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PRINCIPAL INVESTIGATOR: Anne Davidson

CONTRACTING ORGANIZATION: Feinstein Institute for Medical Research, Manhasset, NY

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13. SUPPLEMENTARY NOTES

14. ABSTRACT: Lupus nephritis (LN) is a common manifestation of systemic lupus erythematosus that can lead to irreversible renal impairment. Immunosuppressive therapies fail to reverse disease in more than half of treated patients and the rate of progression to renal failure has plateaued in the USA in the past 20 years. This failure in part reflects disease complexity and heterogeneity. We have previously found in animal models that macrophages are involved in renal injury in LN. Macrophages are highly plastic and can mediate both pro-inflammatory and reparative functions. In human LN the presence of macrophages in renal biopsies correlates with poor prognosis. We propose to identify human-relevant myeloid cell subsets in lupus models that reflect differences in disease pathophysiology, disease stage and responsiveness to treatments; this information will help identify new pathways for therapeutic intervention and direct personalized treatment. In Aim 1 we will use single cell RNASeq analysis to define clusters of myeloid cells in 5 different models of LN and integrate these data with that from AMP Phase 2 to identify both shared and unique macrophage sub-populations and determine whether the presence of any of these correlates with responder status. In Aim 2 we will follow the fate of macrophages as they transition from the blood into the kidneys and harvest them at intervals in order to define how subsets are related to each other and the nephritis specific profile of each subset. In Aim 3 we will follow the fate of renal myeloid cells (in responder and non-responder strains and responder and non-responder mice within strains) in human-relevant mouse models treated with remission induction and to maintenance therapies. These studies are a necessary first step towards mechanistic studies aimed to promote novel approaches and therapies that prevent or treat LN. These studies will also set the stage for development of methods to delete sub-populations of pathogenic cells or modulate their function so that they protect the kidney rather than damage it.

15. SUBJECT TERMS: SLE, macrophages, autophagy, Rubicon, ATG14, PGC1alpha, single cell genomics, lupus nephritis, lysosome associated phagocytosis

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INTRODUCTION:

Lupus nephritis is a devastating complication of Systemic Lupus Erythematosus for which there is currently no satisfactory treatment. The last few years have seen advancements in therapeutics for lupus but there are still only two new drugs approved for lupus nephritis and neither achieves complete remission in more than 60% of patients. Renal macrophages are associated with poorer outcomes in lupus nephritis. Renal macrophages comprise both resident cells and bone marrow derived infiltrating cells. Macrophages are highly heterogeneous and plastic cells and can be involved in both tissue injury and repair. Therefore, a better understanding of the heterogeneity of these cells in the kidneys of patients with lupus nephritis and a determination of their function, whether pathogenic or protective, is needed in order to target them therapeutically.

KEYWORDS: SLE, macrophages, single cell transcriptomics, lupus nephritis

ACCOMPLISHMENTS:

What were the major goals of the project?

1. To identify subsets and functional pathways in renal myeloid cells that are similar in mouse and human LN: We will compare scRNASeq data of renal myeloid cells from 5 different models of LN with myeloid cell data from 160 human LN biopsies (AMP Phase 2) to characterize the full spectrum of renal myeloid cells in LN and to determine which mouse model best reflects heterogeneous human states.

2. To identify functional pathways in myeloid cells that change with renal entry: We will examine the myeloid cell transitions by performing bone marrow transfers in partially irradiated mouse models of interest and performing scRNASeq on transferred congenic renal myeloid cells at intervals after transfer. We will identify similar states of cells in the human samples from the AMP Phase 2 cohort and determine how transition states correlate with disease stage and clinical outcomes.

3. To identify functional pathways in myeloid cells that change with treatment: Using relevant disease models, we will determine which macrophage subsets disappear from the kidneys or alter their function in responders and non-responders to remission induction with or without maintenance therapy with either standard MMF/steroids or with the addition of BAFF-R-Ig or TACI-Ig (belimumab/atacicept equivalents). We will then determine whether the frequency of the analogous myeloid cell subsets or presence of analogous activation pathways in LN can predict treatment outcome by analyzing responders and non-responders in the AMP Phase 2 cohort.

<table>
<tr><td>Major Tasks Specific Aim 1

Molecular characterization of single cells from 5 LN models</td><td>Single cell RNASeq and data processing</td></tr>
<tr><td colspan="2">Tasks accomplished: We completed the analysis of our first four mouse models and have obtained the 5th model and are breeding sufficient mice for this experiment.</td></tr>
<tr><td>Major Tasks Specific Aim 2

To identify pathways in myeloid cells that change with renal entry</td><td>We initially planned to do this by making bone marrow chimeras but this has turned out to be difficult since the degree of chimerism is not sufficient with irradiation of just the tail. We have therefore developed two other approaches

1. Exchange transfusion: For this we needed to breed NZW/Yaa mice that are CD45.1 as these mice have large numbers of circulating macrophages and reliably develop nephritis at a fixed age. These mice are now ready and IACUC approval has been obtained. We have identified a surgical colleague to do the transfusions for us

2. Stem cell transplant. Here we use anti-CD117 and streptavidin toxin to kill stem cells allowing repopulation by transferred CD45.1 stem cells. We have established a collaboration with Dr Czechowicz at Stanford who established this protocol in her lab and are currently optimizing the procedure for our mice.</td></tr>
<tr><td colspan="2">Tasks accomplished: Mice bred and ready for experiments. Protocol optimization in process</td></tr>
<tr><td>Major Task 3 Specific Aim 3

To identify functional pathways in myeloid cells that change with treatment</td><td>Bulk reagents have been generated

Mice are aging for intervention</td></tr>
</table>

What was accomplished under these goals?

Aim 1: Extensive bioinformatic analyses have now been completed on all 4 lupus strains and comparisons made with human data from AMP Phase 1 and AMP Phase 2. This includes trajectory analysis and transcription factor analysis.

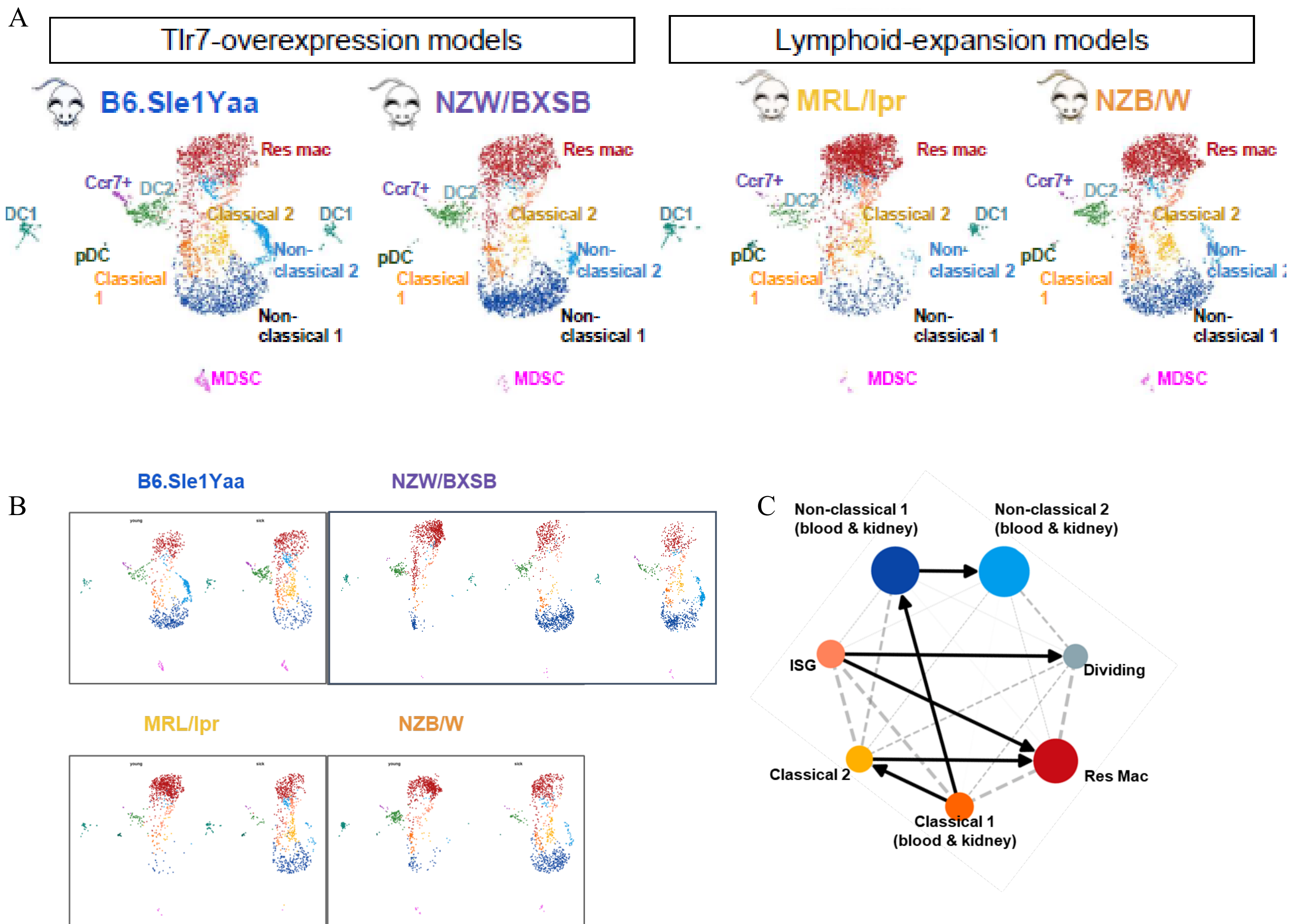


Figure 1: A: UMAPs of myeloid cells from 4 models of lupus nephritis. Corresponding clusters in each strain are colored the same way in each UMAP. B: Comparison of young (left) and nephritic (right) mice of each strain shows cell infiltration over time. C: trajectory analysis shows connections between Classical 1 and Classical 2 and non-classical 1 monocytes.

The AMP data comprises is a very rich dataset and it is clear that there are multiple myeloid subsets in human lupus kidneys. We previously reported using data from Phase 1 of the Accelerating Medicine Partnerships program (23 biopsies studied), there are at least 5 major myeloid subsets in lupus kidneys (CM0 – inflammatory CD16+ monocytes; CM1 - phagocytic monocytes; CM2 – resident population; CM3- dendritic cells; CM4 – alternatively activated reparative monocytes). Myeloid cell subsets have now been analyzed using the data from Phase 2 of the Accelerating Medicine Partnerships program. 155 renal biopsies were processed for the single cell analyses and >20,000 myeloid cells were obtained. These now separate into > 20 clusters with the major subsets being CD14+ monocytes, CD16+ monocytes (inflammatory and phagocytic), resident macrophages, and infiltrating DCs (cDC1 and cDC2). There is substantial overlap with the mouse models.

To complete the cross species analysis, we set up a collaboration with Soumya Ray Chaudhuri from the Brigham. Data has been compared with the human data from the AMP study using 2 tools from the Raychaudhury lab, Harmony and Symphony. Using these tools, the mouse data was mapped onto the human

data and analogous subsets were found. These were then correlated with disease activity and chronicity. We found that disease activity in humans is inversely correlated with the presence of Classical monocytes 1 and strongly correlated with the emergence of Classical Monocytes 2 in the kidneys. By trajectory analysis these cells are derived from Classical 1 monocytes. In mice, Classical Monocytes 2 are not found in the blood and are markedly increased in nephritic compared with young mice suggesting that they are generated locally in response to renal injury. Both these cell types are localized to glomeruli.

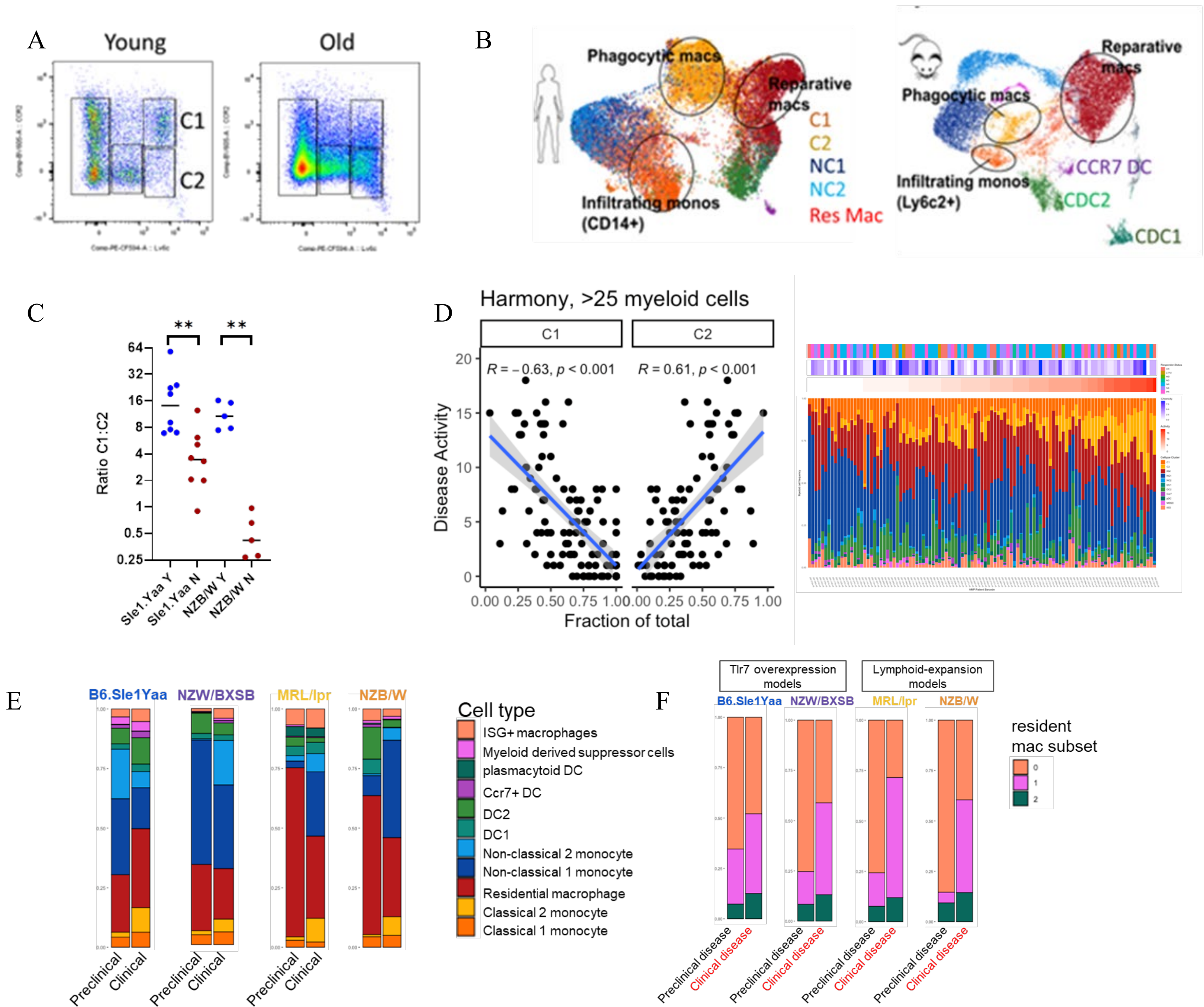


Figure 2: A: Flow cytometry characteristics of classical 1 and classical 2 macrophages. B: Parallel myeloid cell subsets in human and mouse LN kidneys. Corresponding subsets are colored the same way. C: Ratio of classical 1: Classical 2 monocytes in young and nephritic kidneys. D: Association of Classical 2 monocytes (yellow bars) with disease activity in human LN biopsies. E: Distribution of myeloid cell types in mouse kidneys. F: Distribution of resident macrophage subsets in mouse kidneys

We also discovered a novel monocyte subset that we have termed non-classical type 2 to distinguish it from the non-classical type 1 subset found in all 4 strains. This subset is localized in glomeruli and has a unique expression pattern with overrepresentation of genes involved in lipid synthesis and efflux. These cells derive from circulating non-classical Ly6C^{lo} patrolling monocytes by trajectory analysis and both are present in the blood. Our hypothesis, based on prior work by the Geissmann laboratory, is that overexpression of TLR7 in endothelial cells attracts these cells and that the y then adhere to the endothelium respond to ingestion of nucleic acid material from circulating immune cells and local apoptotic material by expressing a program induced by phagocytosis. Strikingly, classical 2 macrophages, but not the other subsets, are associated with

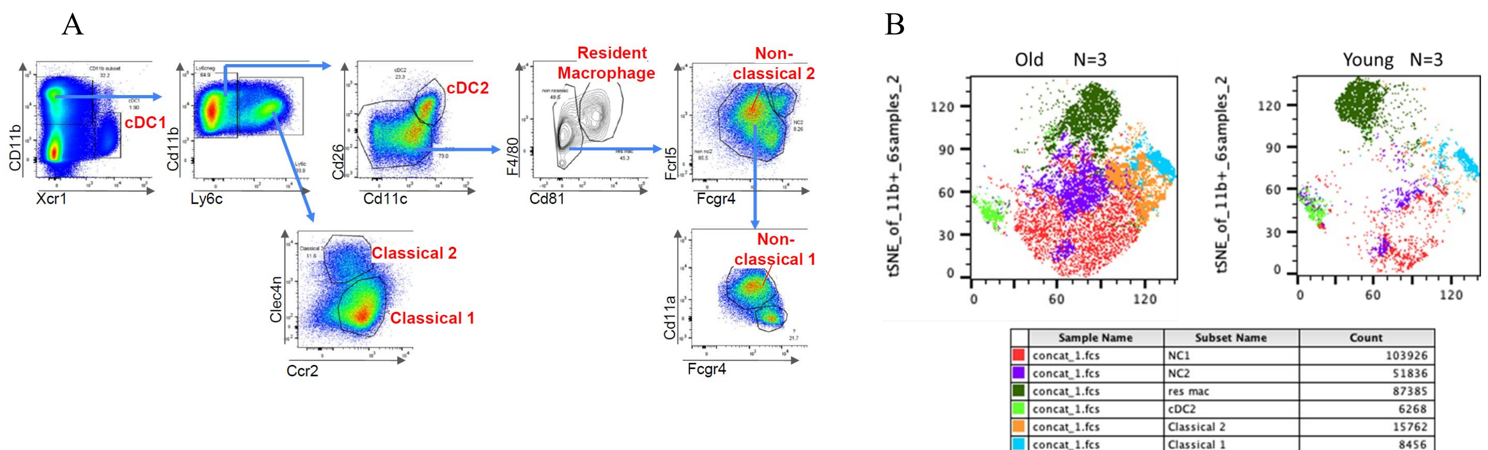
disease activity in both mice and humans, whereas resident macrophages and cDC2 are associated with chronicity.

Resident macrophages are found in the interstitium and peri-glomerular areas. We found that there is a subset of resident macrophages that expands in nephritic mice. In preclinical disease, Subcluster 0 is the predominant sub-cluster and expresses genes associated with anti-fibrotic and immunomodulatory functions. In clinical disease, Subcluster 1 expands and exhibits a mixed pro- and anti-inflammatory gene expression phenotype. This sub-cluster expresses programs for immune cell recruitment and trafficking and ROS production, but also expressed immunomodulatory genes. Recently, Richoz et al identified a resident macrophage subset like our Subcluster 1 that was expanded in aged MRL/lpr mice and expressed a similar chemokine profile (10.1172/jci.insight.159751). Finally, Subcluster 3 is the least frequent residential macrophage population but expand during clinical disease progression. These express genes that modulate kidney inflammation and fibrosis.

The findings from these studies will be reported in a Manuscript that is currently in preparation

The 5th model that we will use is a model that will allow us to later study the effects of BAFF deficiency in vivo. These mice are difficult to breed and needed to be thawed from embryos. We have successfully imported them and they are currently breeding in the laboratory. We expect to be able to harvest some of these mice in the upcoming year.

Aim 2: Here we wish to study the fate of macrophage subpopulations over time. We have extended the scope of these studies to include flow cytometry analysis since with the support of this proposal we developed a 20 color flow cytometry panel to identify all the 7 major myeloid subsets that we could distinguish on our single cell analyses. We will next perform single cell RNA sequencing of the 7 major subsets as a reference data set.



	Sample Name	Subset Name	Count
	concat_1.fcs	NC1	103926
	concat_1.fcs	NC2	51836
	concat_1.fcs	res mac	87385
	concat_1.fcs	cDC2	6268
	concat_1.fcs	Classical 2	15762
	concat_1.fcs	Classical 1	8456

Figure 3: A: Flow cytometry strategy for identifying myeloid cell subpopulations in mouse kidneys. Data from a nephritic mouse is shown. B: TSNE plots of flow cytometry data from NZB/W mice shows infiltration with classical and non-classical monocytes in nephritis mice and a shift in the phenotype of resident macrophages.

We first proposed to use bone marrow transplants to trace myeloid cells newly arriving in the kidney so that these could be analyzed for their gene expression profile. We have optimized our technique in which we shield the kidney during irradiation and then transfer the bone marrow to give us larger numbers of transferred cells. The problem with this method is that since new cells are constantly generated we can't be sure when the transferred cells first enter the kidneys. We therefore have started optimizing an exchange transfusion protocol whereby we transfer cells at a set time and can then follow their fate over time. We are currently optimizing the technology using CD45 congenic Sle1.Yaa mice. To do this more optimally we also generated CD45.1 NZW.Yaa mice that reliably develop renal disease with large numbers of infiltrating cells at about 6 months of age. We are currently aging the donors and recipients. Using our established flow cytometry protocols to identify renal subsets will allow us to determine the half-life and origins of each subset in the chimeric mice as well as to sort the cells for low input RNA sequencing.

Aim 3: In this aim we proposed to determine which macrophage subsets disappear from the kidneys or alter their function after remission induction. We have so far generated large quantities of CTLA4Ig, anti-CD40L and BAFF-R-Ig and have sourced the MMF containing chow for the mice. We are currently aging NZB/W mice for the first intervention experiment. Generating the data for this experiment should be straightforward as we have established protocols for isolation of myeloid cells from the kidneys and generation of single cell libraries.

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

Dr. Paul Hoover is our junior collaborator at the Broad Institute, working with Dr Hacohen. This project is his main project and he submitted a KO8 application that was scored and is being revised. He obtained Bridge funding from the Rheumatology Research Foundation this year. He presented this work at the ACR meeting in 2020 and at an NIH meeting on mouse models of lupus in 2021. He was invited to speak at a symposium at the ACR meeting this year

My student Mr. Chirag Raparia was admitted to the Graduate School at Feinstein and is working on the renal myeloid cell sorting. He attended the AAI and ACR meetings in 2022, and a Keystone macrophage meeting this year. He has also been appointed to the T32 Training Grant at our Institution.

Ananya Kar is a master's level student/technician in the laboratory and is working on Aim 3 of the proposal. She plans applications to Graduate School in 2 years.

How were the results disseminated to communities of interest?

An abstract and invited talk were presented at the ACR meeting in 2022.

One manuscript is in preparation.

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

1. Complete the manuscript related to Aim 1

2. Complete scRNA sequencing of myeloid populations to use as a reference set

3. Complete the first set of exchange transfusions and flow cytometry analyses. If successful we will move to scRNA sequencing.

4. Complete the first treatment experiment

4. IMPACT: What was the impact on the development of the principal discipline(s) of the project?

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

Lupus nephritis affects between 30-60% of adult lupus patients and up to 70% of children with lupus and causes significant health consequences. Kidney damage in occurs in 30-40% of affected LN patients and 10% will need dialysis or transplant. The standard therapy for LN consists of drugs that are toxic and are not sufficiently effective. Despite many advances in biologic drug therapy, only one new therapy, belimumab has emerged as being effective for LN. It is currently not possible to predict the outcome of LN or the response to particular drugs based on the analysis of a kidney biopsy. We have previously found in animal models of LN that a particular subset of immune cells called macrophages are involved in kidney injury in LN. When macrophages are present in large numbers in kidney biopsies this is often associated with a worse outcome for the patient.

Aim 1: Because macrophages have so many functions and programs, one way to begin to understand how macrophages play a role in LN is to examine them at the single cell level. Here we analyzed single cell macrophage datasets from diverse mouse models of LN with different immune and inflammation characteristics to find pathways of interest that are like those seen in human LN. We found remarkable similarity in subsets between mice and humans allowing us to identify subsets that are relevant for further

functional studies. We also identified subsets associated with disease activity and chronicity in humans that are also present in some of our mouse models.

Aim 2: We have seen in humans that kidney macrophages alter their function over time. As we identify new subsets and functions of macrophages over time, we can locate them in the kidneys using specialized visualization techniques. Finally, we will find out whether similar states of cells in human biopsies can help to predict outcome. These studies will set the stage for development of methods to delete sub-populations of harmful cells or curb their function so that they protect the kidney rather than damage it. Here we have developed methods to identify subsets using flow cytometry in mice so that populations can be followed over time.

Aim 3: Less than half of patients respond to standard treatment for LN. It is not possible to predict response to treatment simply by looking at the kidney biopsy. Because there are starting to be new treatments for LN, we need to be able to predict which patients will respond best to each treatment or to design new treatments for those patients who are non-responsive to currently available therapies. Here we are using our mouse models to examine the differences in macrophage subsets and functions in models that respond to either standard treatment with immune suppressing drugs or treatment with novel biologic drugs including belimumab. By comparing the differences between those mice that respond to therapy and those that do not to find those pathways that cause harm and those that might be protective.

What was the impact on other disciplines?

The single cell analysis methods can be used by others to study other organs and diseases. Once our data is published it will provide a resource for others wishing to do different analyses.

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

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

Nothing to report

Changes that had a significant impact on expenditures

Nothing to report.

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

Nothing to report

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS:

Journal publications.

N/A

Books or other non-periodical, one-time publications.

Davidson A. Lupus Nephritis. Dubois Lupus textbook (in press).

Other publications, conference papers, and presentations.

Invited talks (Davidson)

Scleroderma National Meeting Boston July 2022

Lupus 21st Century National Meeting Tucson, AZ September 2022

Invited talks (Hoover)

ACR Meeting Philadelphia 2022

Abstracts accepted

Hoover P, Lieb D, Li S, Raparia C, SLE/RA A, Arazi A, Hacohen N, Davidson A. Differentiation of Injury-associated Macrophages in Lupus Kidneys Is Conserved in Humans and Lupus Mouse Models [abstract]. Arthritis Rheumatol. 2022; 74 (suppl 9). https://acrabstracts.org/abstract/differentiation-of-injury-associated-macrophages-in-lupus-kidneys-is-conserved-in-humans-and-lupus-mouse-models/.

Website(s) or other Internet site(s)
Nothing to report

Technologies or techniques
All techniques will be reported in our manuscripts

Inventions, patent applications, and/or licenses
Nothing to report

Other Products
All molecular data will be deposited in a public database

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Ke Lin

Project Role: *Senior technician*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *3*

Contribution to Project: *Breeding, clinical evaluation, bleeding, ELISAs*
Funding Support: *Also partly funded by Feinstein funds and other grants to the Davidson laboratory*

Chirag Raparia

Project Role: *Student*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *6*

Contribution to Project: *Flow cytometry, data analysis, assay optimization*
Funding Support: *T32 grant and Hofstra Northwell School of Medicine*

Ananya Kar

Project Role: *Technician*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *3*

Contribution to Project: *Generation of reagents for therapeutics experiment*

Funding Support: *Also partly funded by Feinstein funds and other grants to the Davidson laboratory*

Nir Hacohen

Project Role: *Collaborator*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *1*

Contribution to Project: *10X genomics of whole kidney cells in different mouse strains and bioinformatics*
Funding Support: *Effort funded by Lupus Research Alliance*

Paul Hoover

Project Role: *Post-doc in the Hacohen laboratory*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *9*

Contribution to Project: *10X genomics of whole kidney cells in different mouse strains and bioinformatics*
Funding Support: *Lupus Research Alliance, RRF*

Arnon Arazi

Project Role: *Bioinformatics, Aims 2 and 3*

ABSTRACT NUMBER: 1666

Differentiation of Injury-associated Macrophages in Lupus Kidneys Is Conserved in Humans and Lupus Mouse Models

Paul Hoover[1], David Lieb[2], Stephen Li[2], Chirag Raparia[3], Accelerating Medicines Partnership SLE/RA[4], Arnon Arazi[5], Nir Hacohen[2] and Anne Davidson[6], [1]Brigham and Women's Hospital, Boston, MA, [2]Broad Institute, Cambridge, MA, [3]Donald and Barbara Zucker School of Medicine At Hofstra/Northwell, Shoreham, NY, [4]NIH, Bethesda, MD, [5]Feinstein Institutes for Medical Research, Melrose, MA, [6]Feinstein Institutes for Medical Research, Manhasset, NY

Meeting: ACR Convergence 2022

Keywords: genomics, Lupus nephritis, macrophages, Mouse Models, Lupus

SESSION INFORMATION

Date: Monday, November 14, 2022

Session Title: Abstracts: SLE – Animal Models

Session Type: Abstract Session

Session Time: 10:30AM-11:30AM

Background/Purpose: Infiltrating monocytes acquire functions that support kidney remodeling in response to tissue damage in lupus nephritis. This process of monocyte differentiation has been difficult to study due to the inability to characterize immune cells from small human kidney biopsies or to accurately use mouse models to investigate mechanisms relevant to human disease. We previously reported on the identification of comparable kidney macrophage states from 24 patients with lupus nephritis and 4 common lupus mouse models using single cell RNA seq. Here, we compared intrarenal myeloid cells from 155 patients from AMP Phase 2 and our 4 lupus mouse models to discover that injury-associated macrophages and their differentiation from infiltrating monocytes is comparable in humans and mice.

Methods: For humans, we analyzed ~25,000 intrarenal myeloid cell transcriptomes in collaboration with AMP-SLE. For mice, we sorted CD45+ cells from dissociated mouse kidneys with spontaneous adaptive-driven autoimmunity (NZB/W & MRL/lpr) and TLR7-overexpression innate-driven autoimmunity (Sle1.Yaa & NZW/BXSB) in early and nephritic disease. We profiled single cells using 10x Genomics, analyzed ~10,000 transcriptomes with >500 genes and UMIs using Seurat 3.0, and performed Louvain-based clustering. We developed an antibody panel based on the top differentially expressed genes for mouse myeloid clusters for flow-cytometry. To identify similar mouse and human myeloid states we applied classifiers trained on our mouse data to

AMP myeloid data. We used partition-based graph abstraction to compare cellular trajectories from analogous human and mouse myeloid clusters.

Results: Injury-associated Clec4n+ and C3ar1+ macrophage populations emerged in nephritic kidneys from all 4 mouse strains. In humans, we identified comparable macrophage populations that correlated with active kidney injury (Fig. 1). These injury-associated states adopted a similar differentiation path in mice and humans that derived from an analogous infiltrating monocyte (Fig. 2). Similar gene programs and transcription factors shifted expression over this differentiation path and culminated in genes for lipid processing and phagocytosis in the injury-associated states.

Conclusion: Our studies of 155 patients and 4 common lupus mouse models identified comparable injury-associated macrophage states in lupus nephritis. These cells were enriched for lipid processing and phagocytosis gene modules and differentiated through a conserved set of cellular states that expressed similar gene programs and transcription factors. These injury-associated states may be critical for remodeling injured lupus kidneys and can now be studied in future mouse work that will accurately reflect this aspect of human lupus nephritis.

Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *1*

Contribution to Project: *10X genomics of whole kidney cells in different mouse strains and bioinformatics. Experimental design*
Funding Support: *Feinstein Institutes*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

What other organizations were involved as partners?

Organization Name: Broad Institute
Boston MA
Partner's contribution to the project

- *Facilities - 10X genomics of additional mouse strains*
- *Collaboration - Collaboration with Nir Hacohen*

8. SPECIAL REPORTING REQUIREMENTS

N/A

9. APPENDICES:

Abstract