 loss in UVR-induced plasmablast accumulation by lymph node transplantation in context of IMQ model.

a. Subtask 1. Do lymph node transplantations, CCL2 KO→WT and WT→WT (timeline 6-12 months; status: on track). We are currently doing the transplantations.

2. Major Task 2. Understand the extent to which FRCs in IMQ and MRL/lpr mice are more prone to stress-induced apoptosis
   a. Subtask 1. Inject cytokines such as TNF and IL-1 intradermally and examine lymph node FRC apoptosis (timeline 3-15 months; status: on track). We are in the process of doing these experiments.
3. Major Task 3. Map the scope of phenotypic alterations in FRC subsets in SLE model lymph nodes compared to non-lupus controls
   a. Subtask 1 Sort FRCs (timeline 6-12 months; status: on track). We have completed this.
   b. Subtask 2 Make scRNAseq libraries (timeline 12-15 months; status: ahead of schedule). We have completed this.
   c. Subtask 3 Sequencing (timeline 15-18 months; status: ahead of schedule). We have completed this and are just beginning the analysis. Shown is a UMAP of multiple FRC populations in SLE and non-lupus lymph nodes:



C. What opportunities for training and professional development has the project provided? Nothing to Report.

D. How were the results disseminated to communities of interest? We have not yet disseminated the results in this first year of the project, but we will disseminate in the form of publications, conference presentations. That we are working on this project has been disseminated by the DOD in the announcement of the grant recipients and my institution and I have further disseminated this information via social media forums. The publicizing of ongoing research is important in letting the patient communities know that something is being done.

E. What do you plan to do during the next reporting period to accomplish the goals?
   a. Aim 1.
      i. Continue ongoing experiments treating SLE mice with interferon blocking antibodies and examining FRC state. (Major Task 1).
      ii. Complete ongoing experiments assessing sufficiency of IFN in activating FRCs (Major Task 2).
      iii. Complete the OHRO forms and obtain interstitial fluid from collaborator. (Major Task 3, subtask 1).

iv. Assess the role of interferon in skin fluid modulation of FRC phenotype using the murine system. (Major Task 3, subtask 2).
v. Set up the parallel human in vitro skin fluid-FRC culture system. (Major Task 3, subtask 2).
vi. Begin the proteomic assay of human SLE skin interstitial fluid. (Major Task 4, subtask 1).
vii. Begin the proteomic analysis of human SLE skin interstitial fluid. (Major Task 4, subtask 2).

b. Aim 2.
i. We will induce the IMQ model in the lymph node transplanted mice. (Major task 1, subtask 2).
ii. Complete experiments examining the effect of cytokines on FRC apoptosis. (Major Task 2, subtask 1).
iii. Analyze the murine SLE lymph node FRC single cell RNAseq analysis. (Major Task 3, subtask 4).

4. IMPACT

a. What was the impact on the development of the principal disciplines(s) of the project? The impact on the discipline of lupus research will be better understood once we are near completion of our study. However, impact in terms of more discussion in the field of lupus pathogenesis is already being felt as I have been invited to write a review on aspects of photosensitivity and connection to systemic autoimmunity and to co-organize a mini-symposium on lupus skin disease as part of the Lupus 21st Century Meeting. These are all opportunities to raise awareness of both the need and what we are doing about it.

b. What was the impact on other disciplines? The impact on other disciplines will be better understood once we near completion of the study. Our study is interdisciplinary in that it touches upon both the immune system and the lymphatic vascular system (in addition to lupus), and our study is anticipated to impact multiple disciplines.

c. What was the impact on technology transfer? Nothing to report.

d. What was the impact on society beyond science and technology? Because this project has been publicized by the DOD and, subsequently, by my institution and myself on social media, patients and families are aware that this research is going on. When I have heard from lupus patients who hear about our research, they are also grateful that someone is addressing their issue and hopeful for results that will help them. Giving lupus patients hope is immeasurable and we look forward to giving them more hope when our study is complete.

5. CHANGES/PROBLEMS

a. **Changes in approach and reasons for change.** As discussed above, since our original proposal, we have changed the site where we obtain the human interstitial fluid. The original plan was to collect at my home institution at HSS and we changed it to obtaining de-identified samples from co-investigator Dr. Rashighi at University of Massachusetts

(as recommended for approval per Ms. Kimberly Carter, Grants Management Specialist, of 11/14/22). This is because Dr. Rashighi has a center for collecting fluid more efficiently. We have completed the Material Transfer Agreement (MTA) with the University of Massachusetts, and, with the MTA complete, my IRB has just approved the protocol for this exempt study. We will be submitting the OHRO forms within the next week. Collection will start shortly after OHRO review and approval.

b. Actual or anticipated problems or delays and actions or plans to resolve them. Because the change in the source of patient samples necessitated additional paperwork, we are delayed in obtaining patient samples. However, we anticipate sample collection to begin shortly, and that the efficiency of Dr. Rashighi's infrastructure for collecting skin interstitial fluid for research purposes will make up for the delay.

c. **Changes that had a significant impact on expenditures.** None.

d. Significant changes in use or care of human subjects, vertebrate animals, biohazards and /or select agents. None.

e. **Significant changes in use or care of human subjects.** As discussed above, we have changed the site from where we obtain human skin interstitial fluid.

f. Significant changes in use or care of vertebrate animals. None.

g. Significant changes in use or biohazards and/or select agents. None.

6. PRODUCTS

a. Publications, conference papers, and presentations

i. Journal publications. None yet.

ii. **Books or other non-periodical , one-time publications.** None.

iii. Other publications, conference papers, and presentations. None.

b. Website(s) or other Internet site(s). None.

c. Technologoes or techniques. None yet.

d. Inventions, patent applications, and/or licenses. None.

e. Other products. None.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

a. **What individuals have worked on the project?**

Name	Theresa Lu
Role	PI
Nearest person-month worked	1
Contribution to project	Oversees all aspects
Funding support	

Name	Laura Santambrogio
Role	Collaborator
Nearest person-month worked	0
Contribution to project	Proteomic analysis
Funding support	

Name	Hafsa Munir
Role	Postdoctoral fellow
Nearest person-month worked	12
Contribution to project	Murine model work, scRNAsequencing
Funding support	

b. **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?** Nothing to report.

c. **What other organizations were involved as partners?**

i. **Organization Name:** Weill Cornell Medicine
Location of Organization: New York, NY
Partner's contribution to project: In kind support: Collaboration

ii. **Organization Name:** University of Massachusetts
Location of Organization: Worcester, MA
Partner's contribution to project: In kind support: Collaboration

8. SPECIAL REPORTING REQUIREMENTS Not applicable

9. APPENDICES None