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CONTRACTING ORGANIZATION: Hospital for Special Surgery

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14. ABSTRACT

Improper regulation of a specific immune cell called a B cell has been long linked to the development of SLE. A subtype of B cells, termed Autoimmune/Age-associated B cells (ABC), has recently been shown to play a major role in SLE because of their ability to be major producers of the proteins, also known as autoantibodies, that can cause damage in lupus. Expansion of ABCs in SLE is greater in African-American patients and correlates with disease activity and clinical manifestations. The environmental triggers that promote the accumulation of ABCs in lupus patients are largely unknown. Our laboratory has found that the expansion of ABCs in mice is controlled by a small family of two molecules. We have found that deleting both of these molecules in mice (leading to a Double Knock-out=DKO) leads to the spontaneous development of lupus in mice that shares many features with the human disease including the fact that the disease primarily affects female mice. We have recently found that manipulating cholesterol levels in these mice can promote the expansion of ABCs and affect the extent of inflammation in different organs in these mice. In this proposal we will investigate the hypothesis that alterations in cholesterol, as could be driven by a Western-diet rich in cholesterol, can affect the accumulation of ABCs and the ability of inflammatory cells to target specific organs and thus contribute to the heterogeneity of SLE.

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REPORT OUTLINE

1. INTRODUCTION:

A major roadblock to our ability to develop novel treatments for Systemic Lupus Erythematosus (SLE) is the significant heterogeneity that accompanies this disease. The complex cross-talk between an individual's genetic predisposition and exposure to environmental factors like diet is likely to be a major contributor to this heterogeneity. Autoimmune/Age-associated B cells (ABCs) are a novel B cell subset, which exhibit a unique phenotype and preferentially expand in SLE patients. ABCs are major producers of autoantibodies, and their expansion in SLE correlates with disease activity and clinical manifestations like kidney disease. The environmental triggers that promote the generation, function, and differentiation of ABCs in autoimmune settings are largely unknown. Our laboratory previously isolated a protein termed Def6, which exhibits significant homology to only one other protein, SWAP-70. Importantly, mice lacking both Def6 and SWAP-70 (Double-knockout=DKO mice) develop SLE-like disease, which shares several key clinical features with human SLE including its sex-bias. We have previously shown that ABC formation is enhanced in DKO mice. In this proposal we are testing the hypothesis that alterations in lipid homeostasis, such as those promoted by a Western diet, can modulate the accumulation and function of ABCs and impact autoAb responses and end-organ inflammation to a different extent based on host-specific factors and thus contribute to SLE heterogeneity.

2. KEYWORDS:

WD=Western Diet
LDLR= Low-density lipoprotein receptor
ABCs= Autoimmunity/Age-associated B cells
DN2= Double negative 2 B cells
DKO= Double knock-out (mice lacking both Def6 and SWAP-70)

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1: To delineate the mechanisms by which a WD promotes the expansion and differentiation of ABCs

Major Task 1: To assess the transcriptional and epigenetic effects exerted on ABCs by ingestion of a WD (Yr.1)

Subtask 1: RNASEQ chow vs WD

Subtask 2: ATAC-seq chow vs WD

Milestone(s) Achieved: Both sets of experiments have been completed

Major Task 2: To delineate the role of key regulators of cholesterol homeostasis in ABCs. (Yr. 2)

Subtask 1 Analysis of CD23cre SREBP2$^{fl/fl}$DKO post chow or WD-in vivo experiments

Establishment of requirement for SREBP2 in WD- driven effects on humoral autoimmunity

Milestone(s) Achieved: The studies have established a key role for SREBP2 in humoral autoimmunity in the presence and absence of a Western diet as outlined below.

Specific Aim 2: To dissect the mechanisms by which alterations in lipid homeostasis modulate end-organ inflammation in lupus (Yr 3)

Major Task 3: To evaluate the effects of dietary influences on the development of atherosclerosis in DKO mice in the presence/absence of genetic manipulations of the LDLR

Major Task 4: To define the cellular and molecular composition of the inflammatory infiltrates in end- organs of DKO mice with alterations in lipid homeostasis.

What was accomplished under these goals?

Aim 1.1: To assess the transcriptional and epigenetic effects exerted on ABCs by ingestion of a WD. Like human SLE, the expansion and production of autoAbs by DKO ABCs exhibits a striking sex-bias and is primarily observed in DKO female mice (Fig. 1). Remarkably, feeding a WD to DKO males promotes the accumulation of ABCs and autoAb production suggesting that the ability of these cells to accumulate and secrete autoAbs can be regulated not only by genetic and sex-specific factors but also by dietary influences. The major activities for the Yr 1 reporting period were to utilize unbiased genome-wide approaches to gain insights into the mechanisms that underlie the ABC-promoting effects of a WD. The specific objectives were to employ RNA-seq and ATAC-seq to investigate the transcriptional and epigenetic effects exerted on ABCs by ingestion of a WD. To investigate this question, we have performed RNA-seq on ABCs sorted from DKO males or females that were either fed chow or a WD, which was started at approximately 8 wks and continued for 12 wks. Sex-matched and diet-matched wt controls were included as controls for the diet. Analysis of the RNAseq experiments has been completed and has revealed the following: 1) Feeding a WD to DKO females resulted in 576 upregulated and 678 downregulated genes (log_2FC .6; $p<.05$; Fig 1); 2) Feeding a WD to DKO males resulted in 178 upregulated and 92 downregulated genes (log_2FC .6; $p<.05$; Fig. 2); 3) Feeding a WD to either DKO females or DKO males led to upregulation of a common set of pathways. Importantly both DKO females and DKO males fed a WD upregulated TNFα signaling and NF-κB (Red rectangles) and thus exhibited an increased inflammatory profile (Fig. 3); 4) DKO males but not DKO females fed a WD upregulated pathways involved in B cell receptor signaling (blue rectangle, Fig 3) suggesting indeed that feeding a WD can promote enhanced sensitivity of ABCs to BCR engagement and thus increased antigenic responsiveness. This analysis has thus uncovered similarities but also crucial transcriptional differences between ABCs derived from DKO males fed chow versus those obtained from DKO males fed a WD and has revealed that these distinctions/similarities reflect the differential expression of a selected group of pathways. A separate cohort of DKO males and DKO females that were either fed chow or a WD, which was started at approximately 8 wks and continued for 12 wks was also set-up for ATAC-seq. Sex-matched and diet-matched wt controls were included as controls for the diet. ABC cells have been

sorted and processed for ATAC-seq and ATAC-seq has demonstrated that these transcriptional differences are accompanied by differences in the chromatin landscape of these cells with differences observed between DKO females fed a WD (termed HFD in Fig 4) or chow (termed control in Fig.4). Differences were also observed in the ATAC-seq results of DKO males and females fed a WD/HFD (Fig. 5) in line with the transcriptional differences observed by RNA-seq.

Figure 1

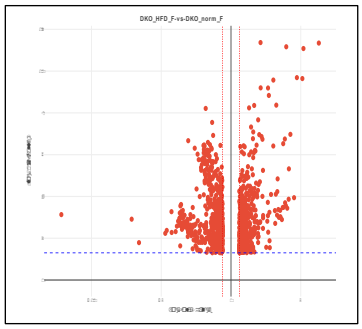


Figure 2

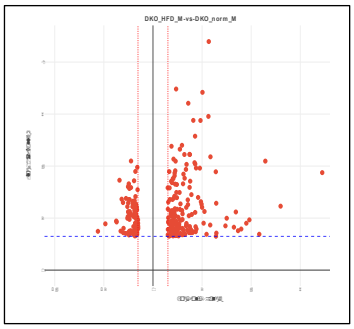


Figure 3

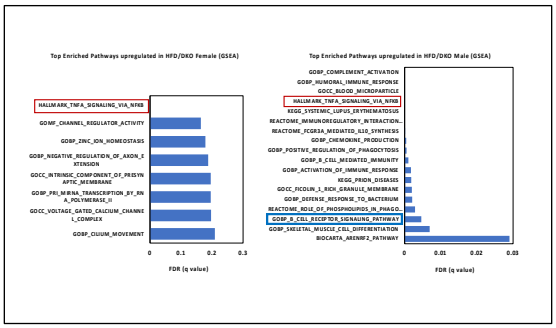


Figure 4

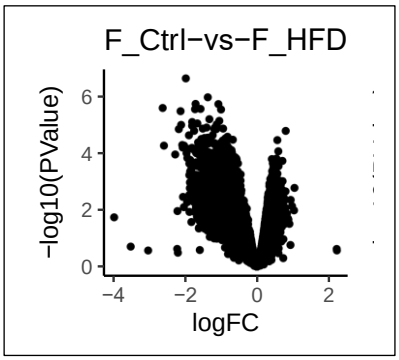
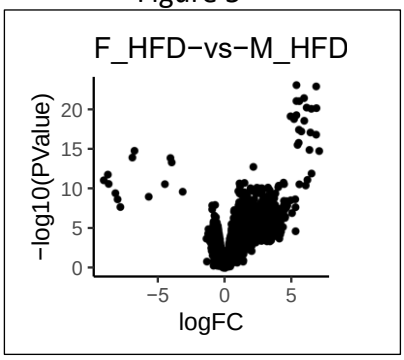


Figure 5



Aim 1.2: To delineate the role of key regulators of cholesterol homeostasis in ABCs. Sterol regulatory element-binding protein 2 (SREBP2) is the major transcriptional regulator of sterol synthesis and directly induces the expression of enzymes involved in the mevalonate pathway. In response to cholesterol starvation, SREBP2 is activated by a complex process involving its transport from the ER to the Golgi where it is cleaved followed by the translocation of transcriptionally active fragments to the nucleus. Interestingly, we have recently observed that, in B cells, the activity of SREBP2 can be regulated by its interaction with IRF family members. The major activities for the Yr 2 reporting period were to investigate the role of SREBP2 in ABC function and whether this role would be altered by ingestion of a WD. To this end we took advantage of SREBP2-deficient DKO mice, which we had previously generated by crossing SREBP2fl/fl mice with DKO mice and then with CD23Cre mice to generate CD23-Cre+ SREBP2fl/flDKO female and male mice. These mice were fed either chow or a WD, which was started at approximately 8 wks and continued for 12 wks as in Aim 1 followed by an extensive analysis of the effects of these manipulations on ABC generation and differentiation in vivo. A detailed assessment of all key B cell populations in CD23-Cre+ SREBP2fl/flDKO female mice (Fig. 6 and data not shown) demonstrated that expression of SREBP2 regulates the expansion of ABCs and even more profoundly their ability to differentiate into a pre-GC B cell population (CD11c+ GCB cells) and subsequently into classical GCs. Differentiation toward PB/PCs was instead not significantly affected suggesting that SREBP2 primarily controls the GC route of ABC

differentiation but not the extrafollicular (EF) route. These effects were lessened by administration of a WD consistent with the idea that SREBP2 controls the production of endogenous cholesterol by B cells and that this step can be bypassed, albeit not completely, by providing exogenous cholesterol. Interestingly, administration of a WD was less effective at ameliorating the GC defect observed in the absence of SREBP2 suggesting that the compensatory mechanisms function in a B-cell stage specific manner. A similar analysis in CD23-Cre^{+}SREBP2$^{fl/fl}$DKO male mice (Fig. 7 and data not shown) indicated a greater impact of SREBP2 deletion on ABC differentiation and GC formation under a WD suggesting that the ABC compartment of males may be less able to compensate for the absence of SREBP2 and that in males SREBP2 may control both GC and EF routes of differentiation.

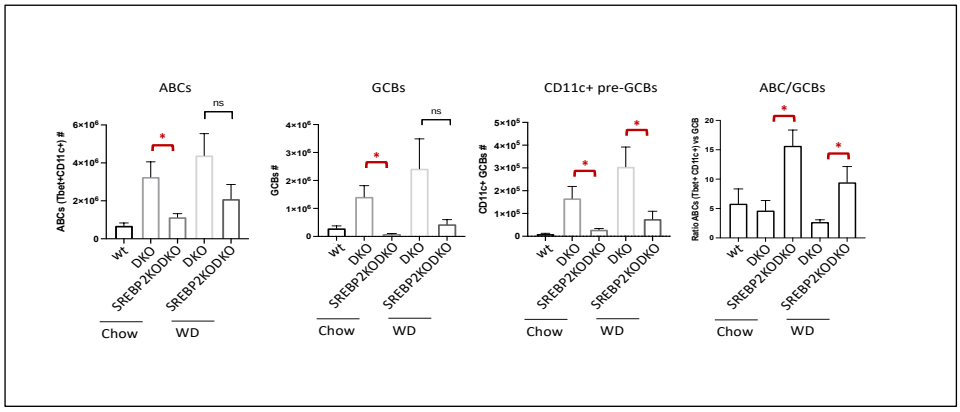


Figure 6 (females)

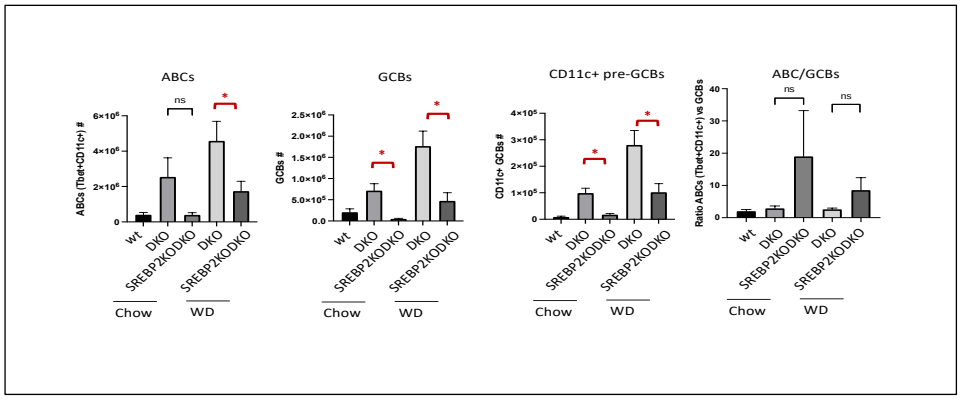


Figure 7 (males)

An an extensive serological analysis (Fig. 8 and Fig. 9 and data not shown) demonstrated sex-specific differences in the production of autoantibodies in the absence of SREBP2 with stronger effects in the males consistent with the greater role of SREBP2 in regulating the ABCs in males than females. We also observed an unexpected increase in the production of SmRNP autoantibodies in males suggesting that, in males, SREBP2 controls not only the ABCs but also the function of specific subsets of PB/PCs. Thes studies in Aim 3 will help determine the role of tissue specific milieus in regulating the ability of cholesterol homeostasis to influence the expansion and differentiation of these B cells.

Figure 8 (females)

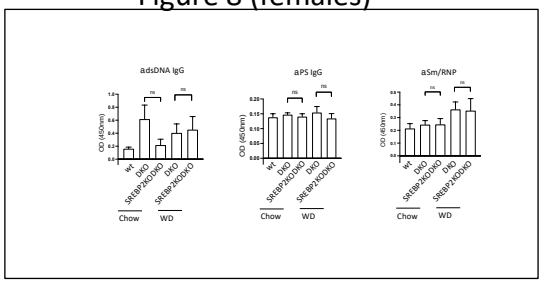
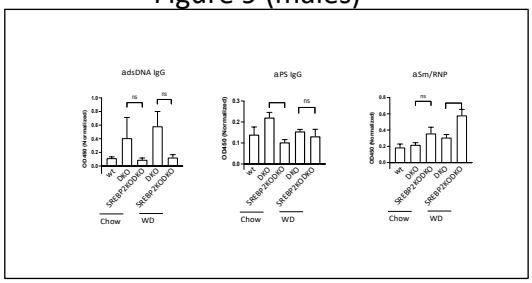


Figure 9 (males)



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

The PI attended the Lupus 21st Century meeting to catch up with recent advances in lupus research. The PI and Dr. Gupta also currently attend virtual Research-in-progress meetings at the Hospital for Special Surgery on a regular basis and closely communicate with other scientists in the field of autoimmunity research.

How were the results disseminated to communities of interest?

Nothing to Report

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

During the next reporting period we will investigate the mechanisms by which a Western life-style can impact the development of atherosclerosis in lupus-prone mice. To this end, we will feed either chow or a WD to DKO mice that also lack the LDLR and assess atherosclerotic plaques to test the hypothesis that the chronic inflammatory milieu of lupus can impart a heightened susceptibility to proatherogenic dietary influences like a WD (Major Task 3). We will also test the hypothesis that dyslipidemia augments and/or modifies the chronic inflammatory infiltrates that accumulate in lupus eventually leading to greater end-organ damage (Major Task 4). To this end, DKO or LDLRDKO female mice will either be fed chow or a WD for 12 weeks starting at approximately 8 weeks of age and the molecular profile of the infiltrates that accumulate in the kidneys of lupus-prone mice in the presence/absence of dyslipidemia will be assessed. Given that we have observed in other studies that significant pathogenic differences between ABCs from mice with different disease severity maps to only few hundred genes, to ensure sufficient sequencing depth to uncover these differences, instead of scRNASeq which only allows for the identification of a limited number of genes, key immune cell populations will be sorted from the kidneys and subjected to bulk RNASeq.

4. IMPACT:

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

Nothing to Report

What was the impact on other disciplines?

Nothing to Report

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:**

Changes in approach and reasons for change

Nothing to Report

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

Nothing to Report

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

Significant changes in use or care of human subjects.

N/A

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:

Publications, conference papers, and presentations

Nothing to report

Journal publications.

Nothing to report

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

Nothing to report

Other publications, conference papers, and presentations.

Nothing to report

Website(s) or other Internet site(s)

Nothing to report

Technologies or techniques

Nothing to report

Inventions, patent applications, and/or licenses

Nothing to report

Other Products

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*Dr. Alessandra Pernis*
Project Role:	*PD/PI*
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0001-8259-1446
Nearest person month worked:	*0.60 calendar months effort, 0.36 calendar months' salary*
Contribution to Project:	*Dr. Pernis guides the studies as well as help with the analysis of the results and the preparation of manuscripts. She has trained in cellular immunology as well as biochemistry and molecular biology. She has sufficient background and expertise for the proposed study.*
Funding Support:	*This award*
Name:	*Dr. Sanjay Gupta*
Project Role:	*Postdoctoral*
Researcher Identifier (e.g. ORCID ID):	*NA*
Nearest person month worked:	*6.0 effort*

Contribution to Project:	*Dr. Gupta worked with Dr. Pernis and helped perform the analysis described in Major Task 2*
Funding Support:	*This award*

Nothing to Report

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

Not Applicable

9. APPENDICES:

Not Applicable