



A Mouse Model of Peanut Allergy Induced by Sensitization Through the Gastrointestinal Tract

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Abstract

Animal models of disease enable the study of the pathology, biomarkers, and treatments for the disease being studied. These models become particularly useful in the study of diseases, such as peanut allergy, that currently have no FDA-approved therapy options. Here, we describe a mouse model of peanut allergy using a peanut extract and cholera toxin that can be applied to both BALB/c and C3H/HeJ mouse strains. Sensitization is induced through the gastrointestinal tract resulting in elevated levels of peanut-specific IgE and anaphylaxis upon challenge with peanut proteins. This model has been used to study the cells and molecules involved in the development of peanut allergy and to evaluate novel immunotherapy approaches and the underlying mechanisms of immunotherapy. Potential utilities of this model are numerous and may include studies on microbial influences on peanut allergy and discovery of biomarkers of anaphylaxis.

Key words Peanut allergy, Food allergy, Mouse model, Anaphylaxis, IgE

1 Introduction

Peanut allergy, a growing public health concern, currently affects about 1% of the US population [1–3]. During an allergic reaction, peanut-specific IgE bound to the high-affinity IgE receptor (FcεRI) on mast cells and basophils is cross-linked by peanut allergen [4]. This results in activation of mast cells and basophils, which release histamine, leukotrienes, and other mediators into tissues. These mediators are ultimately responsible for the allergic symptoms observed in patients, including hives, angioedema, throat tightness, abdominal pain, and vomiting. Allergic patients are advised to avoid the allergen and carry auto-injectable epinephrine in case of accidental exposure [5].

Peanut allergy has drastic implications on the patients' quality of life [6, 7] and can be life-threatening and even fatal [8, 9], yet there remain no FDA-approved therapy options to treat peanut allergies [10]. Furthermore, there are increasingly more questions

surrounding the epidemiology of peanut allergy. Specifically, there is limited knowledge on the causes of food allergies, biomarkers for the disease, and biomarkers for anaphylaxis [11–13]. In order to better understand the disease and to develop new therapeutics, animal models of peanut allergy have been developed. The model described here utilizes cholera toxin as an adjuvant to break oral tolerance in either BALB/c or C3H/HeJ mice and sensitize them to peanut through the gastrointestinal tract. Our group has used this model to investigate novel preventive and therapeutic approaches, among other investigations in similar mouse models [14–22].

2 Materials

Prepare all reagents with Milli-Q Type 1 ultrapure water when possible. All thawed or reconstituted reagents are stored at 4 °C for a maximum of 4 weeks. Other than reagents for ELISAs and preparation of peanut extract, only open and use reagents under a sterile biosafety cabinet. Follow all waste disposal instructions when disposing of reagents. The institution's Division of Laboratory Animal Medicine (DLAM) should be made aware of the use of cholera toxin and informed to not change the cage within 2 days of any treatment with cholera toxin. All animal research should be approved by the Institution's Animal Care and Use Committee (IACUC).

2.1 Peanut Extract

1. Peanut flour: 12% fat light roast, 50% protein (Golden Peanut Co.).
2. Phosphate-buffered saline (PBS) with 1 mol/L NaCl.
3. Microcentrifuge.
4. 0.4 µM syringe filter.
5. 0.2 µM syringe filter.
6. Bicinchoninic acid assay (BCA).
7. NuPAGE 4–12% Bis-Tris gel and protein gel apparatus (Thermo Fisher).

2.2 Mouse Sensitization

1. Cholera toxin: 1 mg/mL cholera toxin from *Vibrio cholerae* in ultrapure water. Resuspended aliquot can be stored at 4 °C for 3–4 weeks.
2. 22 g × 1.5", 1.25 mm straight oral gavage needle.
3. 1 mL syringe.
4. BALB/cJ: female mice (*see* **Note 1**).
5. C3H/HeJ: female mice (*see* **Note 1**).

2.3 Serum Collection, ELISAs for Immunoglobulin Quantification, and Core Temperature Measurements

1. Microtainer TSS serum separator tubes (Becton Dickinson).
2. 0.05 M carbonate-bicarbonate buffer, pH 9.6 (Sigma).
3. 96-well non-treated polystyrene plate.
4. 2,4-dinitrophenyl hapten conjugated to human serum albumin (DNP-HSA): 20 µg/mL in carbonate-bicarbonate buffer (*see Note 2*).
5. ELISA wash solution: 0.05% Tween 20 in PBS.
6. ELISA blocking solution and antibody diluent: 0.05% Tween 20 and 2% bovine serum albumin fraction V in PBS.
7. Detection antibodies: *For specific IgE*, 0.5 µg/mL sheep anti-mouse IgE, 0.5 µg/mL biotinylated donkey anti-sheep IgG, 0.2 µg/mL neutravidin-horseradish peroxidase (HRP). *For specific IgG1*, 10 ng/mL HRP-conjugated goat anti-mouse IgG1.
8. 3,3',5,5'-Tetramethylbenzidine (TMB) substrate solution: SureBlue TMB Microwell Peroxidase Substrate (KPL).
9. TMB stop solution (KPL).
10. Microplate colorimetric spectrophotometer.
11. Rectal probe: Small animal probe to measure core body temperature.

3 Methods

3.1 Peanut Extract

1. Extract peanut proteins by mixing peanut flour in a 1:5 (wt:vol) ratio of phosphate-buffered saline (PBS) with 1 mol/L NaCl.
2. Mix solution with stirring bar on magnetic stir plate for 2 h at room temperature while maintaining an alkaline pH (8.5) (*see Note 3*).
3. Centrifuge solution at $30,000 \times g$ for 45 min at 4 °C.
4. Decant to collect supernatant, and then filter-sterilize it sequentially through a 0.4 µM filter followed by a 0.2 µM filter.
5. Determine protein concentration by bicinchoninic acid assay (BCA) using bovine serum albumin (BSA) as the standard (*see Note 4*).
6. Run collected peanut protein on a NuPAGE gel with a 10–20 µg protein load per well to identify and compare quantities of peanut allergens Ara h 1, 2, 3, and 6 between extract preparations. Use a molecular weight marker for easy identification of key allergenic protein bands (Fig. 1).

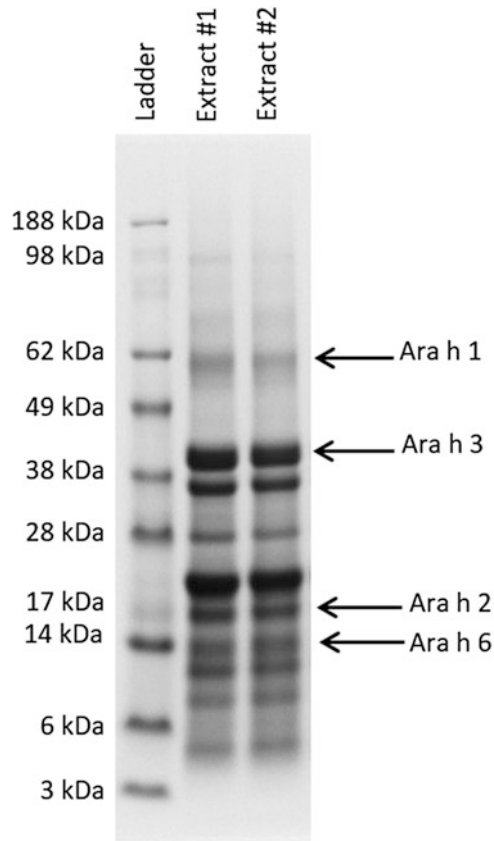


Fig. 1 SDS-PAGE analysis of peanut extracts. Two peanut extracts are shown side by side with 20 μ g of protein loaded per lane. The major peanut allergens, Ara h 1, 2, 3, and 6, are labeled

3.2 Mouse Sensitization

1. Mice can be separated into naïve or sensitized groups.
2. Maintain naïve mice throughout the experiment by treating them with PBS by oral gavage once a week for 4 weeks according to the schedule schematic shown in Fig. 2.
3. Sensitize 4-week-old female mice with 200 μ L containing 2 mg peanut extract and 10 μ g cholera toxin diluted in PBS by oral gavage once a week for 3 weeks. On the fourth week of sensitization, gavage mice with 350 μ L containing 5 mg peanut extract and 10 μ g cholera toxin diluted in PBS.

3.3 Serum Collection and ELISAs for Immunoglobulin Quantification

1. One week after the final sensitization dose, collect 200 μ L whole blood by submandibular bleed into BD Microtainer SST serum separator tubes. Allow blood to clot for at least 20 minutes after collection before centrifuging at 8000 RPM for 15 min. Transfer serum layer to 0.7 microfuge tube, and freeze at -20°C until needed for ELISA.
2. Coat 96 well non-treated polystyrene plate with 20 μ g/mL whole peanut extract for sample wells and 20 μ g/mL DNP-

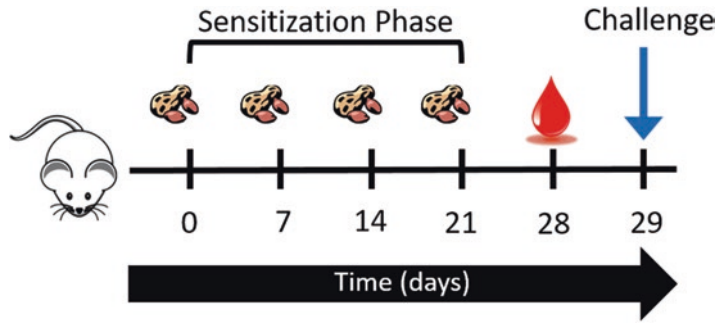


Fig. 2 Schematic of sensitization protocol. Four-week-old female mice are sensitized once weekly for 4 weeks with peanut and cholera toxin by oral gavage. One week later (day 28), mice are bled by submandibular bleed to measure immunoglobulins. The following day, mice can be challenged with peanut extract by intraperitoneal injection

HSA for standard curve wells in carbonate-bicarbonate buffer for 1 h at 37 °C or overnight at 4 °C.

3. Wash plates 3 times with 200 μ L ELISA wash solution.
4. Block plates with ELISA blocking and antibody diluent solution for 2 h at 37 °C or overnight at 4 °C.
5. Repeat plate washing three times with 200 μ L ELISA wash solution.
6. Add serum samples for 1 h at 37 °C. For peanut-specific IgE measurements, use a serum dilution between 1:100 (BALB/c mice) and 1:200 (C3H/HeJ mice). For peanut-specific IgG1 measurements, use a serum dilution between 1:10,000 (BALB/c mice) and 1:20,000 (C3H/HeJ mice).
7. Repeat plate washing three times with 200 μ L PBS containing 0.05% Tween 20.
8. For detection of IgE, add 100 μ L/well sheep anti-mouse IgE (0.5 μ g/mL) for 1 h at 37 °C. Repeat plate washing three times with 200 μ L ELISA wash solution. Add 100 μ L biotinylated donkey anti-sheep IgG (0.5 μ g/mL), and incubate for 1 h at 37 °C. Wash plate three times with 200 μ L ELISA wash solution. Add 100 μ L/well neutravidin-horseradish peroxidase (NA-HRP; 0.2 μ g/mL) for 1 h at 37 °C. Again, wash plate three times with 200 μ L ELISA wash solution. Representative examples of peanut-IgE levels pre- and post-sensitization with peanut extract plus cholera toxin are shown in Fig. 3a.
9. For detection of IgG1, add 100 μ L/well HRP-conjugated goat anti-mouse IgG1 used at 10 ng/mL for 1 h at 37 °C. After incubation, wash plate three times with 200 μ L ELISA wash solution.
10. Measure HRP activity by blue color development of SureBlue TMB Microwell Peroxidase Substrate solution. To do this, add 100 μ L TMB Substrate solution to all wells. Incubate with shaking at room temperature for approximately 10 min.

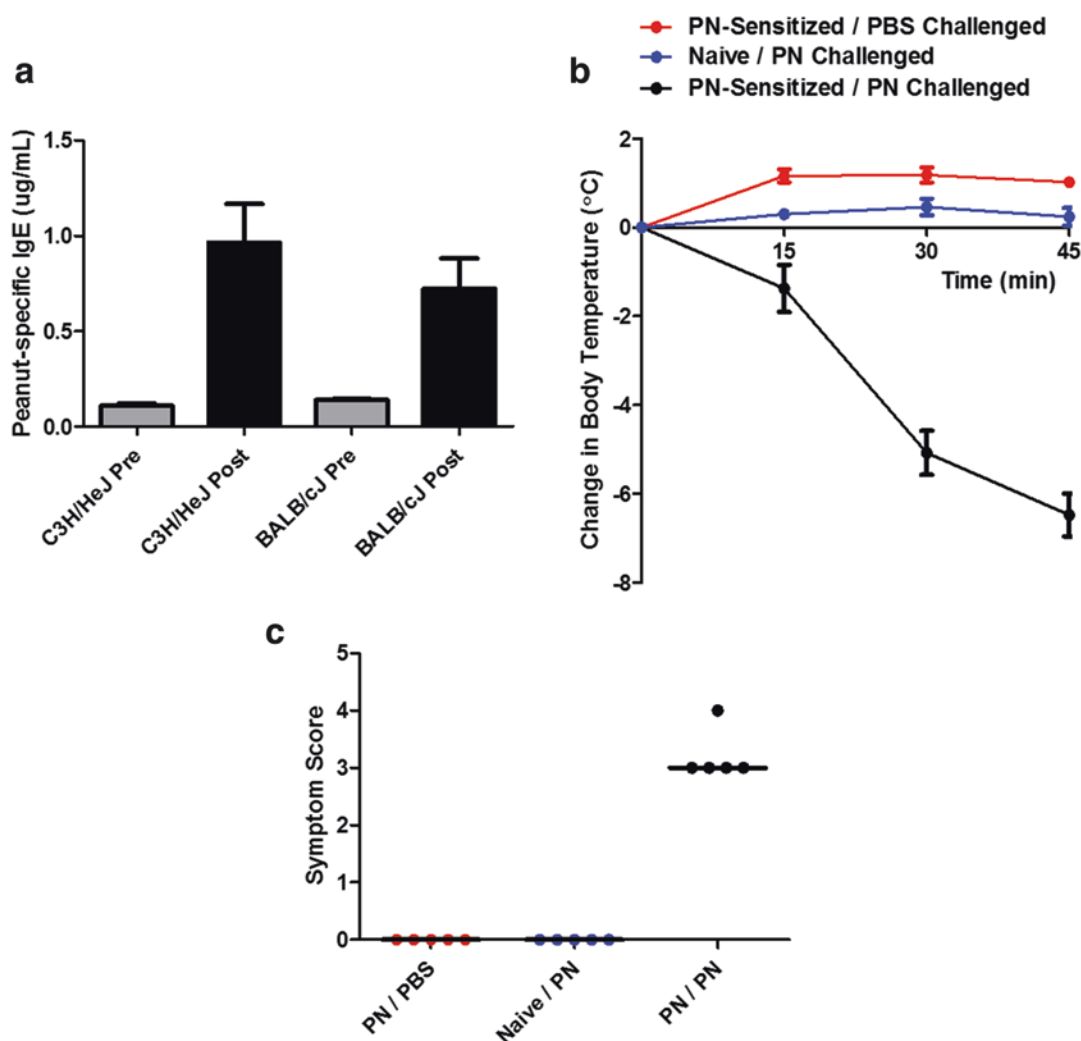


Fig. 3 Representative peanut-specific IgE levels and challenge outcomes. **(a)** Peanut-specific IgE levels in C3H/HeJ and BALB/cJ mice pre- and post-sensitization with peanut extract plus cholera toxin. **(b)** Changes in body temperatures of peanut (PN)-sensitized C3H/HeJ mice after challenge with 200 μ g peanut extract; note that peanut-sensitized mice challenged with an equal volume of PBS do not have decreased body temperature and that naïve (i.e., not sensitized to peanut) mice challenged with peanut extract do not have decreased body temperature. **(c)** Symptom scores in C3H/HeJ mice; note that only peanut-sensitized mice challenged with peanut exhibit allergic symptoms

11. Add 100 μ L of TMB stop solution to each well. Wells that turned blue after TMB addition will turn yellow after the acidic stop solution is added. Avoid causing bubbles in wells as this may interfere with absorbance readings.
12. Read plates on colorimetric spectrophotometer at 450 nm wavelength.

3.4 Intraperitoneal Peanut Challenge

1. Allow animals' body temperature to rise for 15 min prior to beginning the challenge (*see* Note 5).

2. Using a rectal thermometer probe, measure and record the body temperatures of the mice. These values will serve as baseline temperatures.
3. Inject 200 μ L diluted peanut extract in PBS (200 μ g for C3H/HeJ mice and 300 μ g for BALB/c mice) into the intraperitoneal cavity of each animal. Start a timer after the first mouse has been injected (*see Note 6*).
4. Measure and record each animal's body temperature every 15–60 min. Animals experiencing a systemic reaction will have a drop in body temperature, while those experiencing no reaction will maintain a constant body temperature (Fig. 3b).
5. During the 30-min body temperature reading, assign a symptom score to each animal (Fig. 3c). Symptoms are assigned based on the following scale: 0, no symptoms; 1, scratching around the nose and head; 2, puffiness around the eyes and mouth with reduced activity; 3, labored respiration and/or cyanosis around the mouth and tail; 4, no activity after prodding or tremor and convulsion; and 5, death (*see Note 7*).
6. If blood samples are required during the challenge, a submandibular bleed can be performed at the desired time point (*see Note 8*).
7. After 60 min, the housing, tunnels, and food can be returned in the cages, and cages can be returned to their location in the housing facility. If a second challenge is needed, the procedure can be repeated after 1 week of resting the animals (*see Notes 9 and 10*).

4 Notes

1. Female mice are used in this model since male mice do not consistently develop elevated peanut-specific IgE nor experience anaphylaxis upon challenge with peanut extract. Female mice are ordered to arrive at 3 weeks of age. They are allowed to acclimate for 1 week after transfer, and procedures are started at 4 weeks of age. Mice are fed standard chow lacking peanut by the University of North Carolina DLAM personnel and housed under pathogen-free conditions. Mice are housed with four or five animals per cage.
2. Individual peanut allergens (e.g., Ara h 1, 2, 3, and 6) can be assayed and are coated at 5 μ g/mL.
3. pH will drop as peanut proteins enter solution. Check pH every 15 min, and adjust to pH 8.5 using 6 M sodium hydroxide. For more concentrated solution, mix and adjust pH for longer than 2 h.

4. Typical concentrations of peanut extract yielded with this protocol are ~15–20 mg/mL.
5. Animals that have been sleeping will have a lower body temperature. Removing any housing, tunnels, and food will result in the animals running and their body temperatures rising. If this step is not done, the baseline temperature readings will appear lower and may mask initial decreases during the challenge.
6. The concentrations of peanut provided here are those that we have optimized to see maximal reactions while minimizing animal death. Depending on the level of sensitization of the animals, the dose may be increased or decreased to induce more or less reaction severity.
7. Symptom scores may worsen at later time points, and while we make note of these changes for our own records, we only report one symptom score in the literature and not changes in scores over time.
8. During a systemic reaction, mice experience vasodilation, so obtaining more than 100 μ L whole blood can be very difficult. Also, mice tend to experience body temperature decrease following a blood draw, so temperature readings after this point are not useful.
9. Mice should be continuously monitored following challenge until their body temperature stops decreasing. Also, any mouse whose body temperature drops below 30 °C should be euthanized for ethical reasons.
10. C3H/HeJ mice that have been sensitized using this model can then undergo immunotherapy to desensitize the animals.

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