To the EAACI Vice President Science- the EAACI Headquarters,

Project title: Transferring an animal model for the evaluation of allergenicity of novel food from rats to mice

Name: Behnaz shafie, PhD student in the Research Group for Food Allergy at DTU National Food Institute, Denmark.

Type, duration, and location of your Fellowship: Research fellowship from 06.01.2025-06.04.2025 (short stay for 3 months) in Vienna, Austria.

Host Institution and Supervisor name: Experimental Allergy Laboratory, Medical University of Vienna, Department of Dermatology, Dr. Michelle Epstein.

The following questions will be answered:

- What questions were addressed and why?
- What was the nature of the research?
- What was the result?
- How will the findings impact future research?

Introduction:

Food allergy is a prevalent disease and seems to increase at alarming rates. Hence, it is crucial to avoid fuelling this increase by introducing new highly allergenic food to the market. As food allergy is a complex disease and sensitization cannot be studied in humans, animal models may provide a good option for the prediction of sensitization. To develop an animal model with predictive capacity, the first step would be to show that the animals would react and be able to rank foods according to their allergenicity in the same way as humans. Here, we have been developing a predictive oral cavity animal model in Brown Norway rats for studying the sensitizing capacity of novel food, using protein extracts from low-allergenic food pig gelatine, medium-allergenic food pea, and high-allergenic food peanut (at DTU). **This research fellowship aims to transfer the oral cavity Brown Norway rat model (at DTU) to a mouse model (at MedUni Vienna) to confirm the validity of using an oral animal model to predict the sensitizing capacity of novel foods.**

Methods:

An initial attempt to transfer the animal model from rats to mice would involve applying the experimental design of the Brown Norway rat model as closely as possible, with modifications to the specific experimental requirements of the mouse model.

1. **Diet of animal:** The Brown Norway rat diet is based on proteins from rice, potato, and fish.

   Hypothesis: It will be possible to transfer an oral cavity Brown Norway rat model to a mouse model to distinguish between foods using a similar allergen-free diet.

2. **Number of animals:** In the Brown Norway Rat oral cavity models, 12 animals were divided into different groups.

Hypothesis: In the mouse model, it will be possible to perform a pilot study with animals, five animals per group for each food.

3. **Using Adjuvant:**

In the Brown Norway rat model, adjuvant has not been used for the studies.

Hypothesis: It will be possible to develop an oral cavity mouse model to distinguish between foods without using an adjuvant.

4. **Duration of study:**

In the Brown Norway rat model, the oral cavity study for 29 days was performed by administering animals (at an optimal dose of 200 µg (400mg/ml) of peanut, pea, and gelatine protein extracts, 0.5 mL) each day for 21 days and collecting blood on days 0, 7, 14, 21and 28 (up to 7.5% of blood volume/animal) and at sacrifice at dat 29 (figure 1). In addition, an ear swelling test was performed on day 24 or 25, and an oral food challenge was conducted to evaluate clinical reactions before termination of the study.

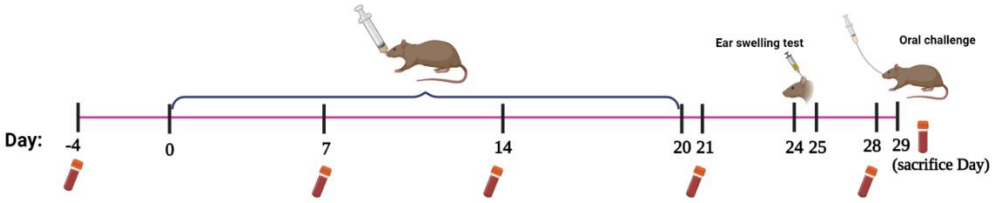

Figure 1. Schematic illustration of the animal experimental design at DTU. Picture created by BioRender.com

Hypothesis: The oral cavity model in Brown Norway rats can be transferred to a mouse model. The duration of the mouse model will likewise be 29 days. Oral cavity dosing will be performed for 21 days with a 32 µg dose of protein extracts (gelatine, pea, and peanut), and the blood will be collected on days 0, 7, 14, 21, 28, and at sacrifice day on day 29. An oral food challenge will be done on day 29 (sacrifice day) to record diarrhea scoring and symptom scoring.

5. **Protein preparation:**

Three different protein extracts have been used in the oral cavity Brown Norway rat model.

Hypothesis: It will be possible to develop an oral cavity mouse model to distinguish between foods using the same three different protein extracts in a mouse model. Gelatine, pea, and peanut protein extracts will be transferred from DTU to MedUni Vienna.

6. **End-point parameters:**

In the Brown Norway rat model, different end-point parameters such as specific IgE and specific IgG1 using the ELISA technique, BAT assay using flow cytometry,

diarrhea scoring, and clinical reactions such as symptom scoring (oral challenge with different protein extracts), as well as the ear swelling test, were performed.
Hypothesis: It will be possible to develop an oral cavity mouse model measuring sIgG1, sIgE ELISA assay (along with establishing ELISA in MedUni Vienna), oral food challenge, and diarrhea scoring.

Animal experimental plan: (MedUni Wien):
Title: Transferring an animal model to evaluate allergenicity of novel food from rats to mice.

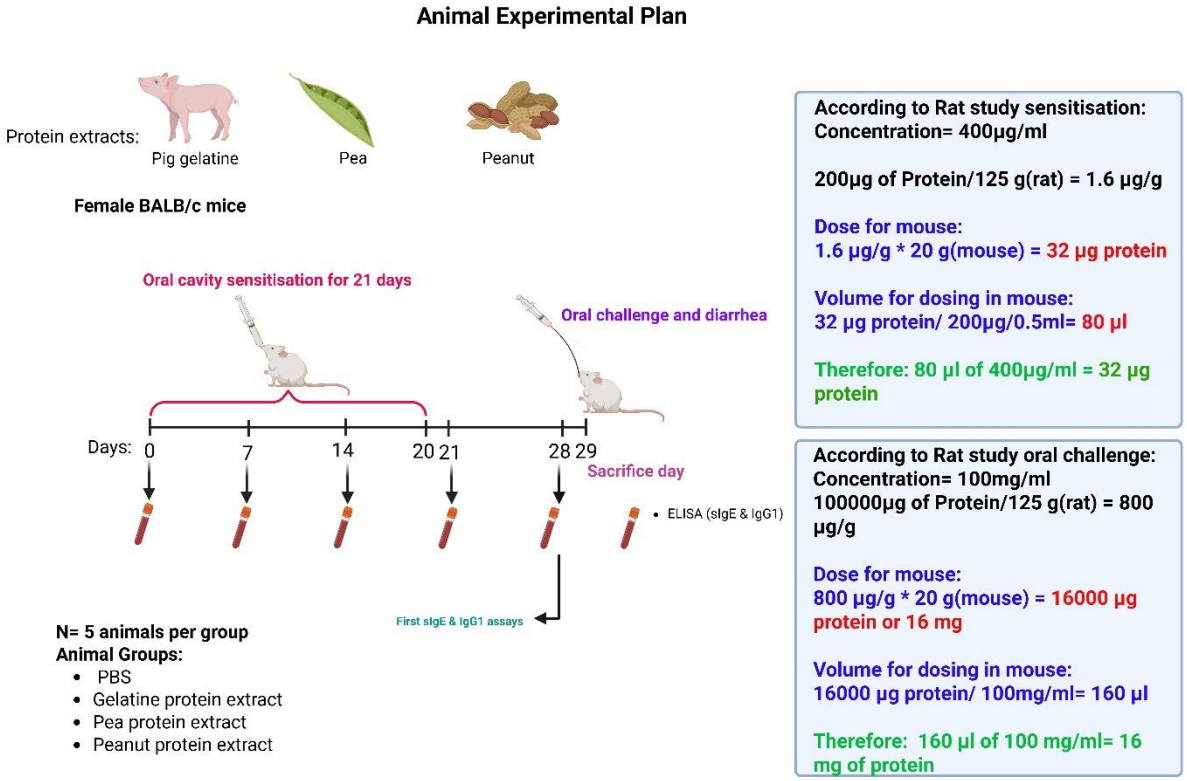

Figure 2. Schematic illustration of the animal experimental design at Med Uni Wien. Picture created by BioRender.com

According to the animal experimental plan (figure 2), animals in each group were given oral cavity administrations each day for 21 days from day 0 to 20 of the experiment.

Before immunization, on days 0, 7, 14, 21, and 28, as well as on the sacrifice day, blood was collected from each animal's tail vein.

On sacrifice day, each group was challenged with 100 mg/ml of gelatine powder, pea powder, and peanut butter powder intragastrically. After the oral challenge, mice were individually observed for 30 minutes, and the clinical allergy score (symptom scoring and diarrhea scoring) was assessed for each group. After 30 minutes, mice were sacrificed, and their blood was collected to do an ELISA assay, and the colon and small intestine were collected to do histology.

Establishing ELISA:

The first attempt was to evaluate the level of sIgG1 and sIgE using ELISA based on the DTU method. First, a pilot i.p study was performed with each protein extract to have a pool af positive control for ELISA assay (as a robust response) (figure 3).

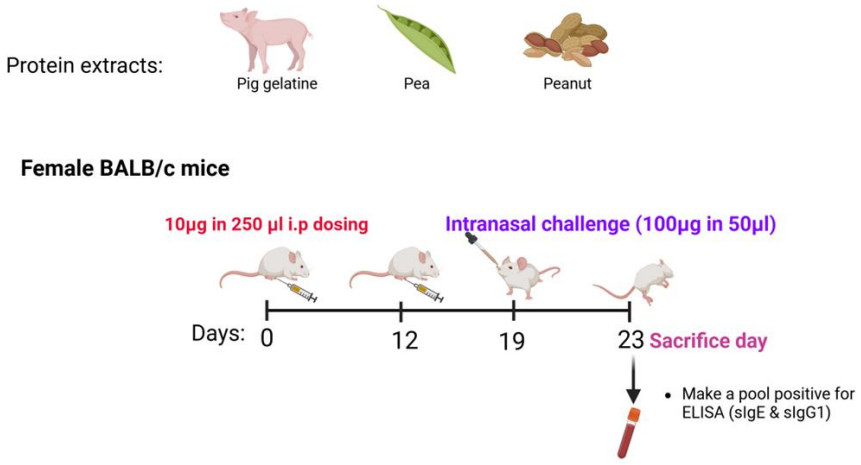

Figure 3. Schematic illustration of the animal experimental design at Med Uni Wien. Picture created by BioRender.com

An Indirect ELISA was optimised for the valuation of sIgG1. The method was set up with different conditions (using different coating concentrations, blocking solutions, and antibody dilutions) for each step and protein extract (figure 4). Due to the time required to evaluate sIgE, the antibody capture ELISA was partially optimised.

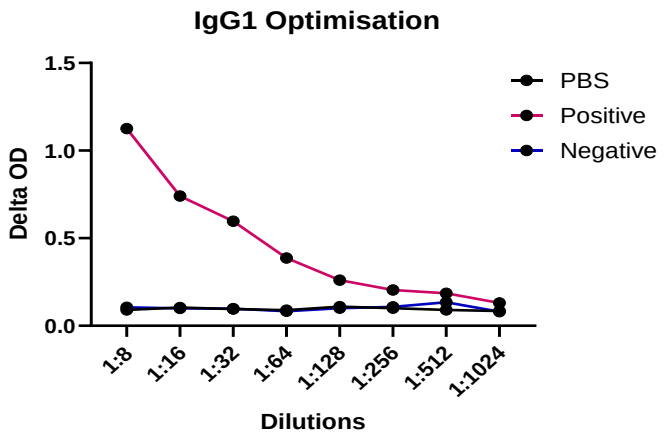

Figure 4. IgG1 optimisation with peanut protein extract.

Results:

In vivo results

Symptom scoring

Symptom scoring was recorded for 30 minutes after the oral food challenge with gelatine, pea, and peanut on day 29 (sacrifice day) to evaluate clinical allergy reaction. The result showed that the manifestation of scratching symptoms (figure 5A) and reduced mobility were statistically significant different in the peanut group compared to the PBS group, whereas there were some scratching symptoms for the pea group, which were not statistically significant different to the PBS group (figure 5B). There were no recorded symptoms for the gelatine group, which did not show any clinical reactions.

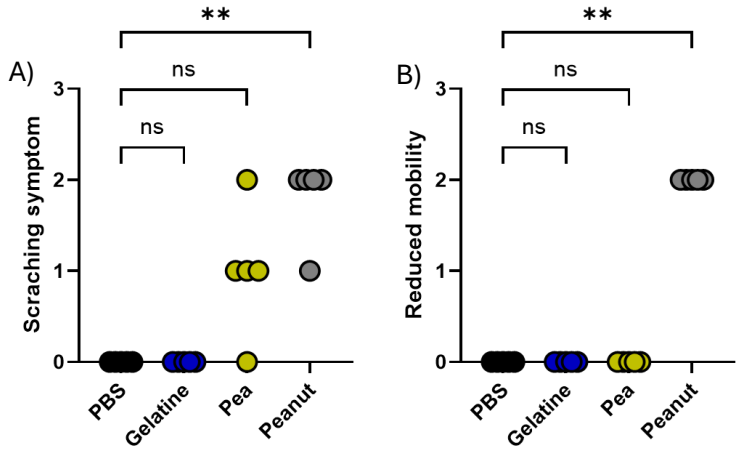

Figure 5. Eliciting capacity is measured by symptom scoring episodes (A & B). Rats were orally challenged with gelatine powder, pea powder, and peanut butter powder with 100mg/ml concentration, and for 30 min, the symptom episode was recorded. Statistically significant differences between foods and PBS groups were determined using the nonparametric Kruskal-Wallis test. Asterisks show as follows: ** p≤0.01.

Diarrhea scoring

Diarrhea scoring was recorded to evaluate clinical allergy reactions. No diarrhea was recorded for the gelatine, pea, and peanut groups compared to the PBS group.

Detection of IgG1 (Immune response)

To evaluate the immune response of gelatine, pea, and peanut, BALB/c mice were administered oral cavity doses for 21 days, with 32 µg doses of each protein extract. Blood was collected at each time point and on the sacrifice day. The level of immune response was detected using indirect ELISA. The level of IgG1 specific for peanut protein extract on different days was shown in the plot graphs (figure 6). As the results show, peanut started to trigger immune response reactions in mice from day 7 to day 29 with an increasing trend over time. Due to the time required to do sIgG1 for pea and gelatine protein extracts, it has been decided to transfer the samples to DTU to carry on doing an ELISA assay with the same ELISA setup as used at DTU.

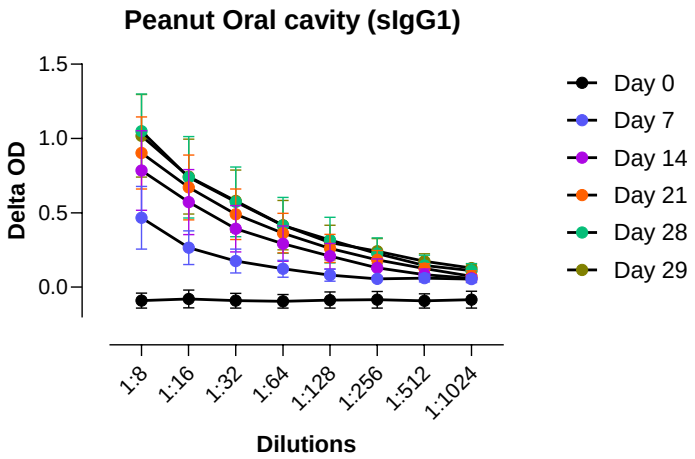

Figure 6. Specific IgG1 antibody response. Comparison of the immunogenicity of peanut in response to 32 µg dose. IgG1-specific antibodies for peanut protein present in mice serum were evaluated by indirect ELISAs at different time points (Day 0, Day 7, Day 14, Day 21, Day 28, and Day 29). The mean and SEM were calculated for each time point.

Discussion

In the present study, the result of clinical reaction and immune response to peanut protein extract showed that it could cause an allergic reaction and trigger an immune response in the oral cavity mouse model, almost the same as the oral cavity Brown Norway rat model, which can verify transferring the model to another lab in terms of validation for peanut protein. Since the validation of sIgG1 for pea and gelatine and the setup of sIgE ELISA would take time, it was decided to transfer the samples from Med Uni Wien to DTU to proceed with the experiments there.

Impact of Findings on Future Research

Although full transfer of the mouse model could not be completed within the secondment period, the results obtained provide strong preliminary support for the feasibility of transferring the established rat model for food allergenicity assessment to mice, allowing to distinguish between low-, medium-, and high-allergenic food. Comparable responses observed with peanut exposure suggest that key immunological features of the model are preserved in another rodent species.

These findings will influence future research in the following ways:

Foundation for model refinement

The data generated serves as a valuable foundation for future studies aiming to complete the validation process of a predictive animal model. Researchers can build on the existing work to refine and optimize the protocol for use in mice, thus accelerating model development and reducing redundant efforts.

Expansion to other allergens

Successful replication of results with peanut, pea, and gelatine paves the way for future testing with additional food allergens. This is critical to determine the broader applicability and reliability of the mouse model across different allergenic foods.

Support for ethical and advanced research

Transferring the rat model to a mouse model enhances research potential through the availability of transgenic strains and advanced immunological parameters. Moreover, it aligns with the 3Rs principles (Replacement, Reduction, and Refinement) by promoting ethical animal use in preclinical testing.

Contribution to protocol harmonization

The experience gained during the transfer process highlights practical aspects of inter-laboratory model replication. This contributes to ongoing efforts to standardize experimental procedures across institutions, enhancing reproducibility and scientific validity in the field of food allergy.

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