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14. ABSTRACT
The objective of this study is to elucidate the mechanisms underlying dysregulated plasmablast and plasma cell homeostasis in SLE, resulting in production of autoantibodies that drive disease pathogenesis. Our preliminary data support a mechanistic link between SLE genetic risk variants in the *BANK1* gene with autophagy and plasmablast development. During year 2 of the grant we performed experiments to test the hypothesis that the SLE-associated risk variants in *BANK1* promote autophagy, leading to increased plasmablast differentiation and immunoglobulin secretion. Using gene editing, we found, expression of the *BANK1* risk or non-risk cDNA elicited a selective disadvantage relative to cells deficient in *BANK1*. We also found that BANK1 protein levels decreased as plasmablast differentiation progressed, which is consistent with the idea that more autophagy is required as plasmablast differentiation progresses and that a reduction in BANK1 levels, as a repressor of autophagy, will enable increased levels of autophagy and plasmablast differentiation. In parallel, we are testing autophagy and plasmablast differentiation using primary human B cells from controls and lupus patients genotyped for the *BANK1* variants. We observed that plasmablast differentiation from naïve B cells was detectable by day 7 of in vitro culture and was followed by differentiation of plasma cells at day 9 expressing IgG. The addition of the TLR9 agonist CpG increased plasmablast and plasma cell differentiation. At the same time points, we saw that autophagy preceded plasmablast differentiation, consistent with autophagy being required for plasmablast differentiation. Conclusions regarding the effect of *BANK1* genotype will be determined upon unblinding at the completion of these experiments. Our results support the hypothesis that BANK1 promotes autophagy and plasmablast development.

15. SUBJECT TERMS
B cells, autophagy, plasmablast, plasma cell, differentiation, SLE genetic variant, BANK1, gene editing

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1. INTRODUCTION:

Systemic erythematosus lupus (SLE) is a chronic autoimmune disease affecting up to 1.5 million Americans, and has an incidence of 158 cases per 100,000 Veterans. Our studies will advance the knowledge of lupus disease mechanism and will provide insight into patient heterogeneity with respect to genetic variation, advancing the goal of personalized medicine for people with SLE. The overarching challenge to be addressed is the significant gap in knowledge about the mechanisms underlying the dysregulation of plasmablast and plasma cell homeostasis in SLE that result in production of autoantibodies driving disease pathogenesis. Recent findings support a role for autophagy in the dysregulation of plasma cell homeostasis in SLE. Furthermore, our preliminary data support a mechanistic link between autophagy, plasmablast development, and the *BANK1* SLE genetic risk variants. Based on these published and preliminary studies, we propose to test the novel hypothesis that the SLE-associated risk variants in *BANK1* promote autophagy, leading to increased plasmablast differentiation and/or immunoglobulin secretion that contributes to lupus susceptibility and disease. We predict that autophagy-targeting agents will limit plasma cell differentiation in B cells and may benefit patients with SLE, especially those with the *BANK1* risk variants. Our approach will utilize primary B cells from well-characterized healthy control subjects and SLE patients carrying the *BANK1* risk and non-risk variants, and cutting-edge gene editing of primary B cells and cell lines. In Specific Aim 1, we will determine if the BANK1 risk protein increases autophagy and leads to increased plasmablast differentiation and immunoglobulin secretion. We will address this *in vitro* using gene editing to express the BANK1 risk or non-risk proteins in an isogenic background in primary human B cells and cell lines, and by comparing *BANK1* risk and non-risk primary B cells from healthy control and SLE subjects. These findings will be correlated with levels of circulating plasmablasts and autoantibodies in SLE subjects at the same time point to determine whether in vitro plasma cell differentiation corresponds to *in vivo* development. Finally, we will investigate the mechanism linking BANK1 to autophagy by carefully characterizing the role of its binding partners, ATG3 and TBC1D2B in *in vitro* plasma cell differentiation. In Specific Aim 2, we will determine if targeting autophagy with small molecule inhibitors limits plasmablast differentiation, survival, or immunoglobulin secretion. We will test the autophagy inhibitors using *BANK1* risk and non-risk B cells from genotyped healthy control and SLE subjects. These outcomes will improve the diagnosis and care of active military personnel and Veterans with lupus, and will offer insight into individuals at risk for developing SLE and possible interventions to prevent development of disease.

2. KEYWORDS:

B cells, SLE genetic variant, BANK1, autophagy, plasmablast, plasma cell, differentiation, gene editing

3. ACCOMPLISHMENTS:

Specific Aim 1(specified in proposal) We will determine if the BANK1 risk protein increases autophagy and leads to increased plasmablast differentiation and Ig secretion.	**Timeline** (Updated to reflect 1y NCE)	**Site 1: Benaroya Research Institute**	**Site 2: Seattle Children's Research Institute**	**Percent completed**
Major Task 1 Obtain HRPO/ACURO Approval.	3/2019	Dr. Cerosaletti	Dr. James	100%

Major Task 2 Determine the effect of *BANK1* risk and non-risk variants on autophagy and plasma cell differentiation in primary human B cells.	**Timeline** (Updated to reflect 1y NCE)	**Site 1: Benaroya Research Institute**	**Site 2: Seattle Children's Research Institute**	**Percent completed**
Subtask 1: Make AAV repair templates for each full-length BANK1 variant for delivery to the *CCR5* locus.	3/2019-9/2019		Dr. James	100%
Subtask 2: Test dual editing strategy to disrupt *BANK1* while also delivering over-expressed *BANK1* variants to the CCR5 locus in primary human B cells. Measure indel and homology directed recombination frequencies. B cells will be isolated from cryopreserved PBMC samples of healthy control subjects.	9/2019-3/2020		Dr. James # of healthy human subjects=10; all samples deidentified	100%
Subtask 3: Determine the effect of *BANK1* variants on macro- and TLR-induced autophagy, and plasma cell differentiation in an isogenic background using gene editing to introduce full length risk or non-risk isoform cDNA using homology directed recombination. Experiments will be performed using primary human B cells isolated from cryopreserved PBMC samples of healthy control subjects.	3/2020-4/2021		Dr. James # of healthy human subjects=10; all samples deidentified	100%
Subtask 4: Determine the effect of *BANK1* variants on macro- and TLR-induced autophagy, and plasma cell differentiation in primary human B cells from PBMC of genotyped healthy control subjects and individuals with SLE. All PBMC are pre-existing cryopreserved samples.	9/2019-11/2021	Dr. Cerosaletti Total # of healthy human subjects=60; # SLE subjects=60; all samples de-identified		100%
Subtask 5: Determine circulating plasmablast in PBMC samples and autoantibody levels (ANA) in serum from genotyped SLE patients and correlate with in vitro plasma cell differentiation. All PBMC and sera are pre-existing cryopreserved samples.	3/2020-11/2021	Dr. Cerosaletti Total# of human SLE subjects=60 (same SLE subjects in subtask 4); all samples deidentified		100%
Milestone(s) Achieved: Impact of the *BANK1* risk locus on macro- and TLR-induced autophagy and plasma cell development in health and in lupus.	11/2021			100%
Major Task 3 Loss- and gain-of-function of BANK1 interacting proteins.				
Subtask 1 Make AAV repair templates for delivery of ATG3 and TBC1D2B to the CCR5 locus. Test ATG3 and TBC1D2B guide RNAs in cell lines.	3/2019-1/2021		Dr. James	30%*

*Major task 3 is ongoing.

Major Task 3 continued	**Timeline** (Updated to reflect 1y NCE)	**Site 1: Benaroya Research Institute**	**Site 2: Seattle Children's Research Institute**	**Percent completed**
Subtask 2: Use gene editing and homology-directed repair in primary human B cells to assess loss- and gain-of-function of TBC1D2B on macro- and TLR-induced autophagy, and plasma cell differentiation. B cells will be isolated from cryopreserved PBMC of healthy control subjects.	9/2019-3/2021		Dr. James # of healthy human subjects=10; all samples deidentified	0%
Subtask 3 Use gene editing and homology-directed repair in primary human B cells to assess loss- and gain-of-function of ATG3 on macro- and TLR-induced autophagy and plasma cell differentiation. B cells will be isolated from cryopreserved PBMC of healthy control subjects.	3/2020-3/2021		Dr. James # of healthy human subjects=10; all samples deidentified	0%
Milestone(s) Achieved: Role of BANK1 interacting proteins on autophagy and plasma cell differentiation.	3/2021			10% see note on page 5
Specific Aim 2 (specified in proposal) We will investigate if small molecules that inhibit autophagy limit plasmablast differentiation, survival, or Ig secretion.				
Major Task 1 Determine autophagy inhibitor concentration in lymphoma cell lines.				
Subtask 1: Dose-response experiments in the HBL1 and TMD8 lymphoma cell lines to identify inhibitor concentrations that results in 90% inhibition of autophagy.	3/2019-9/2021		Dr. James	100%
Milestone(s) Achieved: Inhibitor concentrations.	9/2021			100%
Major Task 2 Assess effect of autophagy inhibitors on macro- and TLR-induced autophagy and plasma cell differentiation in B cells from healthy control subjects.				
Subtask 1: Test autophagy inhibitors alone for their impact on macroautophagy and plasma cell differentiation in B cells from healthy control subjects. B cells will be isolated from pre-existing cryopreserved PBMC samples.	7/2019-9/2021		Dr. James # of healthy human subjects=10; all samples deidentified	85%
Subtask 2: Test autophagy inhibitors in presence of TLR agonists for their impact on TLR induced autophagy and plasma cell differentiation in B cells from healthy control subjects. B cells will be isolated from pre-existing cryopreserved PBMC samples.	7/2019-9/2021		Dr. James # of healthy human subjects=10; all samples deidentified	85%
Milestone(s) Achieved: Impact of autophagy inhibitors on plasma cell differentiation in primary human B cells.	6/2022			85%*

*Experiments ongoing to complete number of subjects

Major Task 3 Assess effect of autophagy inhibitors in B cells from healthy control subjects and SLE subjects carrying the $BANK1^{R/R}$, $BANK1^{R/NR}$ and $BANK1^{NR/NR}$ genotypes.	**Timeline** (Updated to reflect 1y NCE)	**Site 1: Benaroya Research Institute**	**Site 2: Seattle Children's Research Institute**	**Percent completed**
Subtask 1: Test impact of autophagy inhibitors on macro- and TLR-induced autophagy and plasma cell differentiation in B cells from healthy control subjects carrying *BANK1* genotypes. B cells will be isolated from pre-existing cryopreserved PBMC samples.	3/2020-6/2022	Dr. Cerosaletti Total # of healthy human subjects=60 (overlapping with major task 2); all samples deidentified		20%
Subtask 2: Test impact of autophagy inhibitors on macro- and TLR-induced autophagy and plasma cell differentiation in B cells from SLE subjects carrying *BANK1* genotypes. B cells will be isolated from pre-existing cryopreserved PBMC samples	3/2020-6/2022	Dr. Cerosaletti Total # of human SLE subjects=60 (overlapping with major task 2)		20%
Milestone(s) Achieved: Impact of autophagy inhibitors on plasma cell differentiation in healthy subjects and SLE patients in the context of the BANK1 risk variants.	6/2022			20%*

*Experiments ongoing building off the results from major task 2 above.

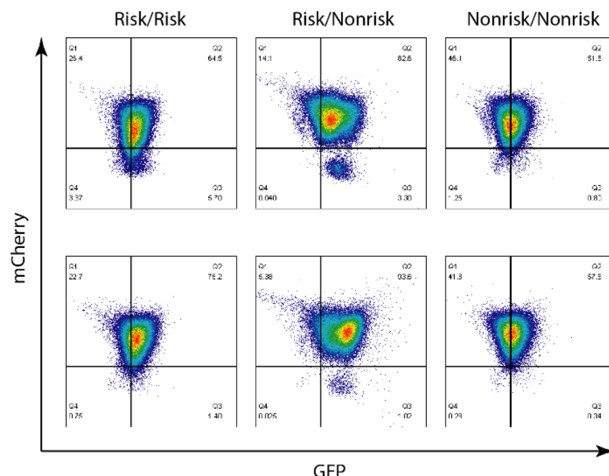
What was accomplished under these goals?

The focus of Year 3 activities was on completing objectives in Aims 1 and 2. Aim 1 is completed except for major task 3 which is ongoing. For Aim 2, major task 2 is nearly complete and task 3 is underway. These tasks are scheduled for completion in the next 6 months for inclusion in a publication of the results of this grant.

Aim 1: Determine the effect of *BANK1* risk and non-risk variants on autophagy and plasma cell differentiation in primary human B cells.

1. *Gene editing of the BANK1 locus*. Repair templates and AAV vectors were made for delivery of full-length risk and non-risk *BANK1* cDNAs into the *BANK1* locus under control of the endogenous promoter. We successfully used gene editing to co-deliver risk and non-risk *BANK1* cDNAs coupled to cis-linked expression of mCherry or GFP into human B cells, enabling delivery to both loci. These templates were expressed and quantified by flow cytometry as shown in **Figure 1.** To make stable lines, cells were sorted

Figure 1: Introduction of BANK1 risk and non-risk sequence into B cells. We designed guide RNAs targeting exon 2 of BANK1 and corresponding repair templates to introduce full-length risk (R) or non-risk (NR) BANK1 coupled to cis-linked expression of mCherry or GFP. In the presence of the guide RNAs and either repair template, we show HDR-mediated integration of cherry into TMD8 lymphoma cells (vs. no template, left). Following cell sorting, we generated polyclonal cell lines expressing the variants and validated HDR using sequencing and digital droplet PCR (data not shown).



based on dual expression of mCherry and GFP. In B cell lines, expression of both variants elicited a selective disadvantage relative to cells deficient in BANK1. We also found that BANK1 protein levels decreased as plasmablast differentiation progressed, which is consistent with the idea that more autophagy is required as plasmablast (PB) differentiation progresses to relieve ER stress and that a reduction in BANK1 levels, as a repressor of autophagy, will enable increased levels of autophagy. These findings add to our data that showed that deletion of *BANK1* resulted in increased autophagy, and expression of the *BANK1* risk cDNA resulted in increased autophagy relative to the non-risk gene. Further, we showed that deletion of *BANK1* in primary human B cells increased PB and plasma cell (PC) numbers compared to *BANK1* sufficient cells, with increased IgG secretion relative to IgM secretion. Our findings support the hypothesis that the SLE risk variants or deficiency in *BANK1* promote autophagy and PC development.

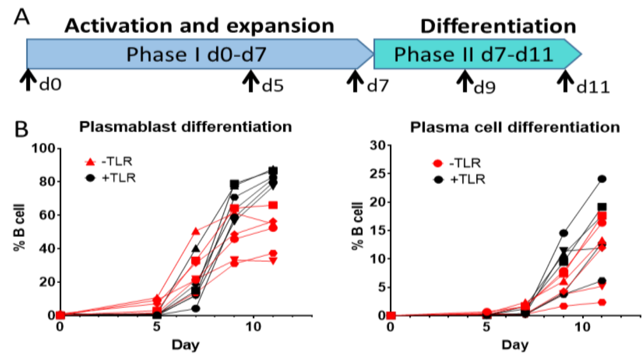


Figure 2. Plasmablast and plasma cell differentiation from naïve primary human B cells. A) Naïve B cells were isolated from BANK1 genotyped healthy control subjects and stimulated with CD40 ligand, IL-2, IL-21 +/- CpG for 7 days (phase I), followed by differentiation for 4 days (phase II/III) in the presence of IL-6, IL-10, and IFNα BAFF. Cultures were analyzed for differentiation and autophagy on days 0, 5, 7, 9, and 11.

2. *PB/PC differentiation in vitro.* We established conditions for in vitro PB/PC development using primary human B cells, and conditions to measure autophagy by flow cytometry for LC3, and p62 flux. We found that PB differentiation from naïve primary human B cells was detectable by day 7 of in vitro culture, peaking at day 9-11, and was followed by differentiation of PCs at days 9 and beyond (**Figure 2A and B**). Similarly, IgM+ PB were detectable by day 7 and plateaued by day 9, whereas IgG+ PB continued in an upward trajectory from day 7 to day 11 (**data not shown**). The addition of the TLR9 agonist CpG increased PB and PC differentiation. At the same time points, we saw that autophagy was detectable by day 5, peaking at day 7, and then declining (**Figure 3**). These results indicate that autophagy precedes PB differentiation.

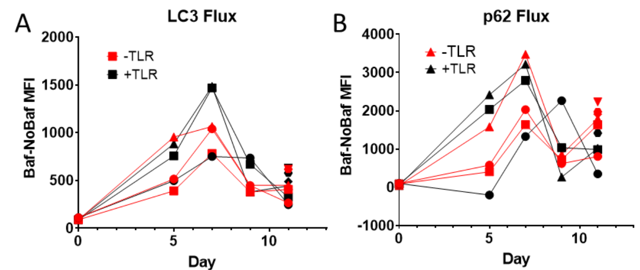


Figure 3. Autophagic flux during PB differentiation of primary human B cells. Autophagy was assessed by treating B cells from differentiation cultures +/- bafilomycin for 4h. Cells were then permeabilized to remove cytoplasmic LC3 and p62 not incorporated into autophagosomes, and then stained for B cell markers, LC3, and p62 and analyzed by flow cytometry. Flux was calculated by subtracting the MFI of LC3 (**A**) or p62 (**B**) in untreated cells from the MFI of LC3 and p62 of cells treated with bafilomycin.

3. *The effect of BANK1 on PB/PC differentiation and autophagy.* We applied our differentiation and autophagy assays described above to study the impact of the *BANK1* risk (GG; rs10516487/rs3733197) and non-risk genotypes (AA or AG) in peripheral blood of healthy controls and patients with lupus. Our first observation was that lupus patients had increased and earlier PB differentiation compared to controls when TLR9 stimulation (CpG) was included in the assay, but not in the absence of TLR9 (**Figure 4)**. This is consistent with increased TLR9 signaling and PB in SLE. In contrast, there was no difference in PC differentiation between controls and SLE patients under any condition (**Figure 4**). When PB differentiation was stratified by *BANK1* genotype, we observed that PB differentiation was increased with

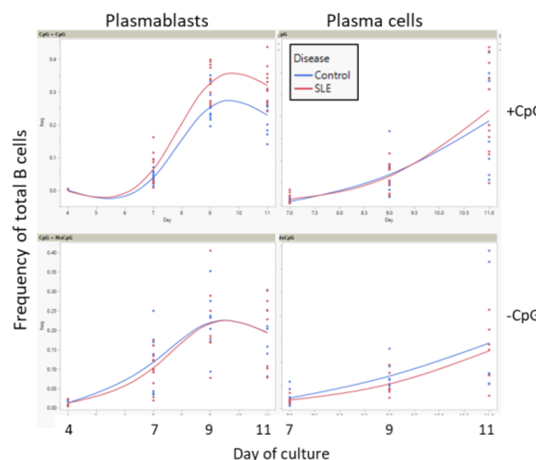


Figure 4. Increased and earlier PB differentiation from SLE B cells versus healthy controls. Naïve B cells were isolated and cultured in PB/PC differentiation conditions for 11 days with (top) and without (bottom) CpG and then analyzed by flow cytometry. SLE (red) B cells had increased PB differentiation than controls in the presence of CpG. No differences were detected in PC differentiation between controls and SLE subjects.

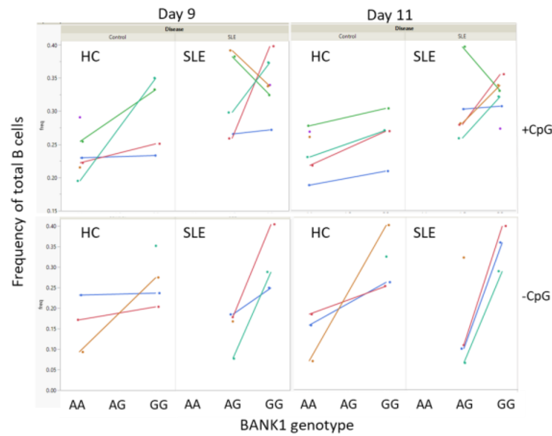


Figure 5. PB differentiation is increased in the presence of the *BANK1* alleles. Naïve B cells were isolated and cultured in PB differentiation conditions for 9 and 11 days with (top) and without (bottom) CpG. PB frequency was compared between subjects carrying one or two copies of the non-risk alleles (AA, AG) with those who were homozygous for the risk alleles (GG). Healthy control subjects are shown on the left (HC) and SLE on the right in each panel. The effect of *BANK1* risk alleles appears to be stronger in SLE patients both with and without TCR stimulation.

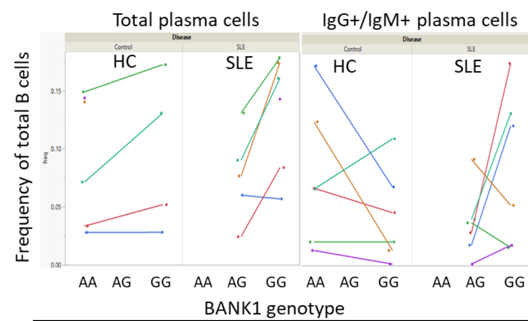


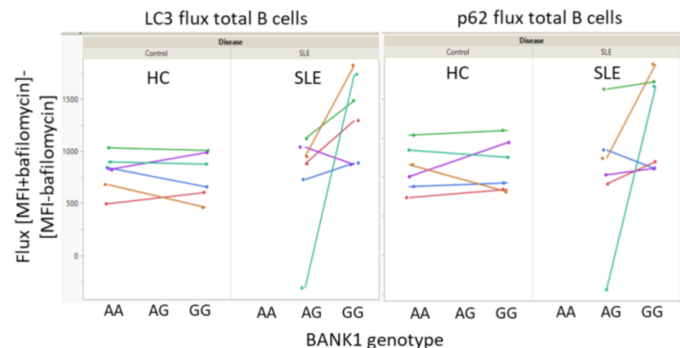
Figure 6. PC differentiation is increased in the presence of the *BANK1* risk alleles. Naïve B cells were isolated and cultured in PB/PC differentiation conditions for 11 days with CpG stimulation. Total PC frequency or the ratio of IgG+/IgM+ PCs was compared between subjects carrying one or two copies of the non-risk alleles (AA, AG) with those who were homozygous for the risk alleles (GG). Healthy control subjects are shown on the left (HC) and SLE on the right in each panel. The *BANK1* risk alleles increased PC differentiation in SLE subjects with TLR9 stimulation, and increased IgG+ PCs compared with IgM+ PCs.

the risk genotype (GG) compared with the non-risk genotype (AA or AG) (**Figure 5**). This was more apparent in SLE patients than control subjects and was detected in the presence or absence of TLR9 stimulation. Similarly, we observed that PC differentiation was increased in SLE patients carrying the *BANK1* risk variants compared to subjects with the non-risk alleles in the presence of TLR9 stimulation (**Figure 6**). Further, the *BANK1* risk genotype favored the development of IgG+ PC relative to IgM+ PC. The effect of genotype on PC development was not detected in control subjects. These findings are consistent with our gene editing results. Taken together, our results support the hypothesis that the SLE risk variants in *BANK1* promote PB and PC development, and favor the development of IgG PC in patients with lupus.

We subsequently evaluated the effect of the *BANK1* risk genotype on autophagy in the same differentiation cultures. At days 4-7 of differentiation, we observed an increase in LC3 flux in all B cells in the culture in the presence of TLR9 stimulation. SLE patients carrying the *BANK1* risk variants had increased LC3 flux compared to the non-risk genotype (**Figure 7**). This effect was not apparent in the healthy control subjects. There was also an increase in p62 flux in total B cells in the differentiation cultures in SLE patients carrying the *BANK1* risk genotype (**Figure 7**). The effect of the risk alleles was even more apparent at day 7. These results mirror the findings in our gene editing experiments demonstrating that the *BANK1* risk variants increase autophagy during PB and PC development. Notably, the effect of the BANK1 risk variants is greatest in lupus patients.

4. *Circulating PB levels.* We analyzed circulating PB in the same donors tested for PB/PC differentiation. We detected low levels of PB in PBMC and did not detect an effect of *BANK1* genotype in healthy control donors, although there was a slight trend in lupus patients towards increased PB in *BANK1* risk subjects. It is important to note that PB levels in peripheral blood are very low, making their detection variable.

Figure 7. Autophagy is increased in the presence of the BANK1 alleles in lupus subjects. Naïve B cells were isolated and cultured in PB/PC differentiation conditions for 4 days with CpG. Autophagy was measured by flow cytometry. LC3 and p62 mean fluorescence intensity (MFI) in the absence or presence of bafilomycin. Flux was calculated as the differenc ein MFIin the presence of bafilomysin minus the MFI in the absence of bafilomycin. Flux was compared between subjects carrying one or two copies of the non-risk alleles (AA, AG) with those who were homozygous for the risk alleles (GG). Healthy control subjects are shown on the left (HC) and SLE on the right in each panel. Autophagy was increased in the presence of the BANK1 risk alleles SLE patients but not controls.



Aim 2: Investigate whether small molecules that inhibit autophagy can limit PB differentiation, survival, or Ig secretion.

1. *Autophagy inhibitor concentration.* We tested three autophagy inhibitors: chloroquine, which is a standard of care for SLE, and Lys05, both of which are lysosomal transfer inhibitors, as well as sar405 which targets the autophagy kinase PIK3C3 to inhibit phagophore formation. In B cell lines we found Lys05 effectively blocked autophagy at 10 uM, a 10-fold lower dose than required for chloroquine to obtain the same level of inhibition. Thus, we tested 2 uM and 10 uM doses. The sar405 inhibitor started blocking LC3B-II accumulation at 80nM, so doses of 100 nM and 500 nM were chosen for testing. We found Lys05 was at 10 uM completely blocked cell growth but 2 uM was not toxic

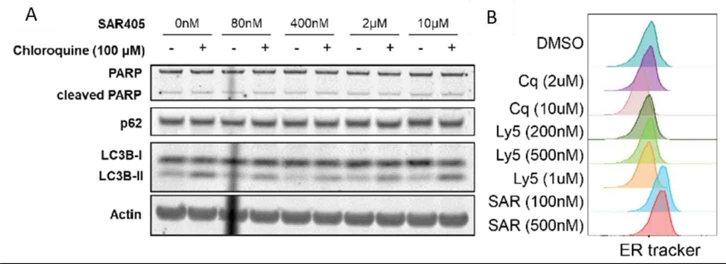


Figure 8. Inhibitors regulate B cell autophagy in a dose dependent manner. Autophagy was inhibited using lysosomal transfer inhibitors (Chloroquine [Cq], and Lys05 [Ly5]) or a PI3KC3 inhibitor (Sar405, [SAR]). Primary B cells were treated with the inhibitors during PB/PC differentiation cultures. Naïve B cells were isolated from healthy control subjects and stimulated with CD40 ligand, IL-2, IL-21 +/- CpG for 7 days (phase I), followed by differentiation for 4 days (phase II/III) in the presence of IL-6, IL-10, and IFNα. A) Cultures treated with the indicated concentrations of SAR405 during phase I were analyzed by western blot and showed decreased buildup of LC3 between 40nM and 2uM. B) Cells were stained for ER tracker and analyzed by flow cytometry to assess abundance of ER. Cultures showed increased ER abundance with SAR405 but decreased abundance with chloroquine and Lys05.

2. *Effect of autophagy inhibitors on macro- and TLR-induced autophagy and PB differentiation in primary B cells from healthy control subjects.* The ability of chloroquine, Lys05, and sar405 to inhibit autophagy was tested in primary B cells (**Figure 8**). LC3B-II accumulation was inhibited by sar405 between 40 nM - 2 uM. However, there was no effect of sar405 on endoplasmic reticulum (ER) abundance (**Figure 8B**) compared to the DMSO control, while chloroquine and Lys05 elicited a dose-dependent reduction in ER abundance. We then tested the ability of the inhibitors to block cell expansion and PB differentiation (**Figure 9**). We found that only Lys05 and chloroquine inhibited cell expansion, with a greater effect of Lys05 compared with chloroquine (**Figure 9A**). The sar405 inhibitor did not reduce cell expansion below levels detected with vehicle alone. Likewise, chloroquine and Lys05 blocked PB differentiation with a stronger effect of Lys05, but sar405 had no effect (**Figure 9B**). <u>Thus, the lysosomal transfer inhibitors chrloroquine and Lys05 can effective inhibit PB differentiation and autophagy.</u> The effect of chloroquine and Lys05 are now being tested in PB/PC differentiation cultures using genotyped primary B cells from healthy control and lupus donors to determine if increased PB differentiation and autophagy with the BANK1 risk variants can be ameliorated with these drugs.

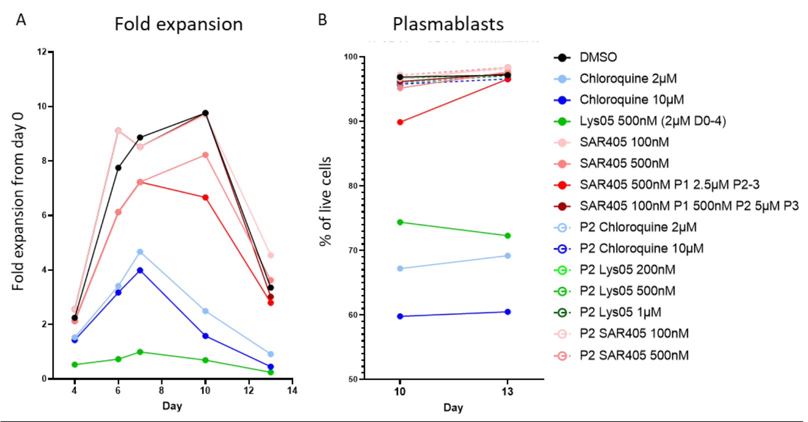


Figure 9. Autophagy inhibitors block cell expansion and PB differentiation. A) Cell expansion relative to the start of the culture (day 0). B) PB frequency on days 10 and 13 after culture initiation was determined by flow cytometry. Chloroquine and Lys05 blocked cell expansion and PB development but sar405 had no detectable effect.

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

While this study was not intended to provide training, it has nonetheless led to the development and refinement of new technologies and skill sets in the Cerosaletti and James labs. Janice Chen in the Cerosaletti lab has refined the plasmablast/plasma cell differentiation assay for use with primary human B cells, and optimized the flow cytometric readouts of differentiation and autophagy. In the James lab, Emma Suchland performed the autophagy and differentiation assays in cell lines and gene edited primary B cells. Iana Meitlis assisted with cloning and guide RNA design in Year 1 of the project. Ms. Meitlis has leveraged her successful period in the James lab for acceptance to medical school at the University of Washington. Professionally, we established a lab meeting between the Cerosaletti and James labs to share results, which provides an opportunity for lab members to present their results in front of peers and receive feedback. We have also established a Slack channel to share lab results in real-time.

How were the results disseminated to communities of interest?

No new communications regarding our results have been presented in Year 3, in part due to cancelation of research seminar series at our institutions and local lupus support groups as a result of COVID-19 restrictions.

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

This is the final technical report. Experiments are ongoing to test the inhibitor Lys05 in primary B cells carrying the risk or non-risk genotype. Additional analysis of BANK1 interacting proteins is ongoing. Final analysis and publication of the results of this study will take place in 2022.

4. IMPACT:

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

The results of this study have an impact on our understanding of the function of the *BANK1* gene in the autophagy pathway in B cells and plasmablast/plasma cell differentiation. Our results demonstrate that genetic variants in *BANK1* that are linked to risk for SLE appear to promote these pathways. Lupus patients carrying the risk variants in the *BANK1* gene had increased plasmablast and plasma cell development (IgG+) in vitro in the presence of TLR9 stimulation, and increased levels of autophagy. We showed that autophagy precedes plasmablast and plasma cell differentiation in vitro, consistent with a requirement for autophagy in these development pathways. This suggests that blocking autophagy could be a viable method to inhibit over-exuberant plasmablast development in lupus. Indeed, we found that the lysosomal transfer inhibitors chloroquine and Lys05 reduced autophagy and plasmablast differentiation in vitro. Notably, the Lys05inhibitor is 10-fold more potent than chloroquine in inhibiting autophagy which has potential implications for treatment of SLE. The gene editing tools used in this study impact study of primary human B cells in general because they allow investigators to change a single gene within the B cells from one individual and then test the impact of that specific change while leaving the remainder of the genome the same.

What was the impact on other disciplines?

Our findings with BANK1 have the potential to advance our understanding of other rheumatic diseases that have been associated with genetic variants in the *BANK1* gene, including rheumatoid arthritis and scleroderma.

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 for Year 3.

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

Nothing to report for Year 3.

Changes that had a significant impact on expenditures

Nothing to report for Year 3.

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

Nothing to report for Year 3.

Significant changes in use or care of vertebrate animals

Not applicable.

Significant changes in use of biohazards and/or select agents

Nothing to report for Year 3.

6. PRODUCTS:

Publications, conference papers, and presentations

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

We established a dual editing strategy to knock out the *BANK1* locus in a B cell lymphoma line and then deliver the BANK1 risk or non-risk cDNA into the *BANK1* locus using homology directed repair in Year 1 of this project. This technology has been shared within our institutions.

Inventions, patent applications, and/or licenses

Nothing to report.

Other Products

We have generated several B cell lymphoma cell lines by gene editing during Years 1 and 2. The HBL1 lymphoma line was edited to delete the *CCR5* locus (control) or the *BANK1* locus. We also generated HBL1 cells with dual editing to delete the *BANK1* locus and simultaneously knock-in the BANK1 risk or non-risk cDNAs into the *BANK1* locus by homology directed repair. DNA reagents generated for these experiments include *BANK1* guide RNAs, and the AAV repair templates for the *BANK1* risk or non-risk cDNA sequences.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project during Year 3?

1. Principal investigators:
Name: Karen Cerosaletti PhD (Benaroya Research Institute)
Project Role: Principal investigator
No change

Name: Rich James PhD (Seattle Children's Research Institute)
Project Role: Co-investigator
No change

2. Other personnel:
Name: Janice Chen MS (Benaroya Research Institute)
Project Role: Research technician III
No change

Name: Emmaline Suchland (Seattle Children's Research Institute)
Project Role: Research scientist
No change

Name: Iana Meitlis (Seattle Children's Research Institute)
Project Role: Research scientist
No change

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

PI: Karen Cerosaletti PhD
1. New awards since year 2 report:
Title: EP407: Immune Profiling for T1GER
Percent effort: 6.1%
Supporting agency: NIH/NIAID 2UM1AI109565-08 (Nepom)
Grants officer: N/A
Performance period: 2/1/2021-1/31/2022
Funding amount (total cost)
Project goals: Phenotypic and transcriptomic profiling of T1GER samples (patients treated with anti-TNFalpha compared to placebo) as well as T1GER samples following antigen stimulation
Overlap: None

Title: Does post-transcriptional control of NLRP3 inflammasome activity impact development of Type 1 Diabetes?
Percent effort: 2%
Supporting agency: NIH/NIAID 1R21AI149171-01A1 (Subramanian)
Grants officer: N/A
Performance period: 5/12/2021-4/30/2023
Funding amount (total cost)
Project goals: In this study, we propose that post-transcriptional regulatory elements in the 3'UTR (untranslated region) of a core inflammasome gene NLRP3 (Nod-Like Receptor Protein 3) control expression of NLRP3 and set the threshold for inflammasome activation and development of type 1 diabetes.
Overlap: None

Title: CRA: T1D Phase 0 study - T cell populations

Percent effort: 15%
Supporting agency: COUR Pharmaceuticals
Grants officer: N/A
Performance period: 11/1/2021-10/31/2022
Funding amount (total cost)
Project goals: Confidentiality agreement in place
Overlap: None

Title: CRA: GentiBio Infrastructure – Edited Tregs
Percent effort: 15%
Supporting agency: GentiBio, Inc. (Buckner)
Grants officer: N/A
Performance period: 04/01/2021-04/01/2023
Funding amount (total cost)
Project goals: Confidentiality agreement in place
Overlap: None

2. <u>Awards completed since year 2 report:</u>
Title: An integrated strategy to define the functional and synergistic impact of T1D causal variants
Percent effort: 10%
Supporting agency: NIH/NIDDK DP3 DK111802 (Rawlings)
Grants officer: N/A
Performance period: 09/30/16– 06/30/2021
Funding amount (total cost)
Project goals: The goal of this project is to determine how genes associated with T1D function individually and in combination to impair immune programs that contribute to T1D.
Overlap: None

Co-investigator Richard James PhD

1. <u>New awards since year 2 report:</u>
Title: Regulation and function of nuclear cGAS
Percent effort: 5%
Supporting Agency: NIH/NIAID R01AI150716-01A1 (Stetson)
Grant Officer: N/A
Performance Period: 10/01/2020 – 08/31/2025
Funding Amount: (Total Cost for All Years)
Major Goals: We have found that the DNA sensor cGAS, long thought to be a cytosolic protein, is a tightly tethered nuclear protein that is carefully regulated to prevent detection of self-DNA. We will define how nuclear tethering prevents cGAS autoreactivity and enables it to respond to foreign DNA.
Overlap: None

Title: Be Bio Sponsored Research Agreement
Percent effort: 7.5%
Supporting Agency: Be Biopharma
Grant Officer: N/A
Performance Period: 01/07/2021 – 01/06/2024
Funding Amount: (Total Cost for All Years)
Major Goals: We propose to develop a therapy to produce protein drugs using antibody-secreting B cells, or drug-secreting B cells. The focus of this research agreement is to further develop the drug-secreting B cell platform. We will do experiments to: 1) build methods for engineering non-human primate B cells, 2) de-risk engraftment of FIX-secreting B cells in an immune competent mouse model of hemophilia B, and 3) implement a suicide switch specific for drug-secreting B cells. Collectively, we envision that these studies will provide proof-of-concept data necessary for an investigational new drug designation for drug-secreting B cells for delivery of recombinant proteins that may eventually lead to a Phase I/II clinical trial.
Overlap: None

Title: The Center for Actionable Variant Analysis; measuring variant function at scale
Percent effort: 5%
Supporting Agency: NIH/NHGRI 1UM1HG011969-01 (Starita)
Grant Officer: N/A
Performance Period: 09/01/2021 – 05/31/2026
Funding Amount: (Total Cost for All Years)
Major Goals: The James Lab will provide support for scale-up of cloning-free saturation genome editing at the UW Brotman-Baty Advanced Technology (BAT) Laboratory. Activities will include support for implementation of cloning-free editing and development of primary cell multiplexed assays of variant effect.
Overlap: None

2. Awards completed since year 2 report:
Title: Approach to CFTR Editing in Differentiated Airway Epithelial Cells
Percent Effort: 10%
Supporting Agency: University of Washington/Cystic Fibrosis Foundation; SINGH15R0 (Singh)
Grant Officer: N/A
Performance period: 09/01/2019 – 09/30/2020
Funding Amount: (Total Cost for All Years)
Project Goals: The goal of this study is to develop gene editing strategies for replacement of the N- or C-terminal half of the CFTR gene, which encodes the protein that is defective in cystic fibrosis. We believe this editing strategy will rescue all possible mutation types in cystic fibrosis. We propose testing the approach in primary human airway epithelia isolated from subjects with cystic fibrosis.
Overlap: None

Title: An integrated strategy to define the functional and synergistic impact of T1D causal variants
Percent effort: 10%
Supporting agency: NIH/NIDDK DP3 DK111802 (Rawlings)
Grants officer: N/A
Performance period: 09/30/16– 06/30/2021
Funding amount (total cost)
Project goals: The goal of this project is to determine how genes associated with T1D function individually and in combination to impair immune programs that contribute to T1D.
Overlap: None

What other organizations were involved as partners?

Organization name: Seattle Children's Research Institute
Location: 1900 Ninth Ave, Seattle, WA 98101
Contribution: Co-investigator (Richard James PhD)

8. SPECIAL REPORTING REQUIREMENTS

Quad chart

Attached file: QuadChart LR170026 Cerosaletti_Final Report

List of equipment purchased with award funds (Final Report)

Nothing to report.

List of residual inventory of unused supplies exceeding $5,000 in value (Final Report)

Nothing to report.

Transition plan

No follow-on grants or CRAs have been proposed yet pending final results.

9. APPENDICES

Attached file: DD882-W81XWH-18-1-0596 Cerosaletti