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TITLE: Exploiting Inhibitory Siglecs to Combat Food Allergies

PRINCIPAL INVESTIGATOR: Michael Kulis, Ph.D.

CONTRACTING ORGANIZATION: University of North Carolina, Chapel Hill, NC

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5b. GRANT NUMBER

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6. AUTHOR(S)
Michael Kulis, PhD – Principal Investigator
James C. Paulson, PhD – Partnering Investigator

E-Mail: mike.kulis@unc.edu; jpaulson@scripps.edu

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5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of North Carolina at Chapel Hill
Department of Microbiology & Immunology, Chapel Hill, NC 27599

The Scripps Research Institute
10550 N. Torrey Pines Road, La Jolla, CA 92037

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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 this award, we have generated and published important data related to targeting of CD22 on B cells and CD33 on mast cells and basophils to abrogate food allergies. For the CD22 approach, we have demonstrated prevention of IgE production by pre-treating mice with a single injection of Ara h 1, 2, and 3 STALs. Furthermore, we demonstrated that peanut allergen STALs can deplete memory B cells in mice. Finally, we went on to show that the human CD22 ligand can be used to deplete human memory B cells in humanized NSG mice. In the project targeting CD33, we have demonstrated in vivo efficacy in mouse models of anaphylaxis and developed a novel approach by conjugating human CD33 ligand directly to anti-human IgE, without the use of liposomes for scaffolding. This molecule is effective in inducing tolerance in humanized mice. Overall, our results move us closer to translating our STALs platform into human studies by focusing now on the use of humanized mouse models and human CD22 and CD33 ligands in our systems. Finally, our team was awarded an Expansion Award to continue our work on both CD22 and CD33 as targets for Food Allergy. The Expansion Award (W81XWH-21-1-0315) is focused on further developing our findings from the original award by performing experiments on additional humanized mouse models and human basophils and mast cells.

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*: **100%**
- ***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*: **100%**
- ***Major Task 3***: Determine translatability of STALs to human CD22 and human B cells
  Target date: Months 5-24; *percentage of completion*: **90%**

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*: **90%**
- ***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*: **100%**
- ***Major Task 4***: Determine therapeutic utility of STALs targeting CD33 and CD22 simultaneously in allergic mice
  Target date: Months 18-36; *percentage of completion*: **75**%

- **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. During the 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.

Ah1, 2, and 3 STALs were prepared, and tested in our mouse models for prevention of peanut allergen responses. Ah1, Ah2, and Ah3 worked well in both BALB/c mice (Dr. Kulis at UNC) and in C57BL/6 mice (Dr. Macauley at UofA). The data from these experiments have been submitted as a manuscript to the *Journal of Allergy and Clinical Immunology*. Additionally, we developed B cell tetramers in collaboration with Justin Taylor (Fred Hutchinson Cancer Center) to track the depletion of Ah1, 2, and 3 B cells in mice following STALs treatment. The tetramer experiments will be continued as part of the Expansion Award.

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

During the award period, we demonstrated the ability to suppress memory B cell responses to Ah1 and Ah2 in an adoptive transfer model. Naïve mice received splenocytes from peanut allergic mice and were then administered STALs to look for depletion of memory responses upon boosting. We were able to demonstrate success using Ah1 and Ah2 in the BALB/c mouse model (Dr. Kulis at UNC) and the C57BL/6 mouse model (Dr. Macauley at UofA). The suppression of IgE and IgG1 against Ah1 and Ah2 was nearly complete and was sustained for at least 3 months following peanut challenges. This prevented anaphylaxis in the animals, indicating successful depletion of memory B cells specific to peanut, a potentially important discovery for applicability of STALs in human peanut allergy. This work has also been submitted for publication in the *Journal of Allergy and Clinical Immunology* in the same manuscript as referenced above.

***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). We then used these mice to demonstrate tolerance induction of peanut allergens through hCD22 is equally as robust as through mCD22 (mCD22). In the final two years of the project, we moved into a humanized mouse model system at UNC to determine the effects of STALs directly on human B cells. We used NSG mice, lacking mouse B and T cells, and repopulated the immune system using human PBMCs from peanut allergic donors. Then, using Ah1 STALs with the *human* CD22 ligand, we found a significant reduction in peanut-specific IgG responses following boost injections with peanut allergens (**Fig. 1**). This concept provides important evidence that we can target human memory B cells and

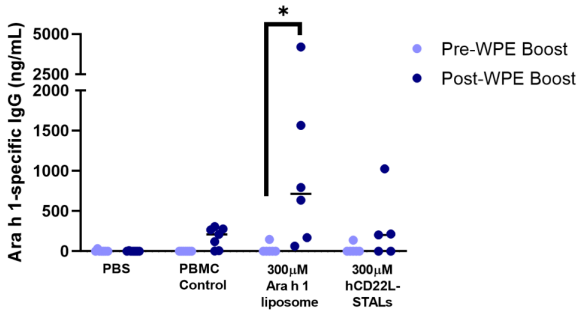

Figure 1. Depletion of human memory B cells specific to Ara h 1. Mice receiving hCD22L-STALs did not have increased Ah1-IgG post boost, whereas mice receiving Ah1 liposomes saw significant increases in Ah1-IgG.

significantly suppress their recall responses. This data was also included in the manuscript submitted to the *Journal of Allergy and Clinical Immunology.*

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:

We optimized the ratio of Ah2 and human CD33L on STALs to generate fully hypoallergenic STALs. At TSRI (Dr. Paulson and Dr. Macauley), several formulations of STALs were prepared and ultimately tested on the human mast cell line, LAD-2. An ideal ratio was found that led to essentially no degranulation from the LAD-2 cells, whereas Ah2 liposomes without the human CD33L led to robust degranulation (**Figure 2A**). We also tested this optimized formulation of Ah2-CD33L STALs on human basophils (Dr. Kulis at UNC) and found a significant reduction in degranulation when cells were pre-treated with STALs (**Figure 2B**). This was an important proof-of-concept demonstrating that the CD33L can inhibit signaling through FcεRI and prevent degranulation.

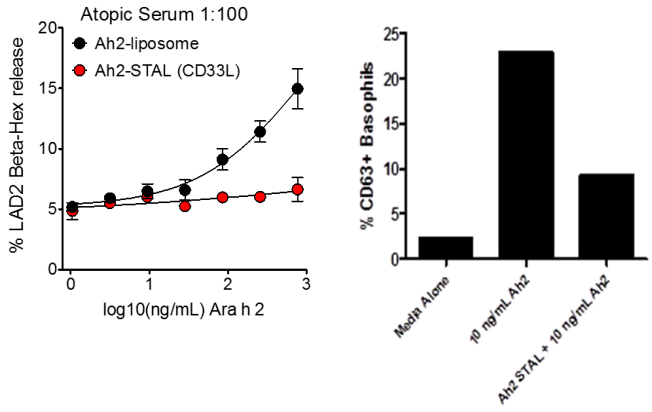

Figure 2. Ah2 STALs targeting CD33 on human mast cells and basophils. Left panel (A) shows Ah2-STALs (red circles) does not cause beta-hex release (i.e. degranulation), whereas Ah2-lioposomes (black circles) cause large amounts of degranulation. Right panel (B) demonstrates that 10 ng/mL Ah2 causes CD63+ degranulation of human basophils whereas when basophils are first treated with the Ah2 STAL then 10 ng/mL Ah2 there is a >50% reduction in degranulation.

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

We have 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. These data are shown below, along with descriptions of the findings. We are still pursuing this line of research and will continue it under the Expansion Award. The limitation here is getting enough blood donors to come in to generate another ~10 data points. As with other parts of the project, this was delayed with COVID-19 and scheduling clinical appointments at the UNC Food Allergy Clinic..

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 3**, 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 4**).

We anticipate reproducing these findings and then writing a manuscript on this data under the Expansion Award.

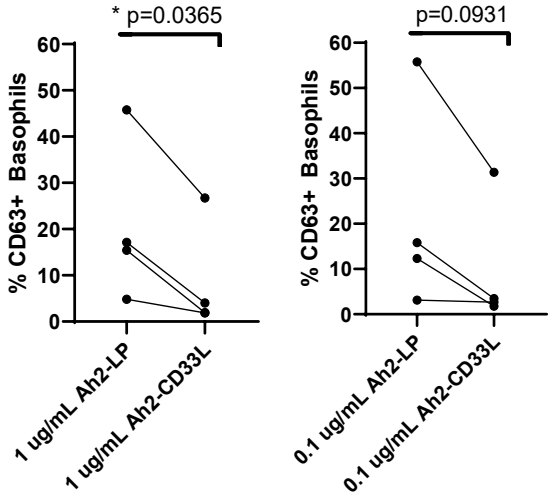

Figure 3. Whole blood basophil activation testing performed with Ah2-LP versus Ah2-CD33L. Data is shown from four individuals with peanut allergy. Ah2-CD33L has significantly lower ability to activate human basophils than Ah2-LP, indicating that CD33 ligand can suppress basophil responses.

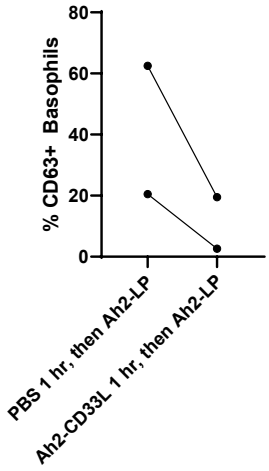

Figure 4. Whole blood human basophil activation testing with pre-treatment of Ah2-CD33L. The data indicate that pre-treatment of human basophils with Ah2-CD33L can suppress subsequent stimulation with Ah2.

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

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

We have subsequently been working towards demonstrating the utility of STALs in actively sensitized mice using human CD33L on humanized mast cells in mice. Additionally, we have created a new reagent by coupling human CD33L to anti-human IgE using a linker (**Figure 5A and 5B**). This reagent binds human IgE as demonstrated by ELISA (**Figure 5C**). We compared the native anti-IgE clone HB121 (ATCC) with the linker modified (N3) and CD33L modified (CD33L) conjugates for their ability to introduce anaphylaxis in NSG-SGM3-CD34+ stem cell humanized mice sensitized 24 hours earlier with human IgE. As shown in **Fig. 6A**, native anti-IgE and N3-anti-IgE produced robust anaphylaxis, while the mice treated with the CD33L-anti-IgE were protected from anaphylaxis. In a separate experiment, mice treated with either buffer (PBS) or CD33L modified anti-IgE showed no anaphylaxis (**Fig 6B**), but when challenged 5 hours later with native anti-IgE, the PBS treated mice did not survive, while the CD33L-anti-IgE treated mice were protected, indicating that the initial treatment desensitized them to the challenge (**Fig. 6C**). In future experiments under the Expansion Award, we will adjust the amount of anti-IgE in the challenge to reduce the severity of anaphylaxis and risk of animal death in the control experiments.

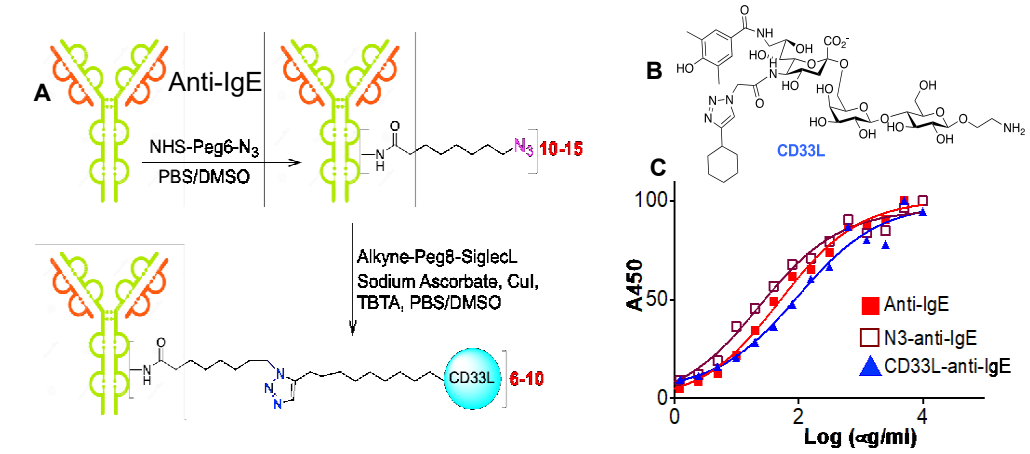

Figure 5. Conjugation of CD33 ligand to human anti-IgE. A) Linkers are chemically conjugated to lysine side chains on anti-IgE (N3-anti-IgE) and then coupled to alkyne modified CD33L (CD33L-anti-IgE). B) Structure of CD33L. C) ELISA analysis of anti-IgE binding to IgE coated on the plate.

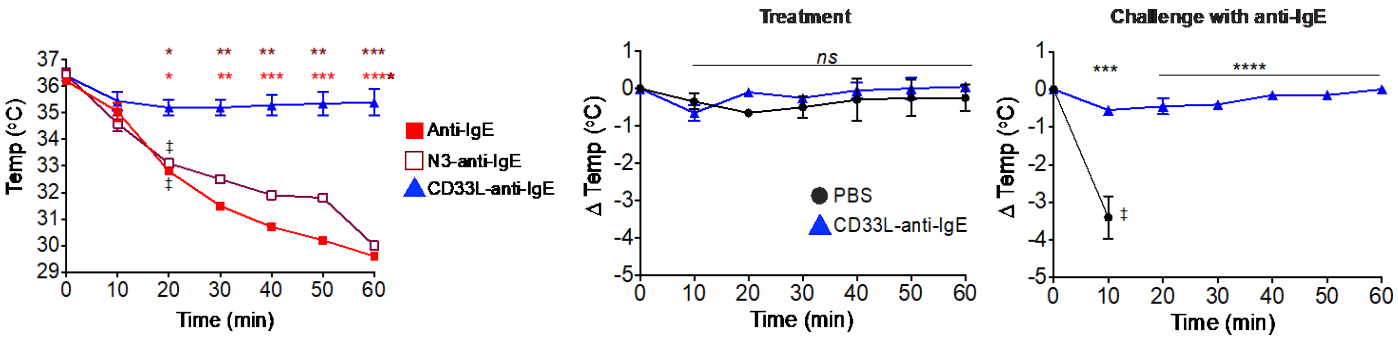

Figure 6. Anti-IgE-CD33L desensitizes NSG-SGM3-CD34+ humanized mice to anti-IgE mediated systemic anaphylaxis. Mice were sensitized with anti-OVA human IgE i.v 24 hours before testig. A) Sensitized mice (N=3) were injected with 5 µg anti IgE ± modification with linker (N3-anti-IgE) or linker and CD33L (CD33L-anti-IgE). B) Sensitized mice were treated with PBS or 5 µg CD33L-anti-IgE (left) and 5 hours later were challenged with 5 µg anti-IgE. Results in (A) were analyzed by Two-way ANOVA followed by Tukey's test (*P<0.05, **P<0.01, ***P<0.001 ****P<0.0001); Results in (B) were analyzed by Two-way ANOVA followed by Sidak's multiple test (***P=0.0002 and ****P<0.0001), ‡ indicates mice found dead.

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. We are still working towards 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 allergens. This part of the original project may be further explored in the Expansion Award period.

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

  At UNC: Lakeya Hardy, a graduate student in Dr. Kulis' lab, has been funded through 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. She is expected to publish her work on CD22 STALs in 2022 and then write her thesis and defend sometime in 2022.

  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, TSRI, and UofA

The project funds have been spent down. We have a manuscript in submission focused on CD22 STALs in mouse models and another in preparation focused on CD33 STALs and human basophils. We have now been awarded an Expansion Award to conduct further experiments to establish the translational nature of STALs.

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. Development of a novel model to investigate human B cells in vivo using NSG mice repopulated with human PBMCs, we are moving closer to demonstrating effects on human cells and moving towards clinical applications.

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. Our development of a novel reagent conjugating the hCD33L to anti-human IgE may prove truly important for clinical utility since no liposomes are used and there is no need for allergen-specific strategies, rather this tolerizing agent may work for mast cell and basophils in a non-allergen specific fashion.

- **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.
  - Development of a novel mouse model in NSG mice to study human CD22 on human B cells in vivo is an important model to study food allergy and other immunologic diseases.
  - 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.
  - Creation of a novel reagent coupling human CD33L to anti-human IgE could be a powerful reagent to study tolerance in a non-allergen specific fashion.

- **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**

  The major delay in the experiments resulted from COVID-19, which shut down our labs for several months and have caused intermittent delays in experiments throughout the past two years. The DoD was gracious enough to award us a no-cost extension to continue the projects after the COVID-19 disruptions. Overall, we were still highly productive during the pandemic and have published several papers with two more in various stages of submission and preparation.

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

  COVID-19 delays caused a slowing of expenditures at UNC. We have been granted a second no-cost extension to continue using the remaining funds. All funds have now been spent down at all three collaborating institutions.

- **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:

Publications: Eight Peer-Reviewed Publications (Two additional manuscripts in preparation).

1. Exploiting CD22 on antigen-specific B cells to prevent allergy to the major peanut allergen Ara h 2 (2017). Orgel KA, Duan S, Wright BL, Maleki SJ, Wolf JC, Vickery BP, Burks AW, Paulson JC, Kulis MD, Macauley MS. J Allergy Clin Immunol 139(1): 366-369.
2. Human CD22 inhibits murine B cell receptor activation in a human CD22 transgenic mouse model (2017). 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. J Immunol 199(9): 3116-3128.
3. Antigenic liposomes for generation of disease-specific antibodies (2018). Bednar KJ, Hardy L, Smeekens J, Raghuwanshi D, Duan S, Kulis MD, Macauley MS. J Vis Exp 140.
4. A mouse model of peanut allergy induced by sensitization through the gastrointestinal tract (2018). Orgel K, Kulis MD. Methods Mol Biol 1799: 39-47.
5. CD33 recruitment inhibits IgE-mediated anaphylaxis and desensitizes mast cells to allergen (2019). Duan S, Koziol-White CJ, Jester WF, Nycholat CM, Macauley MS, Panettieri RA, Paulson JC. J Clin Invest 129(3): 1387-1401.
6. Exploiting CD22 to selectively tolerize autoantibody producing B-cells in Rheumatoid Arthritis (2019). Bednar KJ, Nycholat CM, Rao TS, Paulson JC, Fung WP, Macauley MS. ACS Chem Biol 14(4): 644-654.
7. Coordinated roles for glycans in regulating the inhibitory function of CD22 on B cells (2019). Enterina JR, Jung J, Macauley MS. Biomed J 42(4): 218-232
8. Duan, S.; Paulson, J. C., Siglecs as Immune Cell Checkpoints in Disease. *Annu Rev Immunol* **2020**.

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

- None

Other publications, conference papers, and presentations

- **Abstracts and presentations at conferences:**

2017

1. UNC Pediatric Scholarship Day
   April 20, 2017, UNC, Chapel Hill, NC
   Title: Exploiting CD22 on Ara h 2-Specific B-Cells to Prevent Allergy to the Major Peanut Allergen Ara h 2

Authors: **Kelly Orgel**, Shiteng Duan, Brian Vickery, A. Wesley Burks, James Paulson, Matt Macauley, Michael Kulis

2. Society for Glycobiology Annual Meeting
   November 5-8, 2017, Portland, OR
   Title: Development of a New Human CD22 Transgenic Mouse
   Authors: **Matthew S Macauley**, Kyle J Bednar, Elena Shanina, Romain Ballet, Edward P Connors, Shiteng Duan, Joana Juan, Britni M. Arlian, Mike D Kulis, Eugene C Butcher, Wai-Ping Fung-Leung, Tadimeti S Rao, James C Paulson

2018

3. American Academy of Allergy, Asthma, and Immunology Annual Conference
   March 2-5, 2018, Orlando, FL
   Title: Using Siglec-engaging Tolerance-inducing Antigenic Liposomes (STALs) to reduce memory B cell responses to the major peanut allergen Ara h 2
   Authors: **L. Hardy**, K. Orgel, S. Duan, Soheila Maleki, A.W. Burks, J. Paulson, M. Macauley, M.Kulis

4. Gordon Conference on Food Allergy
   Jan 7-12, 2018, Ventura, CA
   Title: Targeting inhibitory Siglecs to prevent IgE dependent anaphylaxis
   Authors: **Shiteng Duan**, Corwin M. Nycholat, Matthew S. Macauley, Zhou Zhu, Bruce S Bochner, and James C. Paulson

5. American Chemical Society Spring National Meeting
   March 18-22, 2018, New Orleans, LA
   Title: Exploiting the Inhibitory Function of CD22 on B-cells to Prevent Antibody Responses
   Authors: **Matthew S. Macauley**, Britni M. Arlian, Kyle J. Bednar, Shiteng Duan, Wai-Ping Fung-Leung, Mike D. Kulis, Lakeya Hardy, Corwin M. Nycholat, Kelly A. Orgel, Lijuan Pang, James C., Paulson, Tadimeti S Rao

6. American Chemical Society Spring National Meeting
   March 18-22, 2018, New Orleans, LA
   Title: Siglecs: Putting the brakes on the immune system
   Authors: **James C. Paulson**, Britni Arlian, Shiteng Duan, Landon Edgar, Chika Kikuchi, Matthew Macauley, Corwin M. Nycholat, Lijuan Pang, Amrita Srivastiva

7. 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**

8. 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.

9. 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.

10. 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.

11. Oral presentations: James C. Paulson (Partnering PI)

a. International Symposium "Systems Glycobiology and Beyond" – November 16-17, 2017
   Riken University, Wako, Japan
   Title: Siglec targeted nanoparticles for desensitization of mast cells
b. Department of Biochemistry – January 12, 2018
   Duke University, Durham, North Carolina
   Title: Harnessing inhibitory Siglecs to control immune responses
c. 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
d. 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
e. Sialoglyco 2018 – May 10-14, 2018
   Banff, Canada
   Title: Siglec suppression of immune cell responses and home prices: Location, location, location
f. ATS 2018 International Conference – March 18-23, 2018
   San Diego, California
   Glycobiology and Glycomics of Lung Disease
   Title: Targeting siglecs to desensitize mast cells
g. 43rd FEBS Congress – July 7-12, 2018
   Prague, Czech
   Glycans in Health and Disease I
   Title: Exploiting siglecs to modulate immune response
h. 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

12. Poster/Oral presentations: Shiteng Duan (Grad student)

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

13. Oral presentations: Macauley S Macauley (Sub-contract at UofA)

a. Society for Glycobiology – November 7-10, 2017

Portland, Oregon
Title: Development of a new human CD22 mouse model.

b. 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.

c. Sialoglyco – May 14-18, 2018
Banff, Albert, Canada
Title: Changes in Sialic acid during B-cell Differentiation Controls the Germinal Center through CD22

- **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.
- **Abstracts and presentations at conferences: Michael Kulis (PI)**
  - Invited Oral Presentation – March 2020
    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 – March 2020
    American Academy of Allergy, Asthma, Allergy, and Immunology.
    Online (originally planned to be held in Philadelphia, PA)
    Title: Using Siglec-engaging Tolerance-inducing Antigenic Liposomes (STALs) to exploit CD22 on peanut-specific memory B cells to induce tolerance.

14. Website(s) or other Internet site(s)

Nothing to Report

15. 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

16. 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, nothing significant to report.

  Of important note: Dr. Kulis and Dr. Paulson were awarded an Expansion Award from the DoD to continue our efforts on STALs for the treatment of food allergies.

- **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:**
  Not applicable.

- **QUAD CHARTS:**
  Not applicable

9. APPENDICES:
Not applicable