EAACI Research Fellowship 2020: Final Report

Project Title: T-cell cross-reactivity to invertebrate pan-allergen Tropomyosin among house dust mite and shellfish allergic patients

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Type of Fellowship: Short term EAACI Research Fellowship

Host Supervisor: Prof. Heimo Breiteneder and A/Prof. Christine Hafner

Host Institution: Division of Medical Biotechnology, Department of Pathophysiology and Allergy Research, Medical University of Vienna, Vienna, Austria

Home Institution: Australian Institute of Tropical Health and Medicine, James Cook University, Townsville, Australia

Duration: 3 months (February 2021- April 2021)

Addressed research questions and nature of the collaboration

Shellfish allergy is a life-long disease which affects nearly 2% of the world population and is often associated with severe, life-threatening reactions. Tropomyosin, an abundant muscle protein in shellfish, is the major allergen from most edible shellfish species (crustaceans and mollusks). More than 80% of shellfish allergic patients are sensitized to this allergen [1]. Importantly, it is a highly conserved protein among various invertebrate species including crustaceans, mollusks, insects and mites, exhibiting more than 70% amino acid sequence identity. Consequently, the clinical cross-reactivity based on linear IgE epitopes has been shown between tropomyosins from various invertebrate species, particularly shrimp and house dust mite (HDM).

HDM allergy is a serious health condition affecting up to 30% of the general population. Approximately 10% of HDM allergy sufferers exhibit IgE sensitization to HDM tropomyosin [2]. Although previous studies have confirmed IgE cross-reactivity and shared IgE epitopes [3-5], there is a lack of knowledge about the T-cell epitopes and T-cell cross-reactivity between tropomyosins from various species. Investigation of T-cell cross-reactivities between the tropomyosins is of crucial importance for understanding the risks of developing allergy to evolutionary distinct species upon primary sensitization to tropomyosin from one species, such as HDM. It is currently not known whether inhalational exposure to HDM tropomyosin in shrimp allergic patients might sensitize and boost IgE levels to shrimp tropomyosin, even if they are avoiding all types of shellfish-based foods, or vice versa.

Researchers at the Medical University of Vienna have previously argued that ingestion of pollen-related food allergen sources might perennially boost IgE levels to pollen due to their shared T-cell epitopes [6]. The overall aim of this project was to investigate tropomyosin-induced T-cell proliferation in peripheral blood mononuclear cells (PBMCs) collected from

HDM-allergic individuals with no clinical history to shrimp allergy, in order to understand the T-cell cross-reactivity patterns.

Work performed during the fellowship

This project was conducted in four stages;

1) Allergen preparation – Four tropomyosins were used in this study; Pen m 1 (from Black tiger shrimp, *Penaeus monodon*), Der p 10 (House dust mite, *Dermatophagoides pteronyssinus*), Bla g 7 (cockroach, *Blattella germanica*), Ani s 3 (fish parasite, *Anisakis simplex*). These allergens were generated as recombinant proteins in a bacterial expression system and purified using affinity chromatography. The proteins were purified by me at my home institute and sent to Vienna.
   Following the purification, different endotoxin removal protocols were employed including EndoTrap columns, endotoxin removal magnetic beads (Miltenyi Biotech) and triton X-144 detergent treatments of proteins with extensive washing to remove residual detergent. All tropomyosins bound very strongly to the bacterial endotoxins originating from the recombinant expression, as confirmed by the LAL assay. Finally, the triton X-144 detergent removal protocol was successful in reducing the endotoxin content without significant loss of protein, and the endotoxin-free proteins were used in downstream cellular assays.
2) Participant recruitment and isolation of PBMCs – At the Medical University of Vienna and the University Hospital St. Pölten, participants were recruited based on a clinical history of house dust mite allergy and positive IgE binding to house dust mite tropomyosin, Der p 10 on immunoblot. Informed consents were obtained from each participant and the study design explained before proceeding with collection of the blood samples. This study received approval from the Ethics Committee of Lower Austria (GS4-EK-4/242–2013). Approximately 20-30 mL of fresh blood was collected from each participant, and immediately processed for PBMC isolation using Ficol-Paque separation technique and collection of the buffy coat layer. The PBMCs were washed with PBS and total viable cells counted using trypan blue staining. The PBMCs were then either resuspended in 10% FBS in DMEM for subsequent experiments, or in 50% FBS/ 10% DMSO/ 40% DMEM for cryo-freezing. PBMCs from healthy non-atopic donors were collected as a negative control.
3) PBMC proliferation assay – For the main assay, total number of required cells was calculated for the entire experiment (stimulation with 4 tropomyosins, house dust mite extract, shrimp extract, positive and negative controls, and blank wells). Allergens and extracts were used in several concentrations from 7 µg/mL to 80 µg/mL. The cells were incubated with 0.6 mM CFSE stain for 10 minutes, washed and resuspended in medium. 10^6 cells were seeded per well of a 24 well plates. The wells were then exposed to different concentrations of each tropomyosin, extracts and the non-related allergen, Bet v1. Anti-CD3 antibody and IL-2 were used as a positive controls. After incubation for 5 days, the cells were washed and stained with anti-CD3, anti-CD4, cell viability dye and isotype controls. T-cell proliferation on exposure to the different allergens was analyzed using a BD FACS Canto II flow cytometer by gating $CD3^+CD4^+CFSE^-$T cells.

4) Besides the cell proliferation assay, supernatants were collected after cell stimulation and immediately frozen for subsequent cytokine quantification. Quantification of Th2 and Th1 cytokines (IL-4, IL-5, IL-13, IL-2, IFN-g, TNFa) will be performed in near future.

Main outcomes of the study

The PBMC proliferation assay was performed for 4 participants with confirmed house dust mite allergy, positive IgE binding to Der p 10 but no history of shellfish (shrimp) allergy. *In vitro* IgE binding to the four tropomyosins (Pen m 1, Der p 10, Bla g 7, and Ani s 3) was confirmed using serum IgE dot blotting and Western blot. For all 4 participants, $CD4^+$ T cell proliferation was observed for the positive controls (anti-CD3 and IL-2). PBMCs of two out of four donors showed proliferation on exposure to the house dust mite extract but not to shrimp extract indicating low T-cell cross-reactivity. Interestingly, PBMCs of none of the tested participants demonstrated T-cell proliferation on exposure to Der p 10 or other three tropomyosins. This may have resulted from the fact that the tested participants did not have a high number of Der p 10-specific T cells which could have proliferated upon allergen exposure. No proliferation of T cells upon stimulation of PBMCs with any allergens or extracts was observed for healthy non-allergic donors.

Impact of the findings on future research

Clinical cross-reactivity is an important topic to be considered while diagnosing shellfish allergy and/or concurrent house dust mite or insect allergy. Due to the structural conservation of tropomyosins, several IgE binding epitopes elicit IgE cross-reactivity under *in vitro* conditions. This often poses a challenge in finding the primary source of sensitization. Although IgE cross-reactivity is well documented and easy to investigate using patients' sera, cellular T-cell cross-reactivity is currently under-investigated. This project will provide preliminary data for larger cohort studies investigating the potential of exposure to tropomyosin from an unrelated source that can cause cross-sensitization and amplification of a Th2-based IgE response among shellfish allergic patients, and vice versa.

Adaptation of research from the original plan

The original research plan was to recruit 10-15 participants with confirmed HDM allergy but tolerant to edible shellfish in Vienna. However, due to unforeseen challenges, only 4 allergic participants and two healthy donors were recruited during this 3-month fellowship. Due to a limited sample size, it is currently difficult to conclude on T-cell cross reactivity between HDM and shrimp tropomyosins. In the original plan, Th2 cytokines were going to be analyzed in the supernatant collected from the cells after exposure to the allergen source or controls. This will be done at a later stage in Prof. Breiteneder's lab. I will continue the collaboration at my home institute in Australia, and recruit clinically-confirmed shellfish and HDM allergic patients to perform PMBC proliferation assays to the same panel of allergens.

Personal reflections

The EAACI 3-month fellowship provided me an opportunity to enrich my research expertise and foster new professional relationships and collaborations with several researchers at the Medical University of Vienna. I had the opportunity to learn the technique involving flow

cytometry and add to my knowledge in cell proliferation assays, handling primary cell cultures including FACSDiva software and FlowJo software. In addition, I was invited to present my current research activities in Australia to the members of the division. I participated in journals clubs as well as informal get-togethers which was a fantastic opportunity to meet researchers in this field and discuss ideas and possibilities of future grant applications. I learned a lot from the working environment and lab management which I look forward to implement in the lab at my home institution. I had the opportunity to meet and discuss ideas with established researchers, Prof. Barbara Bohle and A/Prof. Eva Untersmayr on future projects and collaborations. I was made to feel very welcomed by everyone in the department, and a very collegial atmosphere enabled me to work smoothly while also enjoying the evening walks around campus and city. Vienna has been rated the most livable city in the world, and I agree with it. The vibrant city offered me a chance to experience the European winter in the natural parks and walking tracks during the weekends.

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