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TITLE: Modeling Lupus Nephritis in a Human Kidney Microphysiological System

PRINCIPAL INVESTIGATOR: Jonathan Himmelfarb, MD

CONTRACTING ORGANIZATION: University of Washington, Seattle

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Fort Detrick, Maryland 21702-5012

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Distribution Unlimited

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6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

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

University of Washington
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4333 Brooklyn Ave NE
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Seattle, WA 98195-9472

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U.S. Army Medical Research and Development Command
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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The kidney is highly susceptible to injury from many systemic inflammatory and autoimmune diseases, including systemic lupus erythematosus (SLE), which is one of the FY19 CDMRP Topic Area. Among people with SLE, kidney involvement (lupus nephritis) affects 60%-80% of SLE patients and is recognized as a main contributor to morbidity and mortality. End stage renal disease occurs in ~25% of SLE patients within 15 years of onset, even with modern day immunosuppressive treatment. We have developed a three-dimensional flow directed "kidney-on-a-chip" populated with human kidney cells, with functional characterization of key component structures of the kidney cortical tubulo-interstitium. In Year 2, we continued our work from Year 1, and focused on major task 1 and major task 2, and have resolved the technical hurdles in the matrix variability to faithfully recapitulate kidney microvessel on a chip. Our overall goal is to use these pre-clinical studies to discover more effective strategies for treating and curing individual patients with SLE, so that clinical studies can then be conducted to improve their quality and quantity of life.

15. SUBJECT TERMS

Systemic Lupus Erythematosus (SLE), Lupus Nephritis, Kidney Disease, End-stage Renal Disease

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
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Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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1) INTRODUCTION:

Background: Kidney disease, i.e. lupus nephritis, affects 60%-80% of SLE patients, and is a main contributor of morbidity and mortality. Development of lupus nephritis increases morbidity and mortality 26-fold compared with age and gender matched healthy people. End stage renal disease occurs in ~25% of patients within 15 years of onset, even with current treatment, and the extent of kidney tubulo-interstitial injury has been strongly correlated with the severity of lupus nephritis and demonstrated to be of great prognostic import in determining lupus nephritis outcomes. Unfortunately, animal models cannot disentangle whether abnormalities identified are cause or effect. Furthermore, they fail to account for the heterogeneity of clinical SLE nephritis because of human genetic variation that contributes both to immune dysfunction as well as end organ susceptibility.
Rationale: *In vitro* models that recapitulate critical aspects of kidney physiology, assess the mechanisms and response to injury, and test reparative mechanisms could substantially enhance therapeutic discovery. We have developed three-dimensional flow directed "kidney-on-a-chip" populated with human kidney cells, with functional characterization of key component structures of the proximal tubule and the peritubular microvascular network as an integrated unit. Strengths of the kidney on a chip include: 1) minimized contact of living cells with artificial materials; 2) living perfusable microvasculature and tubules; 3) interstitial compartments that permit flow and are comprised of modifiable extracellular matrix which can be populated with tissue-specific resident immune cells; 4) proven fidelity to multiple aspects of human renal physiology and pathophysiology. Use of this 'human kidney on a chip' has been successful in developing robust in vitro models of multiple kidney diseases that recapitulate critical aspects of kidney physiology, assess the mechanisms and response to injury, and test reparative mechanisms. We believe that this approach to understanding the pathobiology of lupus nephritis can substantially enhance progress towards disease understanding and ultimately lead to more precise, individual therapeutic approaches, and ultimately to cures.
Hypothesis: The extent of tubule-interstitial injury has been strongly correlated with the severity of lupus nephritis and demonstrated to be of great prognostic import in determining lupus nephritis outcomes (ref 42,77.) The overall hypothesis of this proposal is that the initiating mechanisms leading to kidney damage in SLE can be accurately identified in a kidney on a chip system with defined components that recapitulate in vivo biology.
Specific Aim #1: Modeling microvascular interactions with immune cells and humoral factors as initiating tubulo-interstitial disease in lupus nephritis. We will integrate immune cells and humoral factors into an existing flow directed, three-dimensional human kidney biomimetic microvascular network system in order to model the role of microvascular injury in initiating

tubulo-interstitial disease in lupus nephritis.
Specific Aim #2: Use of a Novel Renal Vascular Tubular Unit to Create an Integrated MPS Model of Lupus Nephritis. We recently reported on the first fully tunable human kidney-on-a-chip platform, which allows the reconstruction of the native architecture of the renal cortical endothelial-epithelial exchange interface using entirely cell-remodelable matrix and patient-derived kidney cells. We will perfuse the tunable platform with the relevant cells, antibodies and / or soluble factors identified in Aim 1 and quantify tubulo-interstitial damage by immunologic, proteomic and RNA transcription profiles as well as biomarkers of kidney injury. We will determine the phenotypes and function of cell types attracted to tubules following injury.
Short-term impact: A better understanding of the pathobiology of human lupus nephritis by (i) creating an integrated microphysiological model of lupus nephritis that is cross validated for clinical-pathological correlation with human clinical findings, and (ii) selectively accounting for the role of immune cells, autoantibodies, complement, immune complexes, chemokines, cytokines and growth factors in initiating kidney injury in SLE.
Long-term impact: Identification of individual pathways and mediators of tubulo-interstitial kidney injury that account for patient heterogeneity and allow rapid translation of the findings from bench to bedside for patients with lupus nephritis.
Relevance of project to FY19 CDMRP Topic Area: Lupus Research Program (LRP) is one of the target areas. The LRP mission is to fund research to understand, prevent, and diagnose lupus and to improve treatments and quality of life of patients, including Service members, Veterans, and beneficiaries. With the overall objective of identifying better treatment strategies, this proposal addresses a critical need in lupus research, exemplified by the focus areas aimed at "understanding disease mechanisms and determining the pathobiology of lupus disease in target human tissues".

2) KEYWORDS: Systemic Lupus Erythematosus (SLE), Lupus Nephritis, Kidney Disease, End-stage Renal Disease, Human kidney microvascular endothelial cells, Microphysiological Systems (MPS), Neutrophils

3) ACCOMPLISHMENTS:

a. What were the major goals of the project?

Specific Aim #1: Modeling microvascular interactions with immune cells and humoral factors as initiating tubulo-interstitial disease in lupus nephritis. We will integrate immune cells and humoral factors into an existing flow directed, three-dimensional human kidney biomimetic microvascular network system in order to model the role of microvascular injury in initiating tubulo-interstitial disease in lupus nephritis.

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and quantify tubulo-interstitial damage by immunologic, proteomic and RNA transcription profiles as well as biomarkers of kidney injury. We will determine the phenotypes and function of cell types attracted to tubules following injury.

b. What was accomplished under these goals?

1) Major Activities:

In this study, we investigated the interactions between neutrophils, isolated from healthy human donors, and human kidney endothelium in both 2D static cultures and 3D engineered kidney micro-vessels. These interactions were examined under various inflammatory stimuli and in the presence or absence of serum from both normal individuals and lupus nephritis patients. Over ten serum samples from lupus nephritis patients and five from healthy subjects have been analyzed through our kidney micro-vessel systems. Despite significant delays due to personnel shortages and inconsistencies in reagents during the COVID-19 pandemic, we successfully developed a framework and methodology to explore human kidney microvascular injury in lupus nephritis using a 3D human kidney microvascular physiological system. Our findings demonstrate that serum from lupus nephritis patients can alter the endothelial lumen, promoting neutrophil adhesion and activation. This effect is analogous to the outcomes observed when pre-activated neutrophils are perfused through the micro-vessels, yet it is distinct from the responses elicited by TNFα-treated micro-vessels.

2) Specific Objectives:

Our objective was to integrate immune cells and humoral factors into an existing flow directed, three-dimensional human kidney biomimetic microvascular network system, and model the role of microvascular injury in initiating tubulo-interstitial disease in lupus nephritis, and study consequent tubulo-interstitial damage after lupus serum is perfused through the micro-vessels.

3) Significant Results/Key Outcomes:

Establishing a framework with kidney microvascular MPS for studying vascular-immune cell interactions in lupus nephritis. In this project, we have developed

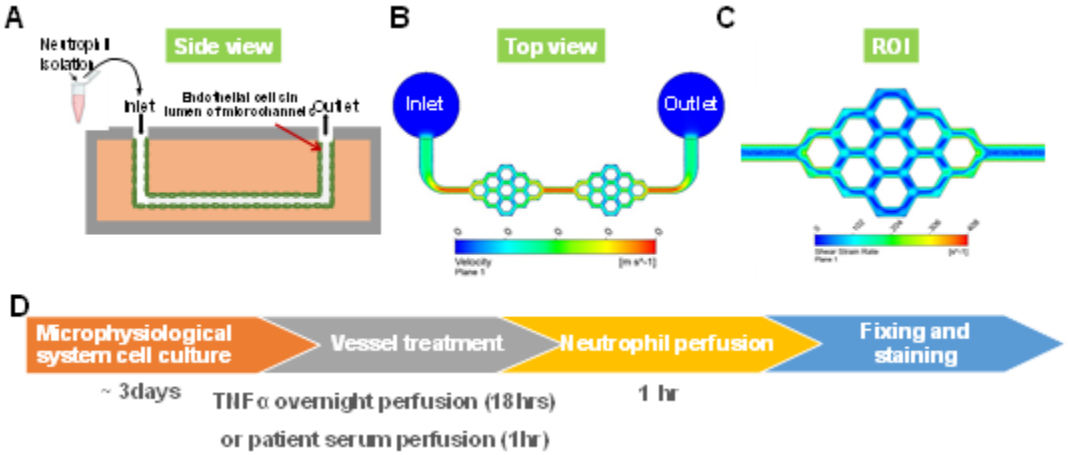

Figure 1. Framework of microphysiological system in the study of immune cell-kidney micro-vessel interactions in the context of lupus nephritis.

methods for perfusing neutrophils into the human kidney microvascular network to monitor cell adhesion and endothelial changes under flow conditions. A comprehensive framework has been established encompassing vessel fabrication, flow and shear simulation, and immune cell-vessel interactions to study the kidney vascular responses in lupus nephritis (Fig. 1).

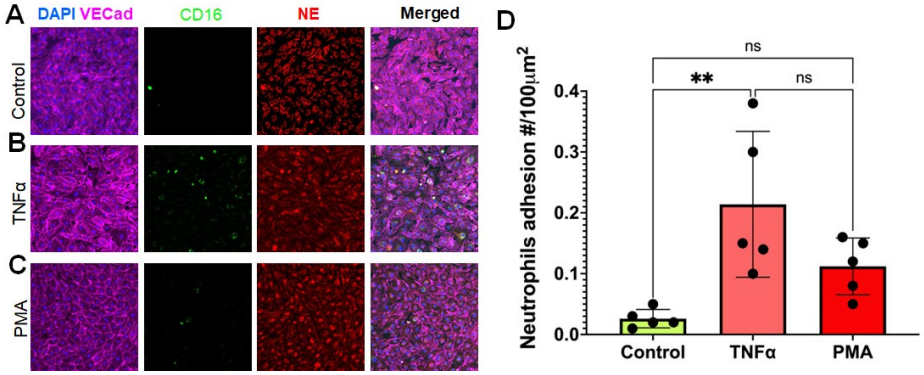

Figure 2. Static culture of kidney endothelial monolayer did not support NETs formation. A. Static condition did not form NETs. B. TNFa treatment promoted neutrophil adhesion. C.PMA treated neutrophil formed NETs but did not interfere with underlying endothelial monolayer. D. Quantification of neutrophil adhesion on HKMEC monolayer.

Our group previously developed techniques to isolate and characterize human kidney microvascular endothelial cells (HKMECs), revealing their distinctive properties. In static culture, these HKMECs form monolayers with robust junctions, as shown with VE-Cadherin staining (Fig. 2A). Treatment with either TNFα (10ng/mL) for 18 hours preserved the integrity of the endothelial monolayer, although it became more adhesive to neutrophils (Fig. 2B). No activation of

neutrophils or formation of neutrophil extracellular traps (NETs) was observed. Even when neutrophils were activated by PMA before plating on the endothelium, NETs were not observed on endothelium, and endothelium maintained robust junctions (Fig. 2C). Neutrophil elastase staining was positive across all endothelial monolayers without discernible difference in expression and morphology. Quantitative analysis of neutrophil adhesion under these conditions revealed a significant increase TNFα treated micro-vessels (Fig. 2D), but no significant differences between control and PMA treated conditions.

Upon perfusing neutrophils through HKMEC-formed micro-vessels, we observed that normal neutrophils exhibited negligible adhesion to the endothelial luminal side after 30 mins of perfusion (Fig. 3A). In vessels treated with TNFα (10ng/mL) for 18 hours, normal neutrophils adhered to the vessel wall with minimal activation (Fig. 3B), similar as what we observed in 2D interactions. However, when PMA-activated neutrophils were perfused (Fig. 3C), we noted significant functional alterations in the vessel lumen, including neutrophil elastase clumps obstructing flow. PMA, a known NET inducer, triggered neutrophil activation and DNA release into micro-vessel lumen, occasionally forming form transluminal structures extending for several millimeters.

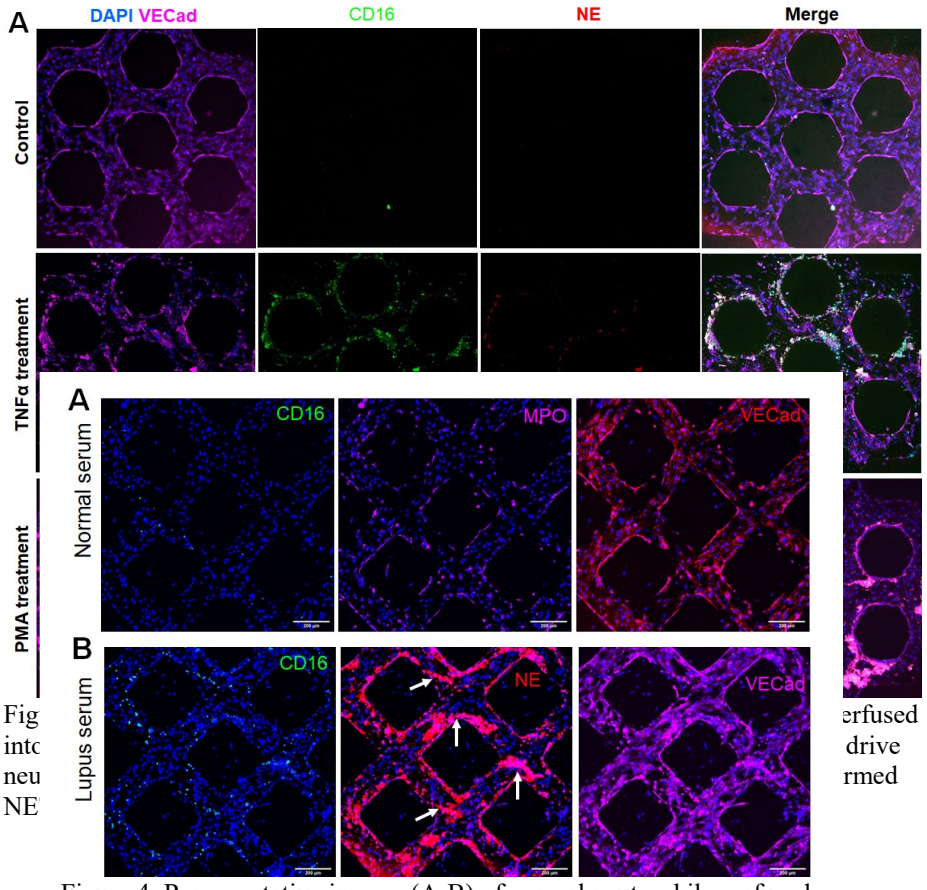

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Figure 4. Representative images (A-B) of normal neutrophils perfused through kidney micro-vessels after perfused with normal (A) and lupus (B) serum.

We also assessed the impact of human sera (11 SLE patients and 5 healthy subjects) on kidney micro-vessels, focusing on neutrophil adhesion and NET formation. Normal serum caused minimal changes to the vessel wall and scant neutrophil adhesion, whereas lupus serum altered the endothelium, leading to NETs formation (Fig. 4) with activated neutrophils interacting with the endothelium. Quantification across these five conditions in 3D micro-vessels highlighted significant differences in neutrophil adhesion in TNFα or PMA conditions to control. Lupus serum treated micro-vessels exhibited notably higher neutrophil adhesion; thus lupus serum directly induced endothelial changes that significantly activated neutrophils. NET quantification confirmed increased NETS in PMA-treated neutrophils within the micro-vessels, however, variability in serum conditions yielded non-significant results, although a trend suggested that lupus serum may increase NETs formation.

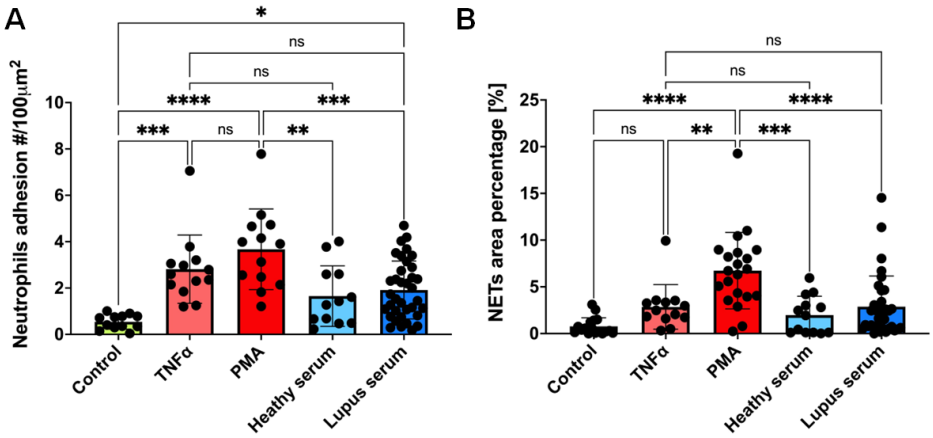

Figure 5. Quantification of NETs formation and neutrophil adhesion comparing all conditions.

In summary, our methods enabled the evaluation of neutrophil interactions with kidney specific micro-vessels, and the development of quantitative methods for NETs formation assessment. Future work can focus on molecular studies to elucidate mechanisms of kidney vascular injuries in these processes, potentially impacting the tubular and interstitial changes.

4) Other Achievements:

Nothing to report

c. What opportunities for training and professional development has the project provided?

In this project, we recruited Dr. Dongjune Kim as a postdoctoral fellow, and provided him with comprehensive training in critical laboratory techniques relevant to both the biology of lupus and to microphysiological systems. This includes the culture and characterization of HKMECs, along with vessel fabrication. Additionally, Dr. Kim was trained in conducting the full suite of experiments and was integral in the development

of our project framework. This opportunity has significantly contributed to his professional development in the field.

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

A manuscript describing the initial findings from this work is currently in preparation.

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

N/A

4) IMPACT:

a. What was the impact on the development of the principal discipline(s) of the project?

We have developed the conditions to analyze the effects of serum on the renal vasculature in the MPS system. We have also shown, to our knowledge for the first time, that we can observe both neutrophil adhesion and NET formation in a human kidney MPS system with organotypic vasculature. These landmark developments will allow us in future work to compare the effects of serum and cells from patients with and without kidney injury as outlined in the proposal. These findings could have a major impact on our understanding of the mechanisms responsible for tubulointerstitial nephritis.

b. What was the impact on other disciplines?

Nothing to report.

c. What was the impact on technology transfer?

Nothing to report.

d. What was the impact on society beyond science and technology?

Nothing to report.

5) CHANGES/PROBLEMS:

a. Changes in approach and reasons for change

We have not made major changes to the approach outlined in the approved SOW updated in December 2020.

b. Actual or anticipated problems or delays and actions or plans to resolve them

In this project, we encountered some delays in making progress due to two major reasons: 1) COVID - personnel sickness. As COVID-19 persisted, University restrictions coupled with personnel sickness hindered our progress. The average number of experiments we could run per unit time was much lower then what we could do without COVID. Also, the COVID pandemic considerably reduced the numbers of patients in clinic making it more difficult to obtain fresh samples from SLE patients. 2) Collagen issues: We had significant technical problems in collagen isolation during the past year which resulted in significant cell death in micro-vessels, a problem which we were eventually able to overcome. Nevertheless, we successfully developed a framework and methodology to explore human kidney microvascular injury in lupus nephritis using a 3D human kidney microvascular physiological system. Our findings demonstrate that serum from lupus nephritis patients can alter the endothelial lumen, promoting neutrophil adhesion and activation. This effect is analogous to the outcomes observed when pre-activated neutrophils are perfused through the micro-vessels, yet it is distinct from the responses elicited by TNFα-treated micro-vessels.

c. Changes that had a significant impact on expenditures

Nothing to report.

d. Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

As requested by Allison McClean, Human Subjects Protection Scientist with the Human Research Protection Office, we obtained UW IRB concurrence that the use of residual samples from Dr. Elkon's existing study, STUDY00001145, does not meet the definition of human subjects activity. This IRB determination letter was provided to Allison and uploaded to eBRAP.

e. Significant changes in use or care of human subjects

The change in the IRB approved study that will serve as the source of human subjects, from Keith Elkon's study to Benjamin Freedman's study, has already been communicated and approved through the updated SOW dated December 2020.

f. Significant changes in use or care of vertebrate animals

Nothing to report.

g. Significant changes in use of biohazards and/or select agents

Nothing to report.

6) PRODUCTS:

a. Publications, conference papers, and presentations

A manuscript describing the model and initial findings is currently in preparation.

b. **Website(s) or other Internet site(s)**

Nothing to report.

c. **Technologies or techniques**

Nothing to report.

d. **Inventions, patent applications, and/or licenses**

Nothing to report.

e. **Other Products**

Nothing to report.

7) PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

a. What individuals have worked on the project?

Name: Jonathan Himmelfarb, MD
Project Role: PI
Research Identifier (eRA Commons): JHIMMELFARB
Nearest Person Months Worked: .48
Contribution to the Project: Dr. Himmelfarb is the PI at the University of Washington. He has coordinated the research, planned experiments, and interpreted data.
Funding Support: this project

Name: Keith Elkon, MD
Project Role: Co-Investigator
Nearest Person Months Worked: .22
Contribution to the Project: Dr. Elkon oversees all the immunological experiments. He has collaborated with all investigators on the project, planned experiments, and interpreted data.
Funding Support: this project

Name: Ying Zheng, PhD
Project Role: Co-Investigator
Nearest Person Months Worked: 0

Contribution to the Project: Dr. Zheng oversees experiments intended to study the interaction of SLE serum and cellular components with kidney endothelium in 2D monolayer and 3D microvessels under perfusion.
Funding Support: this project

Name: Christian Lood, PhD
Project Role: Co-Investigator
Nearest Person Months Worked: 0.2
Contribution to the Project: Dr. Lood assists with experiment design, isolation and characterization of immune cell subsets.
Funding Support: this project

Name: Benjamin Freedman, PhD
Project Role: Co-Investigator
Nearest Person Months Worked: .1
Contribution to the Project: Dr. Freedman is a leading expert in generating kidney organoids as well as directed cell differentiation, in each case from human pluripotent stem cells (hPSCs). His lab will supply hPSC derived kidney cells for the experiments of this project.
Funding Support: this project

Name: Jie An, PhD
Project Role: Senior Staff Scientist
Nearest Person Months Worked: 0.1
Contribution to the Project: Dr. An assists with QPCR, flow cytometry, and performs most of the immunofluorescence staining of immune cells in Keith Elkon's lab.
Funding Support: this project

Name: Xizhang Sun, PhD
Project Role: Research Scientist
Nearest Person Months Worked: .7
Contribution to the Project: Dr. Sun assists Dr. An with QPCR, flow cytometry, and assists with the immunofluorescence staining of immune cells in Keith Elkon's lab.
Funding Support: this project

Name: Ping Luo, PhD
Project Role: Research Scientist
Nearest Person Months Worked: 1.3
Contribution to the Project: Dr. Luo performs the experiments in studying SLE serum and cellular components interacting with kidney endothelium under flow in Dr. Zheng's lab.
Funding Support: this project

b. What other organizations were involved as partners?

None.

8) **TRANSITION PLANS**
We plan to continue to submit the results of this work for peer-reviewed publication.

9) INCLUSION ENROLLMENT REPORT-
Not applicable as this is not considered human subjects research

10) INVENTIONS
No inventions were made under this award.

11) EQUIPMENT & RESIDUAL SUPPLIES
No equipment was purchased on this award.
There are no residual supplies totaling $5,000 or more.

Federal Financial Report

(Follow form Instructions)

1. Federal Agency and Organizational Element to Which Report is Submitted	2. Federal Grant or Other Identifying Number Assigned by Federal Agency (To report multiple grants, use FFR Attachment)
USA MED RESEARCH ACQ ACTIVITY	W81XWH-20-1-0666

3. Recipient Organization (Name and complete address including Zip code)

Recipient Organization Name: University of Washington

Street1: 4300 Roosevelt Way NE

Street2: Box 354966

City: Seattle County:

State: WA Province:

Country: USA: United States ZIP / Postal Code: 98195-4966

4a. UEI	4b. EIN	5. Recipient Account Number or Identifying Number (To report multiple grants, use FFR Attachment)
HD1WMN6945W6	916001537	62-2312

6. Report Type	7. Basis of Accounting	8. Project/Grant Period	9. Reporting Period End Date
☐ Quarterly	☐ Cash	From: 7/15/2020 To: 7/14/2023	7/14/2023
☐ Semi-Annual	☒ Accrual		
☐ Annual			
☒ Final			

10. **Transactions**	Cumulative
(Use lines a-c for single or multiple grant reporting)	
Federal Cash (To report multiple grants, also use FFR attachment):	
a. Cash Receipts	503,437.41
b. Cash Disbursements	503,437.41
c. Cash on Hand (line a minus b)	0.00
(Use lines d-o for single grant reporting)	
Federal Expenditures and Unobligated Balance:	
d. Total Federal funds authorized	525,000.00
e. Federal share of expenditures	503,437.41
f. Federal share of unliquidated obligations	0.00
g. Total Federal share (sum of lines e and f)	503,437.41
h. Unobligated balance of Federal Funds (line d minus g)	21,562.59
Recipient Share:	
i. Total recipient share required	0.00
j. Recipient share of expenditures	0.00
k. Remaining recipient share to be provided (line i minus j)	0.00
Program Income:	
l. Total Federal program income earned	0.00
m. Program Income expended in accordance with the deduction alternative	0.00
n. Program Income expended in accordance with the addition alternative	0.00
o. Unexpended program income (line l minus m or line n)	0.00

11. Indirect Expense

a. Type	b. Rate	c. Period From	Period To	d. Base	e. Amount Charged	f. Federal Share
Provisional	0.765	7/15/2020	7/14/2023	285,233.66	218,203.75	218,203.75
			g. Totals:	285,233.66	218,203.75	218,203.75

12. Remarks

Cash Receipts, line 10a, includes pending final payment(s) due totaling $4,957.61.
Total Exemptions: 0.00

13. Certification: By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the Federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil or administrative penalties for fraud, false statements, false claims or otherwise. (U.S. Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

a. Name and Title of Authorized Certifying Official

Prefix: First Name: Juan Middle Name:

Last Name: Lepez Suffix:

Title: Director, GCA

b. Signature of Authorized Certifying Official

Digitally signed by Juan Lepez
Date: 2023.11.04 09:32:56
-07'00'

c. Telephone (Area code, number and extension)

206-616-9995

d. Email address

gcahelp@uw.edu

e. Date Report Submitted

11/4/2023

14. Agency use only: