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TITLE: mRNA Vaccine for Peanut Allergy

PRINCIPAL INVESTIGATOR: Michael Kulis, PhD

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

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

During Year 1 of this Discovery Award, we have made significant progress on Specific Aim 1. We provided peanut allergen sequence information to Synbio Technologies who then synthesized Ara h 1, 2, 3 and 6 mRNA, as well as mouse IL-12. We transfected HEK 293T cells with the Ara h 1-6 mRNAs and confirmed protein expression by Western Blot with plasma from peanut-allergic subjects. Cell supernatants and lysates have been sent to MS Bioworks for mass spectrometry analysis of protein expression levels. Once the protein expression has been confirmed by mass spectrometry, we will formulate the mRNA in lipid nanoparticles for intramuscular delivery in mice, and we will send the mRNA to Orlance for formulation on gold microbeads for Gene Gun delivery in mice. Overall, our data demonstrates that peanut allergen mRNA can be expressed in mammalian cells, indicating that we are making progress towards the mRNA vaccine.

15. SUBJECT TERMS

Food allergy, peanut allergy, mRNA vaccine, Gene Gun

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1. **INTRODUCTION:**

Peanut allergy is the result of an abnormal immune response to peanut proteins that results in elevated levels of peanut-specific IgE directed against the major peanut allergens Ara h 1, 2, 3 and 6 with Ara h 2 and 6 contributing to the majority of allergic responses. This proposal stems from a partnership between Orlance, which specializes in nucleic acid vaccines, and our team at the UNC Food Allergy Initiative, which specializes in food allergies and treatments. We aim to leverage our combined expertise to create and test a novel peanut allergy immunotherapy (vaccine) that may lead to a clinical therapeutic for peanut allergy.

2. **KEYWORDS:**

Food allergy, peanut allergy, mRNA vaccine, Ara h 1, Ara h 2, Ara h 3, Ara h 6, IgG, Gene Gun, lipid nanoparticles

3. **ACCOMPLISHMENTS:**

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

Specific Aim 1: Construct mRNAs expressing Ara h 1, 2, 3, 6 and mouse IL-12.

- **Major Task 1:** Synthesize individual mRNAs encoding Ah1-6 and mouse IL-12.
  - Target date: months 1-3. Percentage of completion: 100%
- **Major Task 2:** Confirm the expression of Ah1-6 and IL-12.
  - Target date: months 3-6. 80%

Specific Aim 2: Determine the safety and efficacy of the peanut mRNA vaccine in peanut allergic mice.

- **Major Task 1:** Determine whether the mRNA vaccines induce peanut-specific IgG in naïve BALB/cJ and CC027 mice.
  - Target date: months 1-12. Percentage of completion: 20%
- **Major Task 2:** Determine whether the vaccine can safely and effectively treat peanut allergic BALB/cJ and CC027 mice.
  - Target date: months 13-24. Percentage of completion: 0%

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

Specific Aim 1: Construct mRNAs expressing Ara h 1, 2, 3, 6 and mouse IL-12.

- **Major Task 1:** Synthesize individual mRNAs encoding Ah1-6 and mouse IL-12.

  - The Ara h 1, 2, 3, 6 and mouse IL-12 mRNA was synthesized by Synbio Technologies. Our lab received 1 mg of each mRNA and 5 µg of template so that we can generate more mRNA if needed.

- **Major Task 2:** Confirm the expression of Ah1-6 and IL-12.
  - We confirmed expression of each mRNA using HEK-293 FT cells. Briefly, cells were transfected with Ara h 1, 2, 3, 6 and IL-12 mRNA transcripts using Lipofectamine MessengerMAX Transfection Reagent. We also included eGFP as a control during transfection. After 24-28 hours, cells and supernatants were collected for Western blot and mass spectrometry analysis. Western blots were performed using both cell lysates and supernatants, with purified allergens as controls, and incubated with plasma from peanut-allergic subjects containing high levels of peanut-IgE. The Western blot confirmed expression of Ara h 1, 2 and 3, but Ara h 6 was not detectable with this method (Figure 1). Both cell lysates and supernatants have been shipped to MS

Bioworks to identify these proteins by mass spectrometry. We expect Ara h 6 is expressed, but at lower levels than the other peanut allergens, meaning Ara h 6 will be detectable by mass spectrometry.

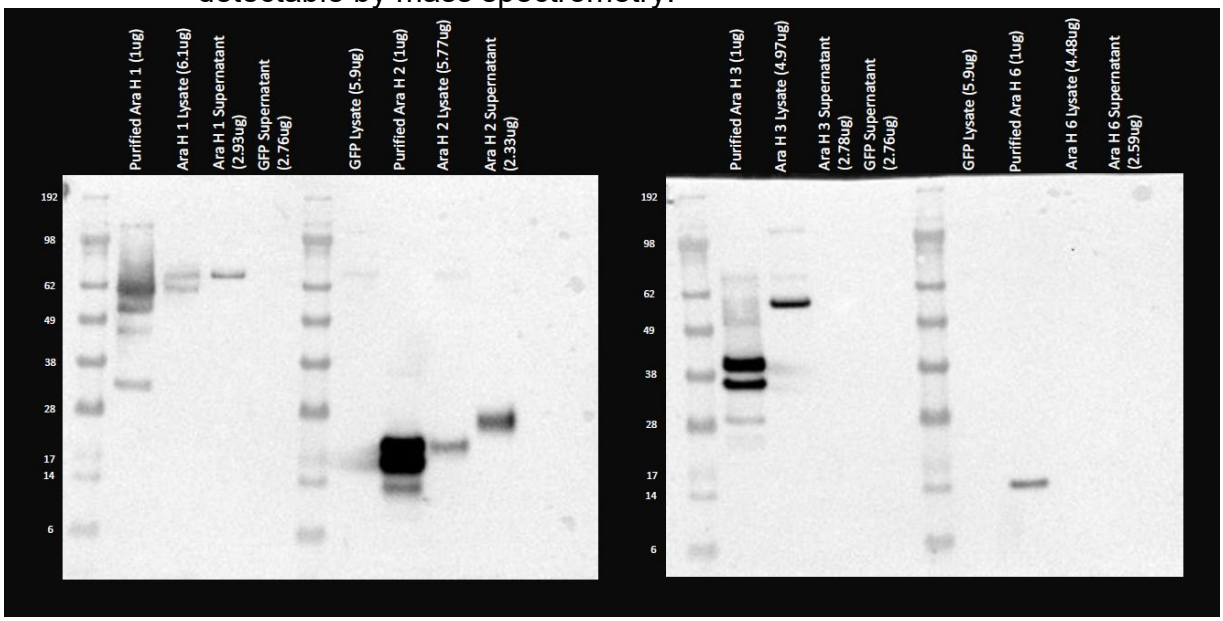

Figure 1. Western blot showing human IgE binding to purified Ara h 1, 2, 3 and 6, and proteins from transfected HEK 293 FT cells.

Specific Aim 2: Determine the safety and efficacy of the peanut mRNA vaccine in peanut allergic mice.

- **Major Task 1:** Determine whether the mRNA vaccines induce peanut-specific IgG in naïve BALB/cJ and CC027 mice.
    - We have received approval from UNC IACUC to perform the proposed experiments in both BALB/cJ and CC027 mice. These experiments will begin following the mass spectrometry analysis described in Aim 1 Task 2.
- **Major Task 2:** Determine whether the vaccine can safely and effectively treat peanut allergic BALB/cJ and CC027 mice.
    - We have received approval from UNC IACUC to perform the proposed experiments. These experiments will begin once we demonstrate immunogenicity of the mRNA vaccines in Aim 2 Task 1.
    - **What opportunities for training and professional development has the project provided?**
        - Nothing to report.
    - **How were the results disseminated to communities of interest?**
        - Nothing to report.
    - **What do you plan to do during the next reporting period to accomplish the goals?**

At UNC: For Specific Aim 1, Task 2, we are awaiting results from the mass spectrometry analysis to confirm protein expression in mammalian cells. Once we receive this data, this task and aim will be complete.

For Specific Aim 2, Task 1, we will next send the mRNA to Orlance for formulation on gold microbeads for Gene Gun delivery in mice. We will formulate the mRNA into lipid nanoparticles for intramuscular injection in mice. Once these particles are formulated, we will begin immunizing mice with the mRNA vaccines. Based on the results from Task 1, we will determine the best mRNA vaccines to use in our treatment model in Task 2.

At Orlance: For Specific Aim 2, Task 1, Orlance will formulate the mRNA on gold microbeads for Gene Gun delivery in mice. They will then ship these particles back to UNC for testing in mice.

4. **IMPACT:**
    - **What was the impact on the development of the principal discipline(s) of the project?**
        - Nothing to report.
    - **What was the impact on other disciplines?**
        - Nothing to report.
    - **What was the impact on technology transfer?**
        - Nothing to report.
    - **What was the impact on society beyond science and technology?**
        - Nothing to report.
5. **CHANGES/PROBLEMS:**
    - **Changes in approach and reasons for change**
        - Nothing to report.
    - **Actual or anticipated problems or delays and actions or plans to resolve them**
        - Nothing to report.
    - **Changes that had a significant impact on expenditures**
        - Nothing to report.
    - **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**
     - Nothing to report.
   - **Website(s) or other Internet site(s)**
     - Nothing to report
   - **Technologies or techniques**
     - Nothing to report.
   - **Inventions, patent applications, and/or licenses**
     - Nothing to report.
   - **Other Products**
     - Nothing to report.

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

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

Name:	*Michael Kulis*
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1.2*
Contribution to Project:	*Supervising the daily activities and progress of the project at UNC.*
Funding Support:	*N/A*

Name:	*Johanna Smeekens*
Project Role:	Co-investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1.8*
Contribution to Project:	*Experimental design and troubleshooting.*
Funding Support:	*N/A*

Name:	*Andrew Turner*
Project Role:	Research Technician
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	0.6
Contribution to Project:	*Transfection of mRNAs and Western blotting.*
Funding Support:	*N/A*

Name:	*Kenneth Bagley*
Project Role:	Co-investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1.2*
Contribution to Project:	*Provided guidance on mRNA synthesis and provided sequences to Synbio Technologies.*
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.*
- **What other organizations were involved as partners?**
  - *Only UNC and Orlance were involved in this project during the reporting period.*

8. **SPECIAL REPORTING REQUIREMENTS**
   - Nothing to report.
9. **APPENDICES**
   - Nothing to report.