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.

PRINCIPLE INVESTIGATOR: James C. Paulson, Ph.D.

CONTRACTING ORGANIZATION: The Scripps Research Institute
La Jolla, CA 92037

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13. SUPPLEMENTARY NOTES At the end of this project period Dr. Paulson replaced Dr. Macauley as the Partnering PI at The Scripps Research Institute (TSRI). 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 second 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. We have developed a new model using adoptive transfer of peanut allergic splenocytes into naïve mice to evaluate depletion of memory B cells in an allergen-specific fashion. We have demonstrated that Ara h 2 STALs targeting CD22 can indeed deplete Ara h 2-specific B cells via IgE and anaphylactic measurements. The tolerance induced by Ara h 2 STALs is long-lasting. We are now poised to evaluate this approach in the humanized CD22 mouse model that we developed last reporting period, along with utilizing human B cells in an assay under development. In terms of targeting CD33 in this past year, we have used the transgenic mouse model with human CD33 expressed on mast cells to evaluate the activity of CD33 ligand (CD33L) STALs on allergen-mediated activation of mast cells <i>in vitro</i> and <i>in vivo</i> . In particular we found that CD33L-STALs profoundly suppress allergen mediated degranulation of bone marrow derived mast cells <i>in vitro</i> and prevent anaphylaxis in mouse models of passive cutaneous and passive systemic anaphylaxis. Animals remain desensitized to subsequent antigen challenge. 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: 75%
- **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: 50%
- **Major Task 3:** Determine translatability of STALs to human CD22 and human B cells
Target date: Months 5-24; percentage of completion: 30%

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: 20%
- **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: 20%

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 Principle Investigator. Dr. Matthew Macauley was originally Partnering PI at TSRI and moved to the University of Alberta (UofA) towards the end of this project period, 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. Finding a source of the four highly purified allergens proved to be challenging, since our collaborator who originally provided Ah2 did not have sufficient quantities of the other allergens. In May 2018, Dr. Kulis and Dr. Macauley acquired 25 mg of each allergen from a reputable European company that specializes in purifying these allergens. The linking of each allergen to lipid, for incorporating into liposomes, was carefully optimized and we have begun studies in both the prophylactic model as well as an adoptive transfer model (see below). Indeed, many studies are ongoing and planned at both UofA and UNC for the use of these allergens in combination, and we will report the results of these in the next project period. Dr. Macauley has also provided the lipid-linked versions of Ah1, Ah2, and Ah3 to TSRI for making CD33-targeted liposomes to test against basophils and mast cells – these liposomes are being shared with Dr. Kulis for tests in human peanut allergic patient blood for effects on basophils and human B-cells.

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

Major progress was made in further developing a mouse model for studying the response of memory B cells, which we introduced in our last progress report. Recently, we published the details of this mouse model (Bednar et al., JoVE, *In Press*). Briefly, mice are sensitized whole peanut extract with cholera toxin in the same way we have reported and published previously. Following sensitization, splenocytes from allergic mice are transferred into naïve recipient mice to confer sensitivity. One day after adoptive transfer, mice are given immunogenic Ah2 liposomes and two weeks later given a boost with low amounts of peanut extra. Two weeks after this boost, mice are challenged with a high dose of the peanut extract. Using this model, we routinely obtain highly reproducible anaphylactic responses in mice on Day 61. The schematic in **Figure 1** outlines the protocol.

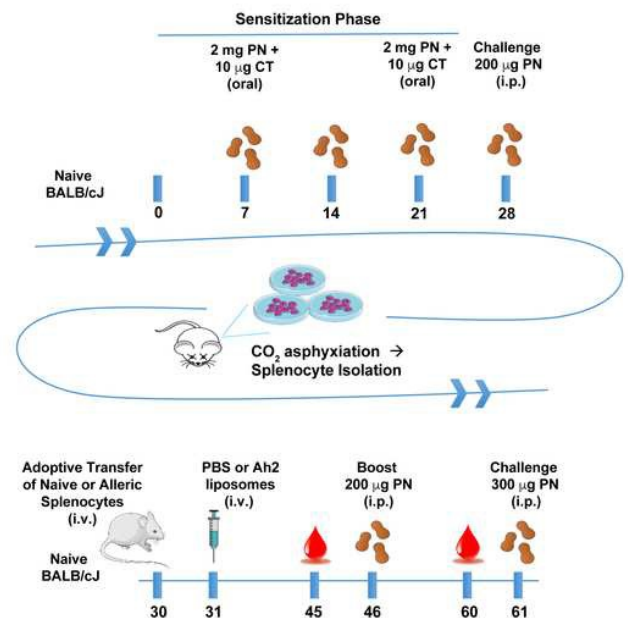


Figure 1. Schematic of adoptive transfer protocol. Mice are sensitized with whole peanut extract (PN) and Cholera toxin (CT), and subsequently challenged with PN. Splenocytes are harvested from mice with confirmed peanut allergy and transferred to naïve mice. Mice with conferred sensitization are primed with Ah2 liposomes, then boosted with peanut, and challenged with peanut. Blood is collected on days 46 and 60 to measure Ah2-specific immunoglobulins.

To assess whether the CD22-targeted Ah2 STALs would be effective in abolishing antibody responses and anaphylaxis upon challenge in this transfer model, we intervened with various doses and numbers of injections of STALs on day 31, which is one day after the adoptive transfer. Mice received either 300 μ M, 600 μ M, or 900 μ M of Ah2 STALs and then underwent boosting and challenge. **Figure 2** demonstrates that 300, 600, and 900 μ M STALs injections significantly reduces the amount of Ah2-specific IgE at day 60 compared to mice that had received liposomes with Ah2 but without the CD22 ligand, indicating that these STALs silenced the memory B cells transferred into the mice on day 30. An Ah2 challenge was conducted on day 61 that demonstrated that mice receiving Ah2 STALs were highly protected from anaphylaxis compared to mice receiving Ah2 liposomes (**Figure 3**). Interestingly, the 600 μ M (**Figure 3B**) dose appears more effective than the 300 μ M dose (**Figure 3A**), demonstrating a dose-response with the STALs.

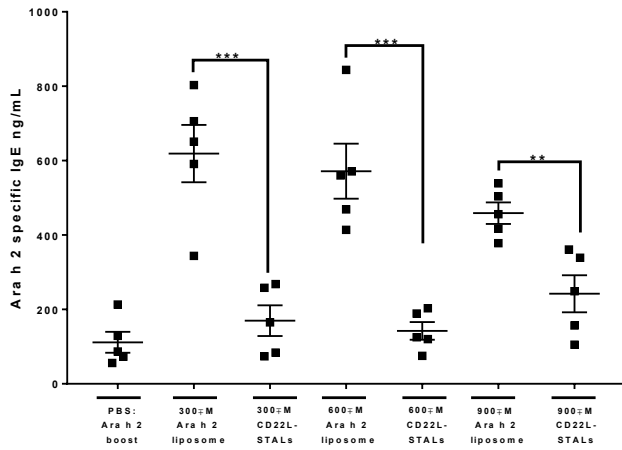


Figure 2. Ara h 2-specific IgE levels on day 60. Groups of mice are shown for those receiving PBS, or 300, 600, and 900 μ M Ah2 liposomes or STALs. ** and *** indicate $p < 0.01$ and $p < 0.001$, respectively.

We next wanted to assess whether additional doses of Ah2 STALs would provide better protection from challenges and how long this tolerance state would persist. Mice were given 300, 600, or 900 μ M Ah2 STALs twice prior to the boost with Ah2 on day 46. As shown in **Figure 4**, two injections of STALs rendered mice completely non-reactive to Ah2 during challenge. This protection from allergic reactions

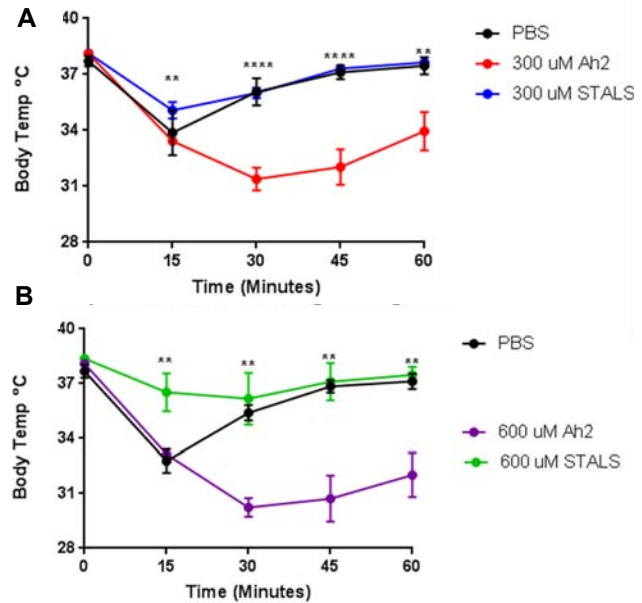


Figure 3. Ah2 challenge in mice receiving 300 or 600 μ M Ah2 STALs. Mice from the adoptive transfer experiment were challenged with 200 μ g of Ah2 on Day 61 and body temperature was measured. Indicated group represented what the mice were given on Day 31 of the protocol.

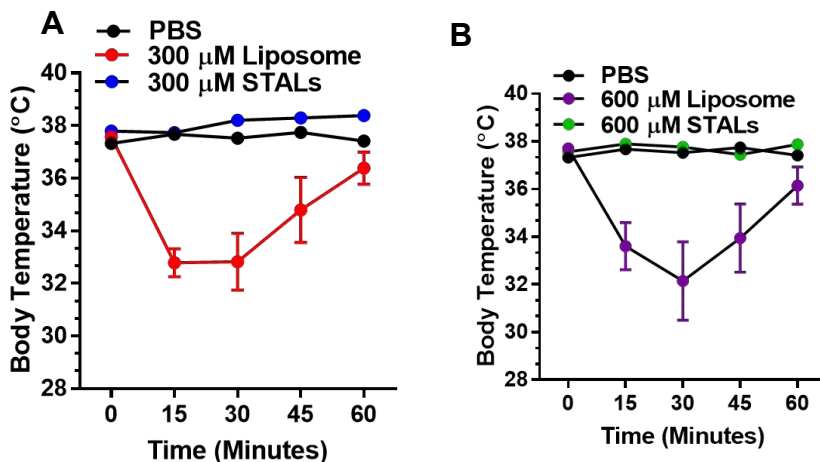


Figure 4. Two injections of Ah2 STALs renders mice non-reactive to Ah2 during challenge. Mice were passively sensitized with peanut-allergic splenocytes and given the indicated treatments on both Day 31 and 45, followed by a boost with peanut extract on Day 60 and 74, with the body temperature measurements taken on Day 74. Indicated groups represent what the mice were given on Day 31 and 45 of the protocol.

was better than that obtained with one injection of STALs as we showed in **Figure 3**. Furthermore, the tolerance was assessed at three and eight weeks after the initial peanut challenge (**Figure 5**). We demonstrate that up to eight weeks later, mice that received two injections of Ah2 STALs are non-reactive to Ah2. This could have major implications in translational approaches using STALs in that long-lasting protection is highly

sought after and not currently attainable by any investigational drug products, such as oral and epicutaneous Immunotherapies (OIT and EPIT, respectively).

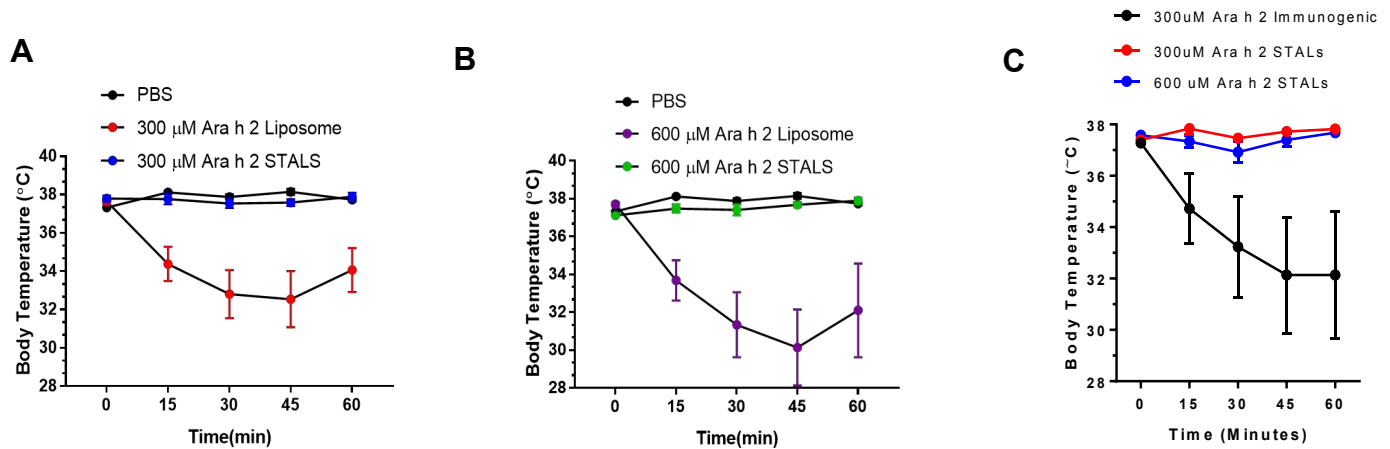


Figure 5. Longevity of tolerance induced by Ah2 STALs. (A) Mice were passively sensitized with peanut-allergic splenocytes and given the indicated treatments on both Day 31 and 45, followed by a boost with peanut extract on Day 60 and 74. (A,B) Three weeks later (Day 105), mice were again challenged with peanut extract. (C) Five weeks later (Day 140), mice given two injections of STALs remained unsensitive.

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

This past year, we published a new human CD22 (hCD22) transgenic mouse (Bednar et al, J Immunol, 2017). Specifically, we demonstrated that this mouse is an appropriate model for studying hCD22 in mouse and that it rescued most of the functions of mouse CD22 (mCD22). 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). Our hCD22 transgenic mice are on a C57Bl/6J (B6) background, unlike the Balb/C mice used for all our previously reported mouse experiments at UNC. This difference in mouse strain has proven to be very significant in terms of the response of the mice to the peanut Allergens. Specifically, we have found that peanut-sensitized B6 mice fail to undergo an anaphylactic response to an Ah2 challenge yet do respond to a challenge with peanut extract. This suggests that Ah2 may not be the major allergen in peanut-sensitized B6 mice. With the other allergens in hand, we tested Ah1, Ah2, Ah3, and Ah6 directly in peanut-sensitized B6 mice and found that Ah1 and Ah3 generated a strong anaphylactic response (**Figure 6A**). This allergic response was consistent with the high levels of serum antibodies against these two allergens, and low levels of antibodies targeting Ah2 and Ah6 (**Figure 6B**). We also tested the same adoptive transfer model in B6 mice as outlined in **Figure 1**. Ah1 and Ah3 liposomes led to strong anaphylaxis responses on Day 31 of the

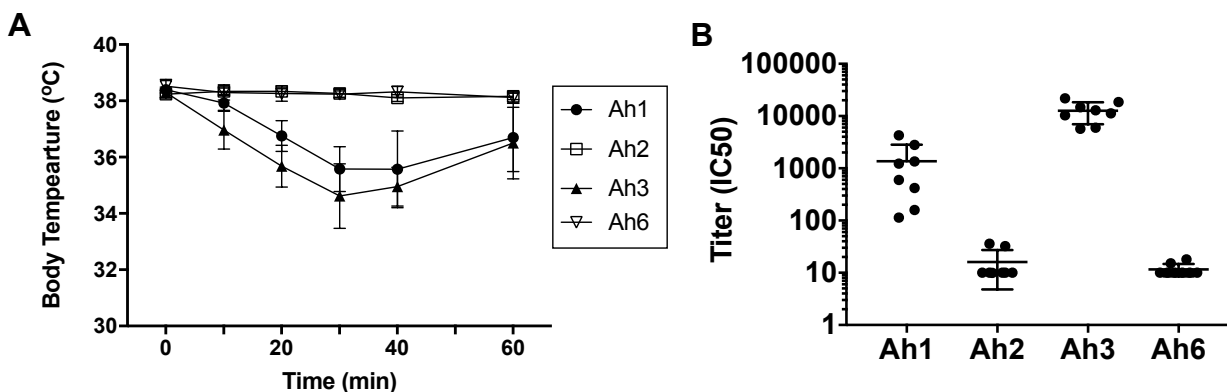


Figure 6. Ah1 and Ah3 are the immunodominant antigens in C57BL/6J mice. WT B6 mice were sensitized with peanut extract and cholera toxin for four consecutive weeks. One week after the last sensitization, mice bled and then challenged with an *i.p.* injection of 250 μg of the indicated allergen. (A) Body temperature of mice were followed for one hour following the allergen challenge. (B) Anti-allergen antibodies (IgG) were measured by ELISA prior to the challenge.

model, while Ah2 liposomes did not (**Figure 7**). Accordingly, Ah2 and Ah6 are poorly immunogenic in B6 mice, which Ah1 and Ah3 strongly immunogenic.

Having established that Ah1 and Ah3 that the immunodominant allergens in B6 mice, the next question we asked was whether we could induce tolerance to Ah1 and Ah3 incorporating them into STALs. For initial experiments, we chose to use WT B6 mice while our colony of hCD22 transgenic mice expanded following arrival from TSRI. These experiments were performed in the adoptive transfer model (Figure 1) given that we got robust response to these allergens in this assay (Figure 7). Ah1 STALs could indeed generate strong tolerance in this system as shown by a lack of a temperature decrease following the challenge (Fig 8A). Similar results were also obtained for Ah3, (Fig 8B). These results demonstrate that Ah1 and Ah3 STALs are effect in tolerizing peanut-specific memory B-cells to either Ah1 or Ah3.

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

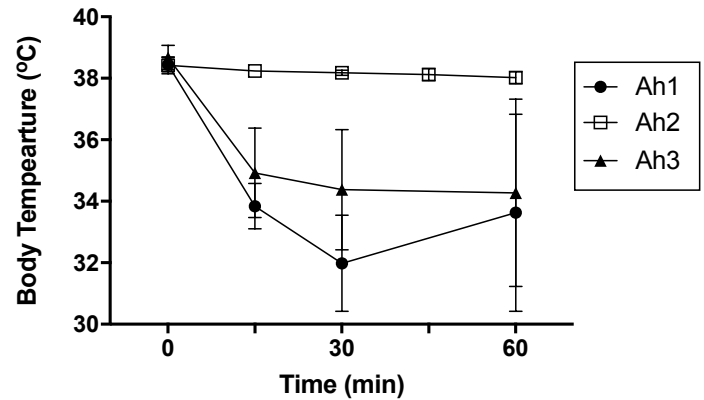


Figure 7. Adoptive transfer model of conferred sensitivity to peanut allergen in B6 mice produces robust responses to Ah1 and Ah3. Following the same schedule as presented in Figure 1, WT B6 mice were given liposomes with the indicated allergen-displaying liposome on Day 31. Following a boost and challenge with peanut extract, body temperatures were monitored over the course of an hour.

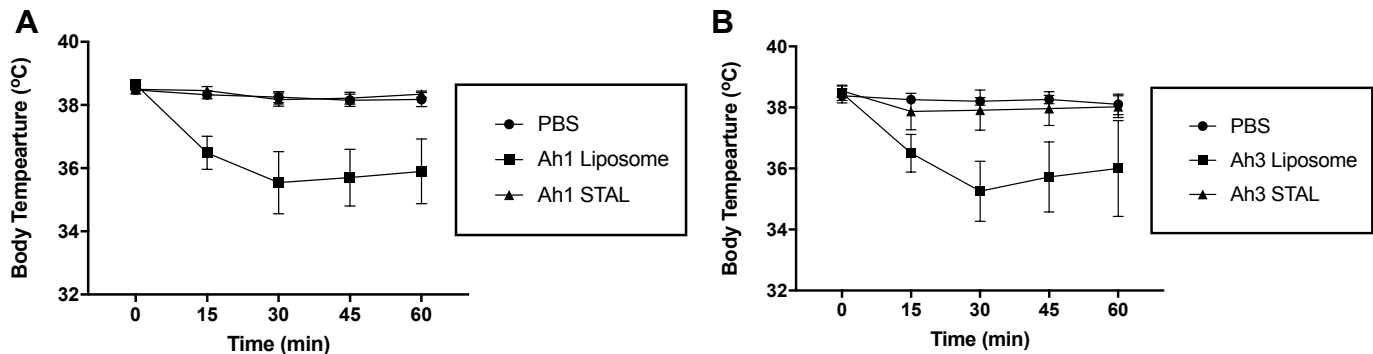


Figure 8. Tolerance induction to Ah1 and Ah3 in the adoptive transfer model of conferred sensitivity to peanut allergen in B6 mice. Mice underwent the same protocol as the experiment presented in Figures 2 and 3, except mice were administered STALs or liposomes displaying (A) Ah1 or (B) Ah3. Body temperatures were measure after the challenge on Day 31.

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. We have begun to develop this assay using healthy control patient B cells. In several experiments, we have now demonstrated that we can isolate B cells using a Miltenyi B cell enrichment kit via negative selection (i.e. no antibody-beads are tagged on the B cells) and can go on to culture these with and without activating factors. After seven days in culture, cell supernatants are collected, and human IgG is quantified by ELISA (R&D Systems kit). **Figure 9** shows that we can indeed detect human IgG when B cells are stimulated but not in unstimulated conditions. This lays the groundwork for our desired assay, in which we will obtain B cells from peanut allergic subjects then determine whether Ah2 STALs can deplete the memory B cells such that Ah2-specific IgG will not be detectable.

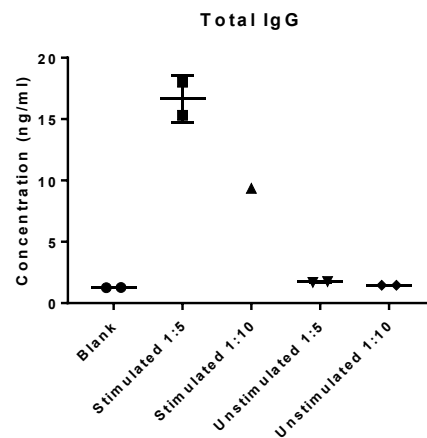


Figure 9. Developing an assay to test tolerance induction in primary human B cells. Human peripheral blood B cells isolated by negative selection and stimulated with a cocktail of factors, including: LPS, IL-21, BAFF, and anti-CD40. After 5 days in culture, the culture was used in ELISA to measure total IgG production.

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 of 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

Significant Results and Achievements: In the previous project period we reported initial results on the impact of Ah2-STALs to suppress peanut antigen mediated degranulation of human basophils. Subsequently, we encountered problems with third party supply of peanut antigen Ah2. Dr. Macauley has now secured alternative sources of purified peanut allergens, and optimized the reaction for coupling PEGylated lipid to Ah1, Ah2, and Ah3. As described in Major Task 3, we have successfully tested the longevity of the desensitization of mast cells, and the mechanism of how desensitization occurs, and we fully expect the same mechanism to apply to human basophils. We intend to complete this task in the coming project period.

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

Significant Results and Achievements: Very significant progress has been made on this task in the last year. In the previous progress report, we reported that we had successfully established human transgenic mice at TSRI with human CD33 expressed on mast cells. This work was done under protocols approved by the IACUC at USAMRMC ACURO. In the last year, these mice have been used to evaluate the preventative effects of STALs targeting CD33 in mouse models of allergic disease at TSRI. For these initial studies, we used commercially available anti-trinitrophenol (anti-TNP) IgE to sensitize mast cells and liposomes (LP) containing TNP (TNP-LP) as the antigen, and STALs co-presenting both CD33 ligand (CD33L) and TNP to test the preventative effects (TNP-LP-CD33L). As illustrated in **Figure 10**, TNP-LP is expected to cause degranulation anti-TNP sensitized mast cells, while TNP-LP-CD33L (CD33 targeted STALs) is expected to inhibit mast cell degranulation.

To demonstrate human CD33 is functional in murine cells, we generated and cultured bone marrow mast cells (BMMCs) from the hCD33-Tg mice. These BMMCs highly express hCD33 (GFP⁺) and fluorescent liposomes with CD33L (LP-CD33L) strongly bound to these cells, but not to mast cells that did not express CD33 (GFP⁻) (**Figure 11A,B**).

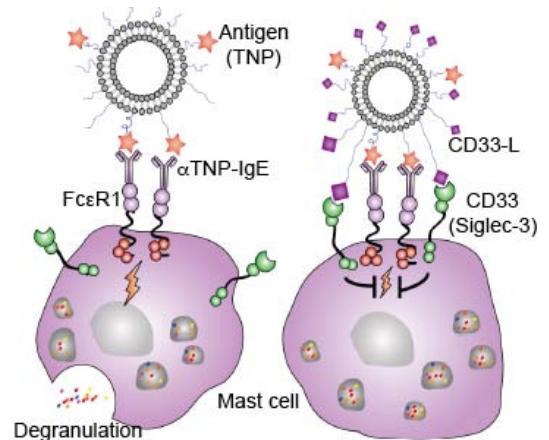


Figure 10. A schematic representation of a TNP-liposome displaying CD33 ligand. (Left) A liposome displaying TNP can crosslink anti-TNP-IgE immobilized on mast cells and trigger mast cell degranulation. (Right) A liposome co-displaying both TNP and CD33 ligand (STALS) can recruit CD33 to the anti-TNP-IgE/FcεRI and prevent mast cell degranulation.

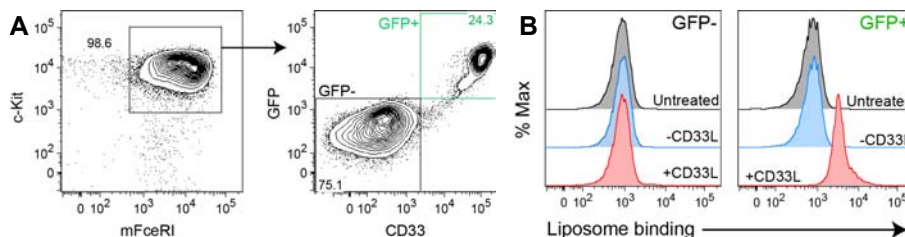


Figure 11. (A) BMMCs cultured from the hCD33-Tg mice highly express human CD33. (B) Fluorescent liposomes formulated with CD33-ligand only binds with hCD33 expressing BMMCs (GFP⁺) but not GFP⁻ cells.

We next investigated the ability of the TNP-liposomes (TNP-LP) to induce degranulation and cytokine production in BMMCs and the ability of the CD33L expressed on the same particle to suppress degranulation. Indeed, TNP-LP strongly induced degranulation (% β -Hexosaminidase release) and cytokine production in BMMCs (TNF- α , IL-4, IL-6, and IL-13) (**Figure 12A,B**), and both degranulation and cytokine production were profoundly inhibited by STALs when CD33L-ligand was co-presented with TNP (TNP-LP-CD33L, **Figure 12A,B**).

To determine if TNP-liposomes with CD33L could suppress mast cell activation *in vivo*, we used a passive cutaneous anaphylaxis (PCA) and a passive systemic anaphylaxis (PSA) model, which are both mast cell dependent. In the PCA model, mice were sensitized in one ear with anti-TNP-IgE, while the other ear received PBS model injection. The next day, mice were given liposomes in Evans's blue dye (**Figure 13A**). In mice with mast cells that do not express CD33 (Control-Tg), both TNP-LP and TNP-LP-CD33L induced vascular leakage in ears sensitized with anti-TNP-IgE (**Figure 13B, C**). In contrast, in CD33-Tg mice, CD33L profoundly inhibited TNP induced ear edema (**Figure 13D, E**).

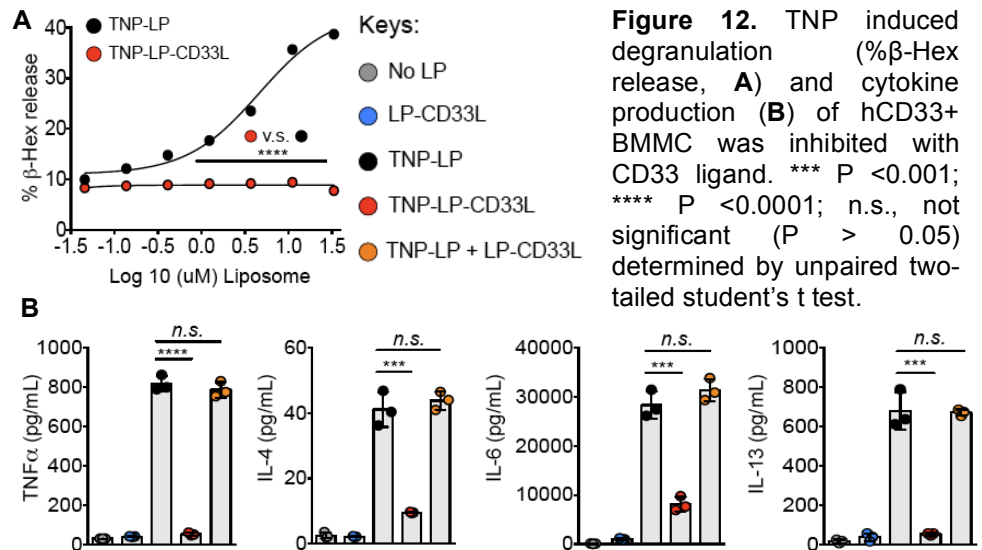


Figure 12. TNP induced degranulation (% β -Hex release, **A**) and cytokine production (**B**) of hCD33+ BMMC was inhibited with CD33 ligand. *** $P < 0.001$; **** $P < 0.0001$; n.s., not significant ($P > 0.05$) determined by unpaired two-tailed student's t test.

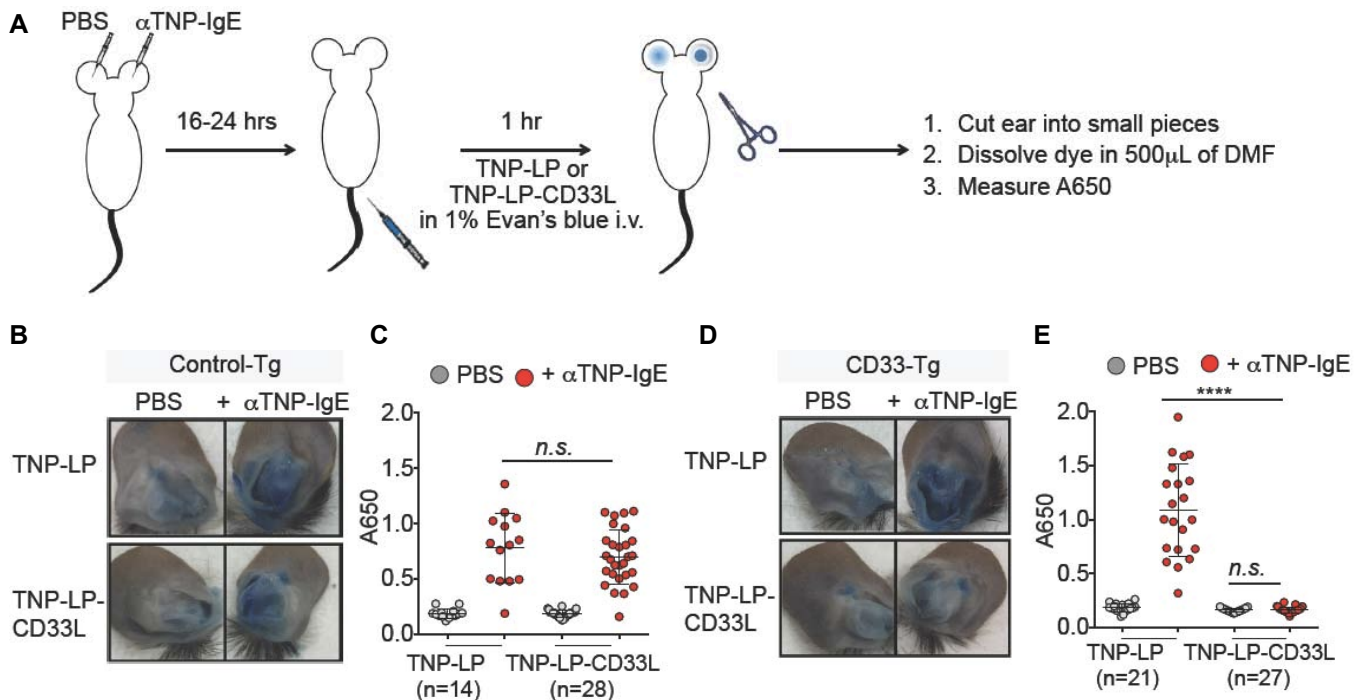


Figure 13. (A) Injection scheme for the passive cutaneous anaphylaxis model. (B) Representative pictures of vascular leakage in Control-Tg mice. (C) Quantification of Evan's blue dye from Control-Tg mice. (D) Representative photos of vascular leakage in CD33-Tg mice. (E) Quantification of Evan's blue dye from CD33-Tg mice. **** $P < 0.0001$; n.s., not significant ($P > 0.05$) determined by unpaired two-tailed student's t test.

For the PSA model, mice were sensitized systemically by tail vein injection with anti-TNP-IgE. The next day, after basal rectal temperatures were measured, mice were given either TNP liposomes with or without CD33L (**Figure 14A**). In Control-Tg mice that do not express hCD33 on mast cells, both TNP-LP and TNP-LP-CD33L

induced profound systemic anaphylaxis (**Figure 14B**). In this case, the CD33L on the liposomes has no effect whatsoever since there is no CD33 expressed on the cells, and as a result no suppression of anaphylaxis.

In contrast, in the hCD33-Tg mice that express hCD33 on mast cells, the TNP-LP induced strong anaphylaxis, but with the ligand present (TNP-LP-CD33L), there is no significant drop in rectal temperature (**Figure 14C**). Accordingly, we have shown that CD33L-STALs can suppress IgE/mast cell mediated anaphylaxis both.

An important aspect of Aim 2-Task 3 is to determine how long the inhibitory effects of the STALs persist. To evaluate this we next assessed if animals treated with TNP-STALs (TNP-LP-CD33L) remained desensitized upon subsequent antigen challenge with allergen (TNP-LP) in the PSA model (**Figure 15A**). As shown in **Figure 15**, TNP-LP-CD33L itself did not induce significant rectal temperature drop comparing to untreated mice (*left panel*). Mice remained desensitized upon subsequent challenges with TNP-LP after 6 hours, and after 24 hours (*middle and right panels*, respectively), while untreated mice exhibited substantial anaphylaxis. Indeed, with the high dose of antigen given in the second challenge, 7 of 9 mice died within 40 minutes (**Figure 15B**). In the coming year, we aim to test if demonstrate that STALs can inhibit peanut antigen (either Ah1 or Ah2) and ovalbumin (OVA) induced anaphylaxis in sensitized mouse models (See also Aim 2, Major Task 4).

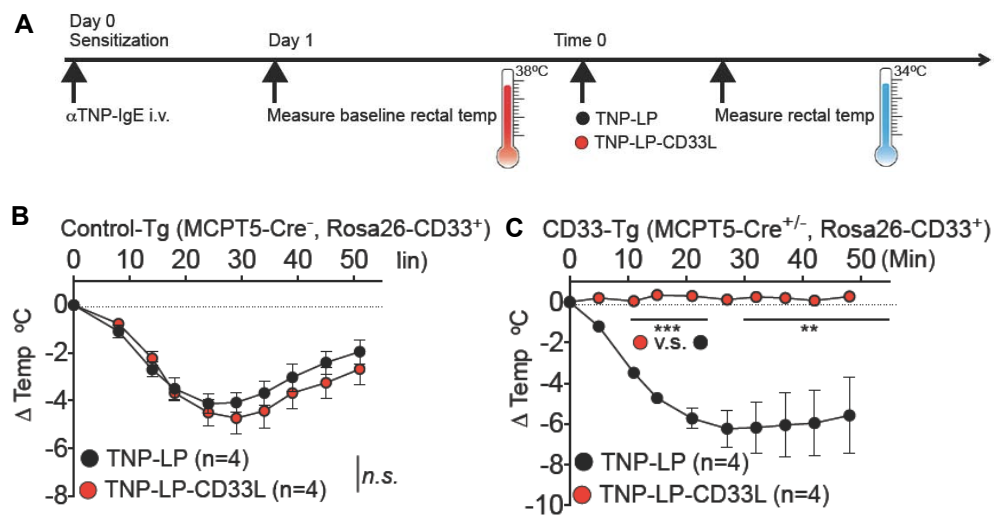


Figure 14. (A) Injection Scheme for the passive systemic anaphylaxis model. (B) Changes of rectal temperature in Control-Tg mice. (C) Changes of rectal temperature in CD33-Tg mice. *** P < 0.001; **** P < 0.0001; n.s., not significant (P > 0.05) determined by unpaired two-tailed student's t test.

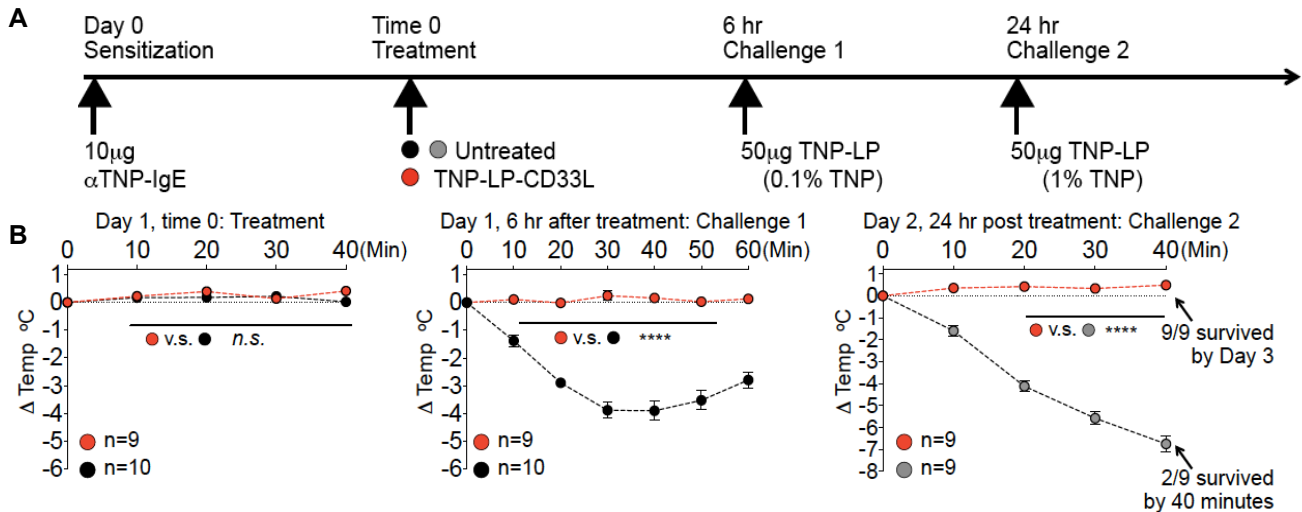


Figure 15. (A) Injection scheme for desensitization and challenge. (B) Rectal temperature induced treatment or challenge. **** P < 0.0001; n.s., not significant (P > 0.05) determined by unpaired two-tailed student's t test.

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

Significant Results and Achievements: The idea behind using both CD33 STALs and CD22 STALs in allergic mice is that antigen bearing CD22 STALs will cause anaphylaxis if the mouse is pre-sensitized. Thus, this task

requires that allergic mice be treated. TSRI has shipped both human CD33-Tg and human CD22-Tg mice to Dr. Macauley at the University of Alberta to set up the model for sensitizing mice to peanut antigen using the protocols developed by Dr. Kulis at the University of North Carolina. In addition, TSRI has imported the transgenic IL-4 'F709 mice' that are well documented to develop IgE mediated peanut allergy and undergo anaphylaxis with peanut antigen challenge. Once allergic mice are obtained, we will assess the impact on simultaneous administration of CD33 and CD22 STALs.

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

At UNC: 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: Individual Development Plans (IDPs) were offered to Shiteng Duan (Grad Student) to help monitoring the training and progress in developing his scientific career. He received one-on-one guidance from Dr. Paulson (Mentor) on a daily basis, in addition to regular lab meetings. He also presented his work in the form of posters/oral presentations at local and international meetings/symposiums. All trainees are encouraged to take advantage of the facilities provided by the Graduate Office with dedicated staff that provide many services for graduate and postdoctoral researchers including peer editing to improve manuscript writing skills, courses in lab management, and grant writing. A significant focus is given to career development from broad career planning to the fundamentals of creating a resume.

At UofA (sub-contract to TSRI): Dr. Macauley met on a daily basis with post-doctoral fellows Dharmendra Rhaguwanshi and Maju Joe and provided hands on training on working with allergens, formulating liposomes, and immunizing mice. Postdoctoral fellows and technician Susmita Sarkar were actively participated in groups meetings are attended 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.

For Aim 1, Major Task 2: We will continue working with the conferred allergy model to better understand how the Ah2 STALs are depleting memory B cells. This will likely occur via the B cell tetramers we developed in the previous reporting period. We are working towards a manuscript utilizing this data as well.

For Aim 1, Major Task 3: We will develop the human B cell assay to quantify Ah2-specific IgG/IgE and utilize Ah2 CD22-STALs to investigate the translational properties of the STALs.

For Aim 2, Major Task 2: We will assess the CD33 STALs in our human basophil activation assay (BAT). The STALs will be prepared by Dr. Paulson's group at TSRI.

At TSRI

For Aim2, Major Task 2: Peanut antigen (Ah1 or Ah2) CD33 targeted STALs will be prepared for testing desensitization of human basophils by Dr. Kullis' group at the University of North Carolina .

For Aim 2, Major Task 3: The mechanism of mast cell desensitization will be further characterized with regards to the impact of CD33 STALs on the signaling pathway involved in desensitization and resensitization by allergen specific IgE. To this end, TSRI has obtained FcεRI-KO and mast cell deficient mice (MCPT5-Cre x SHP-2^{fl^{ox}}) to assess the roles of these key players in mast cell degranulation.

For Aim 2, Major Task 3: We will assess the impact of STALs on peanut antigen (Ah1 or Ah2) mediated mast cell degranulation in peanut sensitized mice. For this purpose we have bred the hCD33 mice with the IL-4R F709 mice to generate peanut sensitized mice that have hCD33 mast cells.

For Aim 2, Major Task 4: TSRI will work with Dr. Macauley's group at the University of Alberta to simultaneously test CD22 and CD33 STALs in peanut sensitized mice.

At UofA

As the next steps, we are doing the following: (1) demonstrate that Ah1 and Ah3 STALs are effective in the prophylactic setting in WT B6 mice; (2) that Ah1 and Ah3 STALs used in combination can more profoundly induce tolerance to peanut extract compared to tolerizing through either allergen alone; and (3) validate that similar results can be achieved through hCD22 in our transgenic mouse model. We have and 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.
 - 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.
 - 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**

We encountered some minor issues leading to delays, however, these have now been addressed as described below.

Aim 2, Major Task 2: This task was aimed at demonstrating the ability of CD33 STALs to desensitize human basophils. While initial results were reported in the last project report, there has been a delay in completing this task due to the low amounts of purified Ah2 peanut antigen to make the STALs. As mentioned above, Dr. Macauley at the University of Alberta is now preparing Ah1 and Ah2. TSRI will prepare peanut antigen STALs (Ah1 or Ah2) and send them to Dr. Kulis at the University of North Carolina for testing with human basophils.

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 have turned to the robust IL-4R-F709 mouse model. These transgenic mouse have a mutant IL-4 receptor on a C57BL/6 background that 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.

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

There was a modest slow down in expenditures at the end of the project period since Dr. Macauley moved to the University of Alberta, requiring winding down work at TSRI and setting up the laboratory at the University of Alberta. This transition is now fully complete. We anticipate a modest carryover as a result of this transition. While it may not be deemed significant, we felt it was worth mentioning.

- **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:

- Bednar KJ, Shanina E, Ballet R, Connors EP, Duan S, Juan J, Arlian BM, Kulis MD, Butcher EC, Fung-Leung WP, Rao TS, Paulson JC, Macauley MS. Human CD22 Inhibits Murine B Cell Receptor Activation in a Human CD22 Transgenic Mouse Model. *J Immunol.* 2017 Nov 1;199(9):3116-3128. [PMID: 28972089]
- Bednar, KJ, Hardy, L, Smeekens J, Raghuwanshi D, Duan S, Kulis MD, Macauley MS. Antigenic Liposomes for Generation of Disease-specific Antibodies. *Journal of Visualized Experiments.* In press.

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

- None

Other publications, conference papers, and presentations

- **Abstracts and presentations at international conferences: Michael Kulis (PI)**
 - Using Siglec-engaging Tolerance-inducing Antigenic Liposomes (STALs) to reduce memory B cell responses to the major peanut allergen Ara h 2. Hardy, L.C., Orgel, K., Duan, S., Maleki, S., Burks, A.W., Paulson, J.C., Macauley, M., **Kulis M.** 2018 AAAAI meeting, Orlando, FL. **Featured Poster Presentation**
 - Adjuvant-free intragastric sensitization to peanut in CC027/GeniUnc mice. Smeekens, J., Orgel, K., Ferris, M.T., Miller, D.R., Burks, A.W., Pardo-Manuel de Villena, **Kulis, M.** 2018 Gordon Research Conference on Food Allergy, Ventura, CA.
 - Suppressing memory B cell responses to the major peanut allergen Ara h 2 using STALs. Hardy, L., Orgel, K., Duan, S., Maleki, S., Burks, A.W., Paulson, J., Macauley, M., **Kulis, M.** 2018 Gordon Research Conference on Food Allergy, Ventura, CA.
 - Exploiting the inhibitory function of CD22 on B-cells to prevent antibody responses. Macauley, M.S., Arlian, B.M., Bednar, K.J., Duan, S., Fung-Leung, W.P., **Kulis, M.D.**, Hardy, L., Nycholat, C.M., Orgel, K.A., Pang, L., Paulson, J.C., Rao, T.S. 2018 ACS meeting, New Orleans, LA.
- **Oral presentations: James C. Paulson (Partnering PI)**
 - Glycomics Week - October 9-13, 2017
Griffith University, Gold Coast, Australia
Title: Harnessing Siglecs to induce immune tolerance
 - International Symposium "Systems Glycobiology and Beyond" – November 16-17, 2017
Riken University, Wako, Japan
Title: Siglec targeted nanoparticles for desensitization of mast cells
 - Department of Biochemistry – January 12, 2018
Duke University, Durham, North Carolina
Title: Harnessing inhibitory Siglecs to control immune responses
 - 255th ACS National Meeting & Exposition – March 18-22, 2018
New Orleans, Louisiana
CELL: Frontiers in Glycoscience, Bridging the Gap Between Carbohydrate & Polysaccharide Chemistries
Title: Siglecs: Putting the brakes on the immune system
 - 2018 World Molecular Engineering Network (WMEN) annual conference – May 5-9, 2018
San Jose del Cabo, Mexico
Title: Putting the brakes on the immune system
 - Sialoglyco 2018 – May 10-14, 2018

Banff, Canada

Title: Siglec suppression of immune cell responses and home prices: Location, location, location

- ATS 2018 International Conference – March 18-23, 2018
San Diego, California
Glycobiology and Glycomics of Lung Disease
Title: Targeting siglecs to desensitize mast cells
- 43rd FEBS Congress – July 7-12, 2018
Prague, Czech
Glycans in Health and Disease I
Title: Exploiting siglecs to modulate immune response
- Benzon Symposium No. 63 – August 27-30, 2018
Copenhagen, Denmark
Glycotherapeutics – Emerging Roles of Glycans in Medicine
Title: Exploiting inhibitory Siglecs to modulate immune response

• **Poster/Oral presentations: Shiteng Duan (Grad student)**

- Food Allergy Gordon Research Conference – January 11-12, 2018
Ventura, California
Title: Targeting inhibitory Siglecs to prevent IgE dependent anaphylaxis
- San Diego Glycobiology Symposium – March 9-10, 2018
San Diego, California
Title: Exploiting CD33 to suppress IgE-dependent anaphylaxis
- InterPEG 2018 – March 10-11, 2018
San Diego, California
Title: Exploiting CD33 to suppress IgE-dependent anaphylaxis
- 3rd Annual Calibr-TSRI Symposium – April 27, 2018
La Jolla, California
Title: Exploiting CD33 to inhibit IgE dependent anaphylaxis

• **Oral presentations: Macauley S Macauley (Sub-contract at UofA)**

- Society for Glycobiology – November 7-10, 2017
Portland, Oregon
Title: Development of a new human CD22 mouse model.
- 255th ACS National Meeting & Exposition – March 18-22, 2018
New Orleans, Louisiana
CELL: Glycosciences Young Investigator Symposium
Title: Exploiting the Inhibitory Function of CD22 on B-cells to Prevent Antibody Responses.
- Sialoglyco – May 14-18, 2018
Banff, Alberta, Canada
Title: Changes in Sialic acid during B-cell Differentiation Controls the Germinal Center through CD22

• **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)

Name:	James C. Paulson
Project Role:	Partnering PI
Researcher Identifier (e.g. ORCID ID):	0000-0003-4589-5322
Nearest person month worked:	0.60
Contribution to Project:	Dr. Paulson is supervising the daily activities of the overall administration and direction of the project at The Scripps Research Institute (TSRI).
Funding Support:	N/A

Shiteng Duan (Grad Student) – unchanged
 Kevin Worrell (Research Assistant) – unchanged
 Joana Juan (Research Assistant) – unchanged

University of Alberta (UofA)

Name:	Matthew Macauley
Project Role:	Subcontractor
Researcher Identifier (e.g. ORCID ID):	0000-0003-4579-1048
Nearest person month worked:	4
Contribution to Project:	Dr. Macauley provided guidance the daily activities of the overall of the project at University of Alberta (UofA)
Funding Support:	N/A

Name:	Maju Joe
Project Role:	Post Doctoral Associate
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6
Contribution to Project:	Linking allergens to lipid
Funding Support:	N/A

Name:	Susmita Sarkar
Project Role:	Research Technician
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Helping with mouse experiment, performing ELISAs
Funding Support:	N/A

Name:	Dharmendra Raghuwanshi
Project Role:	Post Doctoral Associate
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6
Contribution to Project:	carrying out mouse experiments
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 at TSRI,

- NIH grant # R01AI099141 ended 10/31/2017 and
- NIH Grant U19 AI136443 was funded 02/01/2018.
- Dr. Paulson is now the Partnering Investigator at TSRI and Dr. Macauley has moved to the University of Alberta, but otherwise is no significant change in overall effort devoted to the project.

For Dr. Macauley at UofA

- Dr. Macauley is now acting as a sub-contract to Dr. James Paulson, but there is otherwise 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 Principle 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