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13. SUPPLEMENTARY NOTES
Drs. Kulis and Paulson are partnering PIs on this award. Dr. Matthew Macauley, previous Partnering PI, has moved to the University of Alberta (Edmonton, Alberta) and continues to participate under a sub-contract from TSRI.

14. ABSTRACT
During the third year of this award, we have continued to generate important data related to targeting of CD22 on B cells and CD33 on mast cells to abrogate food allergies. With our prophylactic and therapeutic models utilizing CD22 STALs established in the first two years of the award, we have now moved onto mechanistic studies. In particular, we have used peanut allergen tetramers to enrich peanut-specific B cells from mice. Optimization of the tetramer reagents using Ara h 1, 2, 3, and 6 proteins was completed, and initial studies carried out with mouse splenocytes. Using human CD22 transgenic mice, we have been able to demonstrate in our conferred sensitization allergy protocol that full tolerance can be generated to Ah1 through human CD22. In terms of targeting CD33 in this past year, we have moved into human whole blood basophil activation assays (BATs) to demonstrate the peanut proteins coupled with CD33 ligand can suppress degranulation responses ex vivo. Our initial studies indicate that simultaneous engagement of human CD33 and Ara h 2-IgE leads to suppression of further Ara h 2-induced degranulation. The results offer promise for developing therapies for patients with allergies and allergic asthma to desensitize mast cells and improve approaches for inducing tolerance to the offending antigens.

15. SUBJECT TERMS
Food allergy; peanut allergy; Siglec; IgE; Ara h 2; CD22; CD33; mast cell; basophil

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1. INTRODUCTION:

In this pre-clinical, translational project, we will utilize mouse models, human B cells, and human mast cells and basophils to assess the ability of Siglec-engaging Tolerance-inducing Antigenic Liposomes (STALs) to induce immunological tolerance to peanut allergens. STALs are bioengineered nanoparticles that co-display a selected antigen and high affinity Siglec ligand. STALs targeting the Siglec CD22 on B cells induce antigen-specific B cell tolerance through deletion of the B cells recognizing the antigen. Applying this approach to animals with an existing peanut allergy will allow us to deplete memory B cells responsible for producing IgE, and establish a novel therapeutic strategy for food allergies. STALs targeting the human Siglec CD33 will be used to desensitize mast cells. This approach will be investigated as a therapeutic strategy for preventing acute allergic reactions, allowing for tolerizing doses of antigen to be delivered safely. By exploiting the inhibitory functions of CD22 on B cells, and CD33 on mast cells and basophils, our primary objectives are (1) to develop a novel prophylactic and therapeutic approach for peanut allergy and (2) to develop a targeted approach to prevent mast cell and basophil degranulation to peanut allergens.

2. KEYWORDS:

Food allergy; Peanut allergy; Siglec; CD22; CD33; STAL; nanoparticle; Ara h 2; mast cell; basophil; B cell

3. ACCOMPLISHMENTS:

- **What were the major goals of the project?**

Specific Aim 1: **Establish the therapeutic potential of Ara h 2 STALs targeting CD22 to abrogate peanut allergies.**

- ***Major Task 1***: Determine optimal conditions to induce B cell tolerance to Ara h 2 and whole peanut extract in a prophylactic mouse model.
  Target date: Months 1-12; *percentage of completion*: **90%**
- ***Major Task 2***: Use Ara h 2 STALs to induce tolerance by deletion of memory B cells
  Target date: Months 10-30; *percentage of completion*: **75%**
- ***Major Task 3***: Determine translatability of STALs to human CD22 and human B cells
  Target date: Months 5-24; *percentage of completion*: **50%**

Specific Aim 2: **Demonstrate the applicability of Ara h 2 STALs targeting CD33 to prevent mast cell- and basophil-mediated allergic responses to peanut allergen.**

- ***Major Task 1***: Determine inhibitory effects and longevity or inhibition using LAD-2 mast cells
  Target date: Months 1-7; *percentage of completion*: **100%**
- ***Major Task 2***: Determine inhibitory effects and longevity of inhibition using Human Basophils
  Target date: Months 7-18; *percentage of completion*: **40%**
- ***Major Task 3***: Determine preventive effects of STALs targeting CD33 on mast cells in vivo in allergic mice
  Target date: Months 6-30; *percentage of completion*: **80%**
- ***Major Task 4***: Determine therapeutic utility of STALs targeting CD33 and CD22 simultaneously in allergic mice
  Target date: Months 18-36; *percentage of completion*: **25**%

- **What was accomplished under these goals?**

This report is for a Partnering PI project with James C. Paulson at The Scripps Research Institute (TSRI) as Partnering Investigator and Michael Kulis at the University of North Carolina (UNC) as Principal Investigator. Dr. Matthew Macauley was originally Partnering PI at TSRI and moved to the University of Alberta (UofA), where he continues in the project under a subcontract from TSRI. In this current project period, the work was conducted in the laboratories at UNC, TSRI, and UofA and accomplishments listed below we have noted which institution/investigator was involved in the experiments.

Specific Aim 1: **Establish the therapeutic potential of Ara h 2 STALs targeting CD22 to abrogate peanut allergies.**

***Major Task 1*: Determine optimal conditions to induce B cell tolerance to Ara h 2 and whole peanut extract in a prophylactic mouse model.**

Previously, we reported that prophylactic tolerization of BALB/c mice to Ara h 2 (Ah2) using a CD22-targeted approach results in complete tolerance of mice following sensitization with peanut extract followed by a challenge with soluble Ah2 (Orgel et al., JACI, 2017). In that manuscript, we also described that Ah2 STALs-treated mice were also significantly protected from a challenge with peanut extract, however, this protection was not complete. This lack of complete protection was potentially due to minor responses from the other peanut allergens, such as Ah1, Ah3, and Ah6. Accordingly, the most robust form of tolerance to peanuts would need to consider for these other allergens.

As described in our original proposal, we proposed that all four of the major peanut allergens (Ah1, Ah2, Ah3, and Ah6) would be tested either together or separately, with the goal of achieving maximal tolerance to whole peanut extract. Due to the challenges in working with multiple allergens in parallel, we are still working on conditions that can produce tolerance to all 4 simultaneously. Therefore, experiments are ongoing with these STALs in both BALB/c mice (Dr. Kulis at UNC) and in B57BL/6 mice (Dr. Macauley at UofA). The goal is to use multiple allergen STALs to completely protect mice from the whole peanut extract challenges.

***Major Task 2*: Use Ara h 2 STALs to induce tolerance by deletion of memory B cells**

During the previous reporting period, we demonstrated the ability to suppress memory B cell responses to Ah2 in an adoptive transfer model. We showed that CD22-targeted Ah2 STALs was effective in abolishing antibody responses and anaphylaxis upon challenge in this transfer model, and that there was a dose-response with the STALs. During the current reporting period, we have now moved onto a mechanistic approach to better understand how exactly the STALs elicit their effects. As we discussed in our original proposal, we want to utilize peanut allergen tetramers to identify and quantify the number of peanut allergen B cells in mice treated with STALs or the antigenic liposomes. To do this, we sent purified peanut allergens (Ah1, 2, 3, and 6) to Justin Taylor at the Fred Hutchinson Cancer Research Center who then optimized the construction of tetramers for each allergen. **Figure 1** below shows our approach to enriching the peanut allergen-specific B cells from mouse spleens. Briefly, mice are first sensitized to peanut (PN) or left unexposed to peanut (naïve) following our typical sensitization protocol. Next, mice are euthanized and spleens removed to prepare single cell preparations.

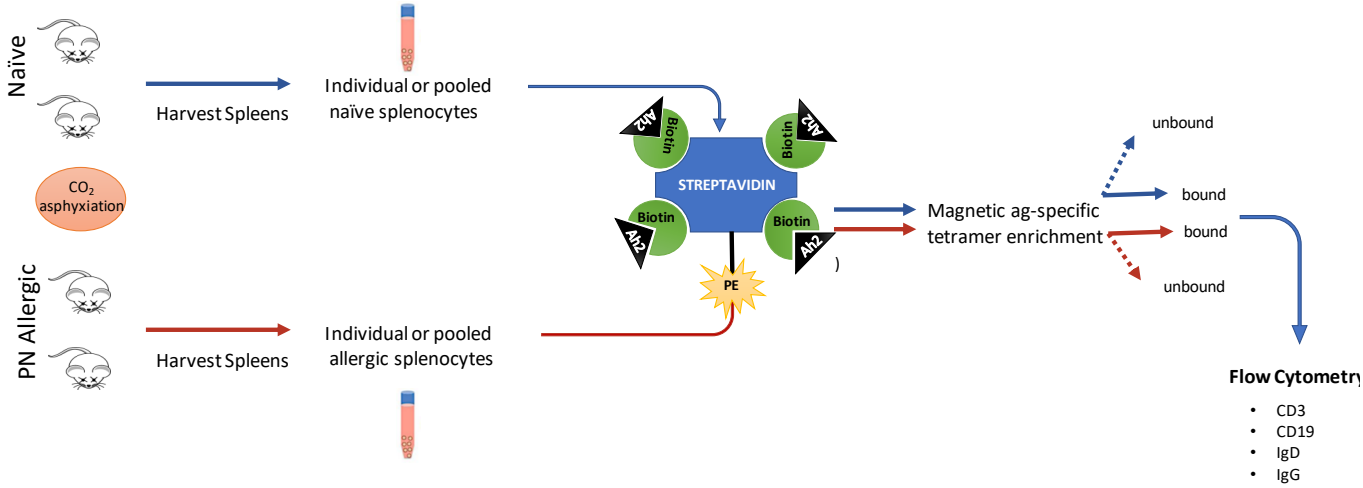

Figure 1. Experimental design for enriching peanut-specific B cells from mouse splenocytes.

Tetramers are added to the cell suspension and then separated using magnetic enrichment columns with anti-PE beads. Finally, flow cytometry is used to demonstrate mouse B cells that are bound to the tetramers, i.e. allergen-specific B cells.

After several pilot experiments using the tetramers, we investigated how these reagents performed in naïve and peanut allergic BALB/cJ mice. **Figure 2** shows our gating strategy for both the magnetically unbound fraction (i.e. flow-through) and the bound fraction. We were able to enrich B cells specific for peanut by pooling all four tetramers (Ah1, 2, 3, and 6) as well as enriching B cells specific for each peanut allergen. A summary of our data from four naïve and four PN allergic mice is shown in **Figure 3**.

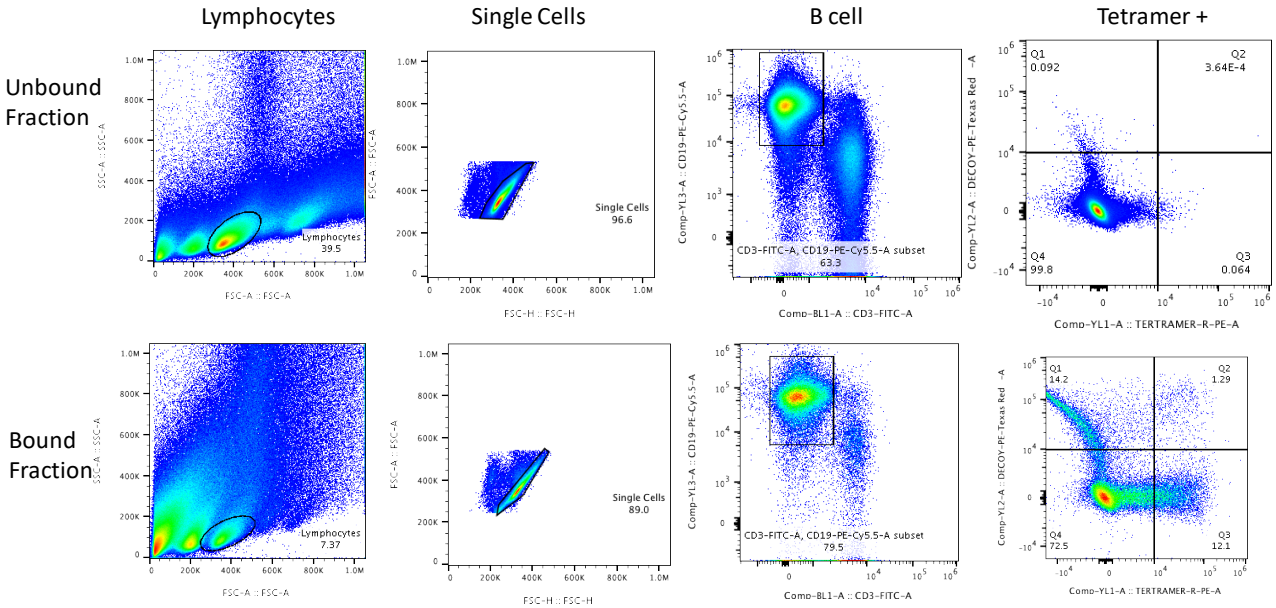

Figure 2. Gating strategy for the identification of tetramer-binding B cells. Lymphocytes are gated first, followed by single cells, then B cells, and finally peanut and decoy tetramers. In this example, the Ara h 1-specific B cells appear in the lower right quadrant of the Tetramer+ gate. While the unbound fractions contains few cells, there is a clear population in the bound fraction.

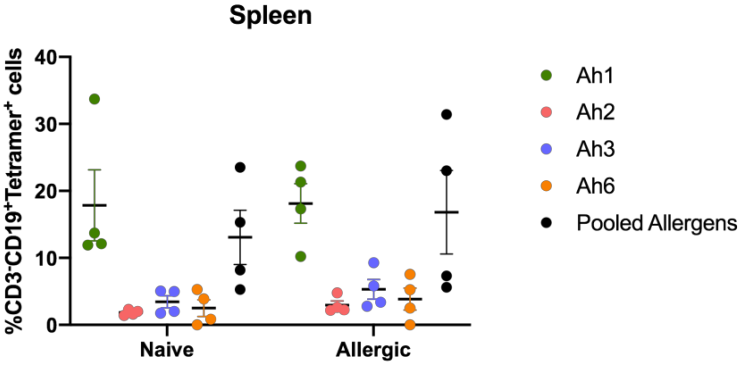

Figure 3. Data from individual naïve and peanut allergic mice showing that individual Ah1, 2, 3, and 6 allergen tetramers can identify these specific B cells in mice. Pooled allergen tetramers also work well and were used in the initial optimization experiments.

***Major Task 3*:** Determine translatability of STALs to human CD22 and human B cells

Previously, we reported the development of a new human CD22 (hCD22) transgenic mouse (Bednar et al, J Immunol, 2017). In our original proposal, we planned to use these mice to determine if tolerance induction of peanut allergens through hCD22 is equally as robust as through mCD22 (mCD22). However, we found that Ah2 (and Ah6) are poorly immunogenic in B6 mice, while Ah1 and Ah3 strongly immunogenic. Last year we reported that tolerance could be generated by STALs displaying Ah1 and Ah3. In this past year, we have taken these finding and applied them to our hCD22 transgenic line. Specifically, we carried out our conferred sensitivity model in these mice that express hCD22 (but not mCD22) exclusively on B cells. To accomplish this task required working with a different glycan ligand to target hCD22 since its specificity differs compared to mCD22. The hCD22 ligand was provided by the Paulson lab for these studies. Using Ah1, we find that Ah1 STALs induce full tolerance in the adoptive transfer model. Specifically, hCD22 mice adoptively transferred with peanut-sensitized splenocytes failed to respond to a subsequent challenge with Ah1 if administered Ah1 STALs, but not Ah1 liposomes (**Figure 4**). Examining titers to Ah1 in these mice, we see that mice were strongly protected from developing anti-Ah1 titers (**Figure 5**).

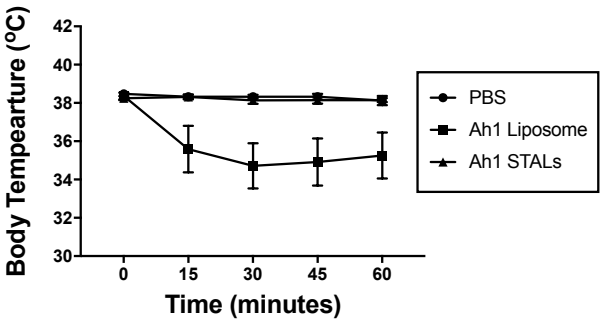

Figure 4. hCD22 transgenic mice are protected from a challenge with Ah1 in the conferred model of peanut allergy. hCD22 mice were sensitized with peanut extract and pooled splenocytes were adoptively transferred into recipient naïve hCD22 mice. One day after transfer, mice were mice were administered PBS, Ah1 liposomes, or Ah1 STALs. Two weeks later, mice were challenged Ah1 and body temperature was measured every 15 minutes.

We are also invested in testing the Ah2 STALs targeting CD22 on human B cells from peanut allergic patients *in vitro*. To do this, we need to develop an assay wherein we obtain blood from an allergic patient, separate out the B cells, stimulate with B cell activating factors, and measure IgG against Ah2. In our previous report, we described the develop of an assay using healthy control patient B cells. We are now very comfortable isolating B cells via negative selection. While initial data was promising, we were unsuccessful in producing detectable levels of Ara h 2-specific IgG from peanut allergic donors. We believe that the cell frequency is exceedingly low and does not allow for much, if any, production of allergen-specific IgG. Drs. Macauley and Kulis have been in contact with a leading expert in this area - Dr. Manel Jordana, McMaster University - who has an unpublished method that is robust. We are currently working on adopting Dr. Jordana's approaches for similar assays.

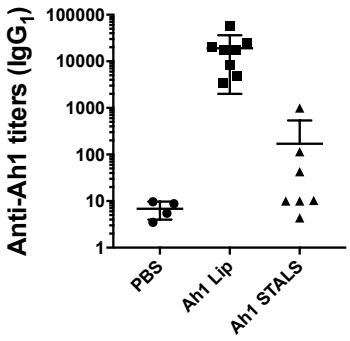

Figure 5. Ah1 STALs prevent the development of anti-Ah1 titers in the conferred allergy model. Mice from Fig 4 were bled at the end of the experiment and levels of anti-Ah1 were determined in the serum.

Specific Aim 2: Demonstrate the applicability of Ara h 2 STALs targeting CD33 to prevent mast cell- and basophil-mediated allergic responses to peanut allergen.

Major Task 1*: *Determine inhibitory effects and longevity or inhibition using LAD-2 mast cells.

Significant Results and Achievements: This task was completed in the first year of the project as reported in the progress report last year.

Major Task 2: Determine inhibitory effects and longevity of inhibition using Human Basophils

In this reporting period, we made significant advances in applying the CD33 STALs platform to human basophil assays. Dr. Macauley provided PEGylated lipid to Ah2 and shipped this to Dr. Paulson. Dr. Paulson's team then coupled the Ah2 and human CD33 ligand to liposomes, thus generating the Ah2 CD33 STALs.

Dr. Kulis tested the Ah2 CD33 STALs in two types of assays from four different peanut allergic patients. The first assay was a straight-forward test of applying either Ah2-liposomes (Ah2-LP) or Ah2 CD33 STALs (Ah2-CD33L) to whole blood from humans with peanut allergy. Basophil activation was then assessed by staining the blood for markers to identify basophils (CD123 and CD203c) and looking at CD63 as a marker of degranulation. As

shown in **Figure 6**, Ah2-LP led to significantly more degranulation of basophils (%CD63+) than the Ah2-CD33L. We tested 1 µg/mL and 0.1 µg/mL doses and found similar trends for both doses. These data indicate that co-displaying CD33 ligand with Ah2 drastically decreases the amount of basophil degranulation. This finding is encouraging for further translational development of CD33 STALs in the treatment of food allergy.

The second assay was designed to test whether pre-incubation with Ah2-CD33L STALs could prevent further responses to Ah2 stimulation. We first added either PBS as a sham control, or Ah2-CD33L to whole blood from peanut allergic donors for 1 hour, then stimulated with Ara h 2 in the form of Ah2-LP. The data demonstrates that Ah2-CD33L pre-treatment dramatically reduces further degranulation in response to Ah2 (**Figure 7**). We plan to repeat this assay using several more peanut allergic donors.

We anticipate reproducing these findings and then writing a manuscript on this data in the next reporting period.

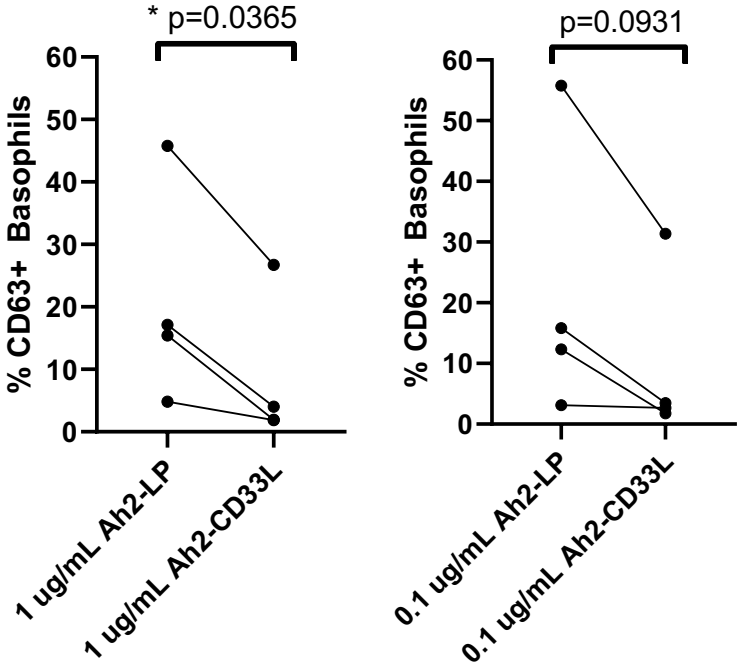

Figure 6. Whole blood basophil activation testing performed with Ah2-LP versus Ah2-CD33L. Data is shown from four individuals with peanut allergy. Ah2-CD33L has significantly lower ability to activate human basophils than Ah2-LP, indicating that CD33 ligand can suppress basophil

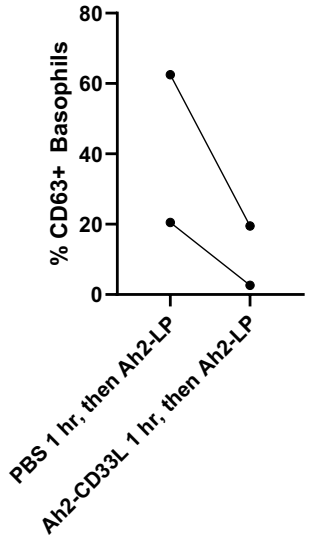

Figure 7. Whole blood human basophil activation testing with pre-treatment of Ah2-CD33L. The data indicate that pre-treatment of human basophils with Ah2-CD33L can suppress subsequent stimulation with Ah2.

Major Task 3: Determine preventive effects of STALs targeting CD33 on mast cells in vivo in allergic mice.

Significant Results and Achievements: Last year we presented major progress in demonstrating desensitization of mast cells using STALs co-presenting both CD33 ligand (CD33L) and TNP in transgenic mice expressing hCD33 on mast cells. This work is now published as indicated below (Duan et al (2019) J. Clinical Invest. 129, 1387).

We have subsequently been working towards demonstrating the utility of STALs in actively sensitized mice. To this end we have acquired several strains of mice. One is a mouse with human FcεRI receptor replacing the murine FcεRI receptor so we could use human anti-allergen IgE, and the other is a mutant IL-4 receptor mouse (F709) that has been reported to generate IgE mediated anaphylaxis upon oral challenge following sensitization by gavage with ovalbumin or peanut butter.[1]

We have now demonstrated that STALs suppress IgE mediated anaphylaxis in the hFcεRI x hCD33 mice sensitized with anti-OVA hIgE (**Figure 8**).

Day 0, 6 PM: Sensitized with 5 ug human anti-OVA IgE clone 11B6, i.v.
Day 1, 9 AM: Treated with PBS or 1 mM 0.1%OVA-LP-3%CD33L (200 ul), i.v.
Day 1, 4 PM: Challenged with 1 mM 0.1%OVA-LP (200 ul), i.v.

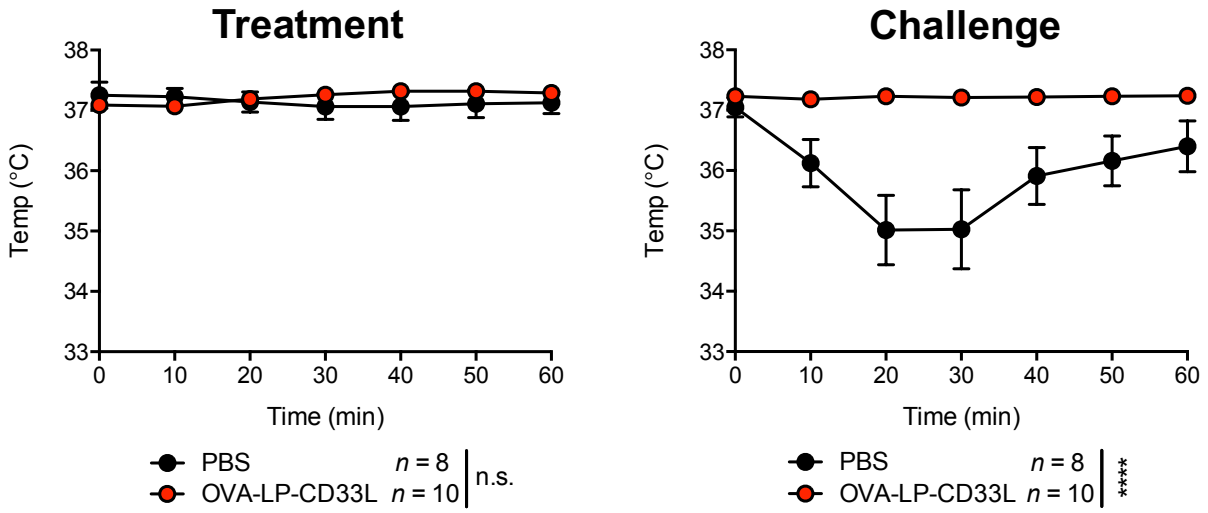

Figure 8. Desensitization of hFcεRI+ hCD33+ mice to IgE mediated anaphylaxis upon OVA challenge.

We have also bred the F709 mice to obtain a on wild type C57Bl/6 and C57Bl/6 x mFcεR1 KO background to allow testing IgE dependent anaphylaxis in sensitized mice, given the known problem of IgG mediated anaphylaxis. To date, however, we have been unable to generate IgE mediated anaphylaxis. This is illustrated in tests with mice sensitized by gavage (i.g.) with Ovalbumin and Staphylococcyl enterotoxin B (SEB) according to the published procedures. No anaphylaxis is observed after 8 weeks sensitization when challenged with oral OVA. When challenged with OVA-liposomes, anaphylaxis is observed, but it is not IgE dependent since the same anaphylaxis is seen in the FcεRI KO mice (**Figure 9**). Direct assessment of the anti-OVA IgE and total IgE responses following oral sensitization reveals that the lack of IgE mediated anaphylaxis is due to a complete lack of the induction of anti-OVA IgE.

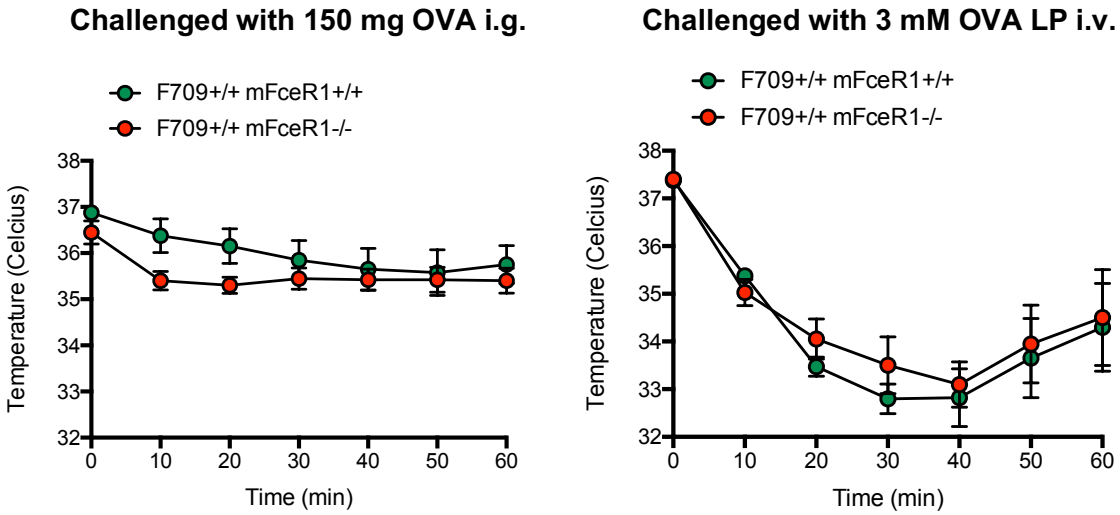

Figure 9. Systemic anaphylaxis in IL-4 F709 mice immunized with oral OVA (250 µg OVA and 10 µg SEB) for 8 weeks is not observed with oral OVA challenge, and while seen with i.v. challenge using OVA-LP and is not IgE/FcεRI dependent.

We are now in the process of doing more intensive sensitization with intragastric feeding with OVA and peanut butter using both SEB and cholera toxin as adjuvants. Ultimately, we expect to be successful in raising IgE responses during sensitization, but we remain concerned about distinguishing between IgE and IgE mediated anaphylaxis. To directly address this, we have requested and are in the process of obtaining FcγRIII KO and FcγRI/RII/RIII/RIV KO mice from Sjef Verbeek in the Netherlands. Since the FcγRIII receptor is responsible for IgG dependent anaphylaxis, we expect that adding this mutation to our existing strains will provide a mouse that will only undergo IgE mediated anaphylaxis even if anti-IgG is produced.

Reference cited:

1. Mathias, C.B. et al. IgE-mediated systemic anaphylaxis and impaired tolerance to food antigens in mice with enhanced IL-4 receptor signaling. *J Allergy Clin Immunol* **127**, 795-805 e1-6 (2011).

Major Task 4: Determine therapeutic utility of STALs targeting CD33 and CD22 simultaneously in allergic mice .

Significant Results and Achievements: This Task requires sensitized mice that undergo IgE mediated anaphylaxis. We are also constrained to the C57Bl/6 strain since hCD33 is on a C57Bl/6 background. As described above, we do not yet have a robust protocol for sensitizing mice to allergen that will produce IgE mediated anaphylaxis. This is absolutely required since it is needed to show that the CD22 STALs will prevent increased production of the IgE, and that the CD33 will prevent anaphylaxis resulting from the exposure to

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

  At UNC: During the current reporting period, Lakeya Hardy, a graduate student in Dr. Kulis' lab, was awarded a Graduate Diversity Enrichment Program (GDEP) fellowship sponsored by the Burroughs Wellcome Fund. The title of her project sponsored under the award is: Using STALs to exploit CD22 on peanut-specific memory B cells to induce tolerance.

  As in previous reporting periods, this work has also contributed to the development of other trainees in Dr. Kulis' group. A postdoctoral researcher (Johanna Smeekens) and two graduate students (Lakeya Hardy and Jada Suber) have been involved with the projects at UNC. Dr. Kulis is responsible for mentoring the three trainees and meets with them individually on a weekly basis to go over experimental progress. This project has allowed for training in various experimental techniques, including mouse procedures, working with human B cells, ELISA, and flow cytometry. Additionally, all three trainees have attended two major conferences, the Gordon Research Conference on Food Allergies (January 2018) and the American Academy of Allergy, Asthma, and Immunology (March 2018), presenting their research. Other career development gained by this project have included opportunities to network with investigators at TSRI and UofA and opportunities to discuss their findings at departmental seminars at UNC.

  At TSRI: An Individual Development Plan (IDPs) was updated for Shiteng Duan (Grad Student) to continue monitoring the training and progress in developing his scientific career. He received one-on-one guidance from Dr. Paulson (Mentor) on a daily basis and presented several times at regular lab meetings. He also presented his work in the form of posters/oral presentations at local and international meetings/symposiums. In May 2019 Shiteng graduated with his PhD at Scripps Research, and in August 2019 he accepted a position at the Genomics Institute of the Novartis Research Foundation in San Diego, CA.

  At UofA (sub-contract to TSRI): Dr. Macauley met on a daily basis with post-doctoral fellow Gour Daskan and graduate student Kelli McCord and has provided hands on training on working with allergens, formulating liposomes, and immunizing mice. All members of our team actively participate in groups meetings and attend local GlycoNet meetings. The postdoctoral and graduate students' offices at UofA facilities many courses to assist in writing in communicating, and all trainees in the Macauley lab are encouraged to take advantage of these resources.

- **How were the results disseminated to communities of interest?**

  Scientific meetings/conference/symposia and publications (see below).

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

  At UNC

  For Aim 1, Major Task 1: We will test Ah1, 2, 3, and 6 in a prophylactic model using BALB/cJ mice. We will quantify allergen-specific IgE and IgG1 and will assess anaphylaxis during challenges with whole peanut extract. The STALs will be prepared by Dr. Macauley's group at UofA with CD22 ligand provided by Dr. Paulson. We will work with the peanut tetramers to study effects of CD22 STALs on the number of peanut-specific B cells.

  For Aim 1, Major Task 2: We will continue working with the conferred allergy model to better understand how the Ah1, 2, 3, and 6 STALs are depleting memory B cells. We will utilize the B cell tetramers we optimized in this reporting period. We anticipate publishing these data once we have a clearer picture of mechanistic correlates with the CD STALs in vivo findings.

  For Aim 1, Major Task 3: We will develop the human B cell assay to quantify Ah2-speicific IgG/IgE and utilize Ah2 CD22-STALs to investigate the translational properties of the STALs. This work will be done with input and guidance from Dr. Manel Jordana's team that have optimized a similar assay.

  For Aim 2, Major Task 2: We will assess the CD33 STALs in our human basophil activation assay (BAT). Ah1, 2, 3, and 6 STALs will be prepared by Dr. Paulson's group at TSRI and tested in the BAT assay by Dr. Kulis at UNC. We aim to demonstrate that the combined Ah1, 2, 3, and 6 CD33 STALs can prevent degranulation to whole peanut extract. Once we have obtained more replicates and completed further testing with additional peanut allergens, we plan to write a manuscript and publish our findings.

  At TSRI

  The majority of the remaining funds for in the Partnering Award are at the University of Alberta in Edmonton, under the direction of Dr. Mathew Macauley. The major emphasis at TSRI will be to establish the sensitized allergen model. TSRI will continue to prepare and provide peanut antigen liposomes/STALs for Dr. Kullis at UNC (Aim2, Major Task 2: Peanut antigen (Ah1, Ah2, Ah3, and Ah6)) and Dr. Macauley with reagents as needed.

  In other work directly related to this project (Aim2, Major Task2) we have discovered that CD22 and CD33 ligands conjugated directly to anti-bodies that bind to the BCR or to the IgE/FcεRI complex can replace liposomes for suppression of B cell and mast cell activation. We intend to file a patent on this work, and if so, will report on it next year.

  At UofA

  As the next steps, we are doing the following: (1) continuing to optimize using different allergens simultaneous with the goal of fully protecting against a challenge with peanut extract; (2) carry out more experiments in hCD22 transgenic mice to, for example, demonstrate that tolerance can be induced to Ah3. We will continue to provide TSRI and UNC with lipid-linked peanut allergens.

4. IMPACT:

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

  Aim 1: Demonstration that STALs targeting CD22 can deplete memory B cells in an adoptive transfer model indicates that utilizing these nanoparticles could be a viable approach to deplete the allergy-causing B cells in allergic individuals. Furthermore, studies with peanut tetramers will allow us to study the effects that STALs have on B cells in vivo (mouse) and ex vivo (humans). The tetramers will be a powerful tool to study peanut-specific B cells in other allergy-related projects, such as clinical trials with oral and sublingual immunotherapy.

Aim 2: Demonstration that STALs can suppress antigen-mediated activation of mast cells and basophils and desensitize mice to subsequent response to antigen challenge suggests the potential for translation to managing treatment of patients exposed to allergens for 'allergen shots' to develop tolerance, or administration of medicines to allergic individuals.

- **What was the impact on other disciplines?**

  - Generation of a novel mouse model to study memory B and T cell responses in the absence of circulating antibodies is a valuable model to the field of food allergy research.
  - Generation and optimization of peanut allergen tetramers provides an important set of reagents to track B cell responses to therapies in mouse models and in human patients.
  - Creation of a novel transgenic mouse with hCD33 expressed on microglial cells will be a valuable tool to study the importance of CD33 as a risk factor in Alzheimer's disease.

- **What was the impact on technology transfer?**

  Licensing the hCD33-Tg mice to pharma companies will facilitate the development of new medicines to treat allergy and Alzheimer's disease

- **What was the impact on society beyond science and technology?**

  Nothing to Report

5. CHANGES/PROBLEMS:

- **Changes in approach and reasons for change**

  There were no significant changes to the approach during the reporting period

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

  Aim 2, Major Task 3 and 4: To complete Major Tasks 3 and 4, the impact of STALs need to be tested in peanut sensitized mice that exhibit IgE mediated mast cell degranulation and anaphylaxis upon peanut antigen challenge. The peanut sensitized model in Dr. Kulis' group uses BalbC mice. Since both hCD22 and hCD33-Tg mice are on a C57BL/6 background, Dr. Macauley at the Univ. of Alberta found that the same sensitization protocol with the C57BL/6 strain resulted in sensitivity to Ah1 and Ah3, but not to Ah2. Another concern is that allergen induced anaphylaxis in C57BL/6 can be mediated by IgG instead of IgE. To insure that we will get mice sensitized to peanut antigen that results in IgE/mast cell mediated anaphylaxis, we will focus on the robust IL-4R-F709 mouse model, which we now have in hand, and we are optimistic we will be successful since these transgenic mice have been found to produce an anti-peanut antigen IgE upon oral gavage with peanut butter, and produces IgE mediated anaphylaxis in response to challenge with peanut antigen. This model will provide a back-up to the sensitized mouse models being evaluated by Dr. Macauley. We have also discussed this in the achievement section for Aim2: Major Task 3.

- **Changes that had a significant impact on expenditures**

  Since Dr. Macauley moved to the University of Alberta in year 2 of this award, there was some slow-down in the experimental work. Therefore, we have requested and been granted a no-cost extension for the next 12 months.

- **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:

- **Publications, conference papers, and presentations**

  Journal publications:

  - Duan S, Koziol-White CJ, Jester WF Jr, Nycholat CM, Macauley MS, Panettieri RA Jr, Paulson JC. (2019) CD33 recruitment inhibits IgE-mediated anaphylaxis and desensitizes mast cells to allergen. J Clin Invest. 129(3):1387-1401. [PMCID: PMC6391081]
  - Bednar KJ, Nycholat CM, Rao TS, Paulson JC, Fung-Leung WP, Macauley MS. (2019) Exploiting CD22 to Selectively Tolerize Autoantibody Producing B-cells in Rheumatoid Arthritis. ACS Chem Biol. 14(4):644-654. [PMID: 30835424].
  - Enterina JR, Jung J, Macauley MS. (2019) Coordinated roles for glycans in regulating the inhibitory function of CD22 on B cells. Biomedical Journal. 42(4):218-232.

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

  - None

  Other publications, conference papers, and presentations

  - **Abstracts and presentations at conferences: Michael Kulis (PI)**
    - Invited Oral Speaker – March 2019
      NIH B.E.S.T. Partnership, Raleigh, NC
      Broadening **E**xperiences in **S**cientific **T**raining - Shaw University
      Title: Using Siglec-engaging Tolerance-inducing Antigenic Liposomes (STALs) to exploit CD22 on peanut-specific memory B cells to induce tolerance.
    - Invited Oral Presentation – April 2019
      Department of Biology, Fayetteville, NC
      Fayetteville State University
      Title: Using Siglec-engaging Tolerance-inducing Antigenic Liposomes (STALs) to exploit CD22 on peanut-specific memory B cells to induce tolerance.
    - Invited Oral Presentation – September 2019
      UNC Translational Medicine Program, Chapel Hill, NC
      The University of North Carolina at Chapel Hill
      Title: Using Siglec-engaging Tolerance-inducing Antigenic Liposomes (STALs) to exploit CD22 on peanut-specific memory B cells to induce tolerance.
    - Poster Presentation – October 2019
      Graduate Diversity Enrichment Fellowship, Durham, NC

Burroughs Welcome Fund
Title: Using Siglec-engaging Tolerance-inducing Antigenic Liposomes (STALs) to exploit CD22 on peanut-specific memory B cells to induce tolerance.

- **Oral presentations: James C. Paulson (Partnering PI)**
  - Lecture – April 1, 2019
    University of Missouri, St. Louis, Missouri
    Title: Glycan Recognition of Self and non-Self
  - Lecture – April 10, 2019
    ETH University, Zurich
    Title: Exploiting inhibitory Siglecs to modulate immune responses
  - Lecture – April 11, 2019
    Geneva University, Switzerland
    Title: Exploiting inhibitory Siglecs to modulate immune responses
  - Lecture – April 12, 2019
    Universität Basel, Switzerland
    Title: Exploiting inhibitory Siglecs to modulate immune responses
  - Beilstein Glyco-Bioinformatics Symposium – June 25-27, 2019
    Dom Hotel Limburg, Germany
    Title: Targeting Siglecs to suppress allergies
  - International Glycoconjugates (Glyco25) – August 25-31, 2019
    University of Milano, Italy
    Title: Siglecs as checkpoints in regulation of immune responses

- **Poster/Oral presentations: Shiteng Duan (Grad student)**
  - San Diego Glycobiology Symposium – February 1-2, 2019
    San Diego, California
    Poster title: Exploiting CD33 to suppress IgE-dependent anaphylaxis
  - FASEB: The IgE and Allergy Conference – July 7-12, 2019
    Scottsdale, Arizona
    Poster title: CD33 recruitment inhibits IgE-mediated anaphylaxis and desensitizes mast cells to allergen

- **Oral presentations: Macauley S Macauley (Sub-contract at UofA)**
  - Society for Glycobiology – Nov 7, 2018
    New Orleans, USA
    Title: New Reagents and Approaches for the Discovery of Siglec Ligands.
  - Lecture – Feb 23, 2019
    Academia Sinica, Taipei, Taiwan
    Title: Probing the Biological Roles of Siglecs using Chemical and Genetic Approaches.
  - Lecture – April 14, 2019
    Laval Univeristy, Quebec City, Quebec
    Title: New Approaches and Tools to Study Siglecs.

- **Website(s) or other Internet site(s)**

  Nothing to Report

- **Technologies or techniques**

  At TSRI an additional novel strain of transgenic mice was created using the Rosa26-hCD33 strain we had used to develop the strain with hCD33 expressed in mast cells described in Aim 2, Task 3. The new strain of mice has hCD33 expressed in brain microglial cells, and is of particular utility for the study of the roles of microglial cells in Alzheimer's disease

- **Inventions, patent applications, and/or licenses**

  TSRI has licensed the novel strain of transgenic mice with hCD33 expressed in microglial cells to one pharmaceutical company, and is in the process of licensing these mice to a second pharmaceutical company

- **Other Products**

  Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

- **What individuals have worked on the project?**

University of North Carolina – Chapel Hill (UNC)

Mike Kulis, PhD (Initiating PI) – unchanged

Rishu Guo, PhD (Research Scientist) – unchanged

Johanna Smeekens, PhD (Postdoc Scientist) – Unchanged

Lakeya Hardy (Grad Student) – unchanged

Kelly Orgel (Grad Student) – unchanged

Jada Suber (Grad Student) – unchanged

Xiaohong Yue (Research Associate) – unchanged

The Scripps Research Institute (TSRI)

James C. Paulson (Partnering PI) – unchanged

Shiteng Duan (Grad Student) – unchanged (last day worked July 26, 2019)

Kevin Worrell (Research Assistant) – unchanged

Joana Juan (Research Assistant) – unchanged (last day worked April 26, 2019)

Name:	Jasmine Stamps
Project Role:	Research Assistant
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Replacement for Joana - performed all the mouse genotyping and helped setup the appropriate mouse breeders. Also performed retro-orbital bleeds and analyzed antibody titers by ELISA
Funding Support:	N/A

University of Alberta (UofA)

Matthew Macauley (Subcontractor) – unchanged

Susmita Sarkar (Research Technician) – unchanged

Maju Joe (Post Doctoral Associate) – last day worked September 1, 2018

Dharmendra Raghuwanshi (Post Doctoral Associate) – last day worked October 1, 2018

Name:	Kelli McCord
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6
Contribution to Project:	Preparing antigen and liposomes
Funding Support:	N/A

Name:	Gour Daskan
Project Role:	Postdoctoral fellow
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6
Contribution to Project:	Linking glycans (provided by Paulson lab) to lipid
Funding Support:	N/A

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

  For Dr. Kulis at UNC, nothing significant to report

  For Dr. Paulson is the Partnering Investigator at TSRI
  - NIH grant #R01AI050143 ended 09/30/2019; #P01HL107151 ended 05/30/2019; UM1AI100663 ended 06/30/2019
  - NIH Grant R01AI132790 was funded 09/24/2018; UM1AI144462 was funded 07/01/2019

  For Dr. Macauley at UofA a sub-contract to Dr. James Paulson, no significant change in overall effort devoted to the project.

- **What other organizations were involved as partners?**

  Organization Name: University of Alberta (UofA)
  11227 Saskatchewan Drive, Edmonton, Alberta, Canada T6G 2G2 (foreign)
  Subcontractor PI: Matthew Macauley, PhD

8. SPECIAL REPORTING REQUIREMENTS:

- **COLLABORATIVE AWARDS:**
  Duplicate reports will be submitted by the Principal Investigator and Partnering Investigator as tasks have been clearly marked with the responsible PI and research site**.**

- **QUAD CHARTS:**
  Not applicable

9. APPENDICES:
Not applicable