Final Report EAACI Research Fellowship 2024

Metabolomic targeted characterization of key drivers in food allergy using a dietary intervention model with pectins in patients allergic to lipid transfer proteins (LTPs)

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Type of Fellowship: Short Term Research Fellowship

Home Institution: San Pablo-CEU University (Madrid, Spain)

Host Supervisor: Prof. Craig. E Wheelock

Host Institution: Institute of Environmental Medicine, Karolinska Institutet (Stockholm, Sweden)

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1. BACKGROUND

Food allergy to lipid-transfer proteins (LTPs)

Food allergy (**FA**) refers to immunologically mediated adverse reactions occurring after the ingestion of certain foods and involves the production of specific type 2 T helper (Th2) lymphocytes in the peripheral blood and intestinal mucosa[1]. In the Mediterranean area, fruit allergy due to sensitization to **lipid transfer proteins** (**LTPs**) is the main cause of IgE-mediated FA in adolescents and adults[2,3]. LTPs are very stable, highly allergenic, and widely distributed allergens with a moderate-to-high homology between different vegetal species (35-95%)[4]. Allergy to LTPs has important clinical implications, as it is characterized by the induction of mild symptoms, such as oral allergy syndrome (OAS) or urticaria, to severe FA and anaphylaxis[5–7]. Currently, the strict avoidance of trigger foods is the main strategy to prevent this type of allergic reactions. However, this does not protect patients against inadvertent ingestion[7]. On the other hand, sublingual immunotherapy has shown to be safe and clinically effective in patients with mild and moderate FA by modulating cellular immune-specific responses[8–11]. Yet, there is a percentage of patients in whom this treatment does not induce tolerance, nor it controls the disease, and could even produce FA symptoms during administration[5]. Overall, these implications can have a significant impact on the quality of life of patients and result in high socioeconomic costs[12], so new treatment approaches are urgently needed.

The potential role of the gut microbiome and pectins in LTP allergy

The **gut microbiota** is thought to play an essential role in the primary sensitization to food allergens and the immune effector response[13]. Indeed, alterations in the composition and function of the gut microbiome have recently been associated with the risk of developing FA[14,15]. This so-called intestinal dysbiosis has been linked to a variety of environmental factors, especially diet[15]. Therefore, dietary

changes – e.g. interventions with **prebiotics** that restore the gut microbiome – may be beneficial in FA[16,17].

Prebiotics are defined as non-digestible food components that stimulate the growth and activity of certain microorganisms[18]. Among them, there is currently great interest in the potential of plant-derived dietary fibers (DFs) as protective components against allergies, based on the promising results that have been obtained in asthma[19–21] and naïve models[22]. In this context, soluble DFs, such as **pectins**, are thought to provide health benefits by promoting the growth of beneficial bacterial strains (e.g. *Bifidobacteria*, *Lactobacilli*, or *Bacteroides* species)[23–25]. In addition, pectins could regulate the inflammatory response through the production of **microbiota-derived metabolites** upon fermentation[26], including **short-chain fatty acids** (**SCFAs**) – which mediate immunomodulatory effects and trigger anti-inflammatory processes[27–29] – and **bile acids** (**BAs**) – which act as mediators of the host-microbiota interaction and have recently been shown to play a role in the regulation of type 2 inflammation[30–32]. However, it is likely that other inflammatory mediators and key players in FA (e.g. bioactive lipids such as **oxylipins** and **sphingolipids**, respectively), may also be involved in the effects of pectins on the immune system and therefore need to be further investigated[33–35].

Pectins are natural polysaccharides derived from fruits, mainly from their peel and pulp, whose chemical structure can vary depending on the source material and the preparation method[36]. In general, the dominant component of pectins is a linear chain of galacturonic acid units (GalA) in which a proportion of the carboxyl groups are presented as methyl esters. The proportion of methyl-esterified GalA units to the total GalA groups is called the **degree of esterification** (**DE**), and this parameter allows for the classification of pectins into **high methoxyl pectins** (DE >50%, **DE^{high}**) and **low methoxyl pectins** (DE <50%, **DE$_{low}$**)[37,38]. Although both DE^{high} and DE$_{low}$ pectins seem to have immunomodulatory effects, it has been suggested that different structural features, such as DE, determine their effect on the immune system[26].

2. AIM AND OBJECTIVES

Considering previous beneficial and promising health effects of DFs in FA, the aim of this project was to analyze the effect of **two different pectins** on the **lipidic systemic profile of LTP-allergic patients**, focusing on **sphingolipids** and **oxylipins**, using **targeted metabolomics**.

To achieve this, the following specific objectives were proposed:

1. To acquire experience on two novel liquid chromatography coupled to triple quadrupole mass spectrometry (LC-QqQ-MS) targeted methodologies for sphingolipids[39] (n = 139) and oxylipins[40] (n = 108), develop by Prof. Craig E. Wheelock's group at Karolinska Institutet.

2. To apply these analytical platforms to 68 serum samples from LTP-allergic patients who had been treated with placebo or one of two pectin varieties during an intervention of two months.
3. To perform data treatment and statistical analysis of the obtained results.

Adaptations from the research plan

In addition, although not included in the original research plan, the award of the EAACI Short Term Research Fellowship allowed the use of an **additional targeted method** for the analysis of **small polar metabolites**[41], such as amino acids or energy metabolism derivatives, which have also been shown to play a key role in the development of allergic reactions[42]. This method was measured in two different polarities (positive and negative modes) in order to increase the coverage of metabolites.

Overall, the results of this project will help us to understand how the interaction between dietary components and the gut microbiota modulates the immune system in order to **identify potential new strategies for intervention in FA**.

3. METHODOLOGY

Patients and sample collection

A randomized double-blind placebo-controlled clinical trial (DBPCCT) was developed at the Hospital Regional Universitario in Málaga including 34 LTP allergic patients with clinical history of FA, and both positive skin prick test and specific IgE to LTPs. These patients were divided into three groups and were orally administered twice a day for two months with placebo (n = 9) or one of two pectin varieties with different DE: Active 1 (blue color, n = 13) and Active 2 (orange color, n = 12) (**Figure 1**).

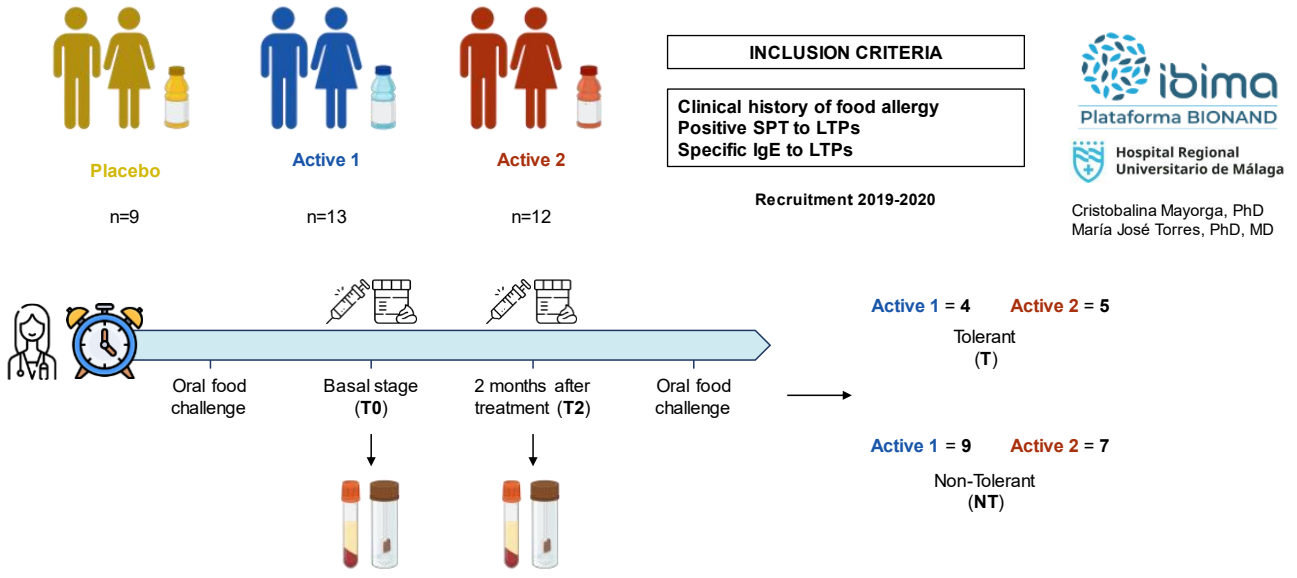

Figure 1. Schematic representation of the double-blind placebo-controlled clinical trial (DBPCCT) performed for this study, including the type and timepoints of sample collection.

Paired serum and fecal samples were collected before (T_0) and after (T_2) the intervention for further evaluation. Lastly, oral food challenge was also performed before and after the intervention to assess tolerance.

Previous analyses

Before the mobility period, the obtained samples were screened for multi-omics analyses including **proteomics and metabolomics** (**Figure 2**). On the one hand, sera samples were analyzed to perform targeted proteomics on a panel of 92 proteins associated with inflammatory and immune response processes using OLINK's Target 96 Inflammation Panel at the Institute of Applied Molecular Medicine (IMMA) from San Pablo-CEU University (Madrid, Spain). On the other hand, both serum and fecal samples have been analyzed using targeted metabolomics methodologies for SCFAs[43] and BAs (adapted from Sarafian et al.)[44] by LC-QqQ-MS at the Center for Metabolomics and Bioanalysis (CEMBIO) in the same university.

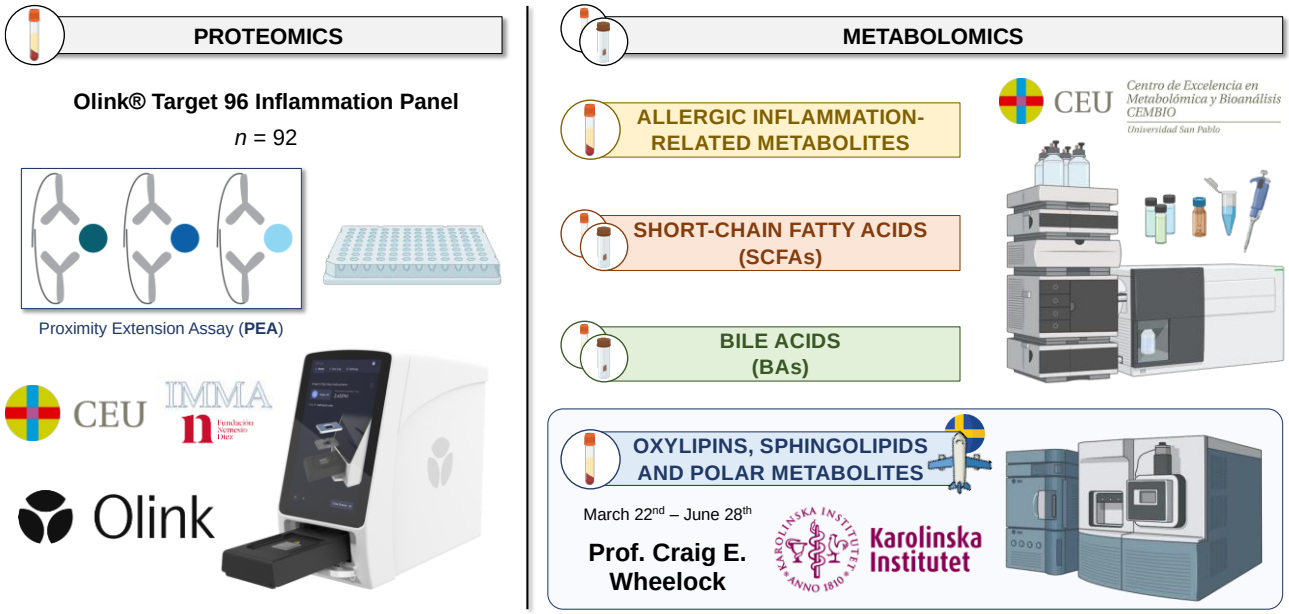

Figure 2. Schematic representation of the work that has already been done with the serum and fecal samples from this project at the home institution (San Pablo-CEU University from Madrid, Spain), and the work to be done at the host institution (The Karolinska Institutet from Stockholm, Sweden).

Sample preparation

A total of 68 samples with the following distribution were sent to Prof. Wheelock's laboratory via international shipping to ensure cold transportation:

- 18 samples from the Placebo group (n_{T0} = 9, n_{T2} = 9).
- 26 samples from the Active 1 group (n_{T0} = 13, n_{T2} = 13).
- 24 samples from the Active 2 group (n_{T0} = 12, n_{T2} = 12).

Samples were aliquoted for each analytical platform, and they were stored at -80ºC for optimal preservation. Then, the four analytical methodologies were applied following previously published

procedures[39,40]. Briefly, the day of sample preparation, the aliquoted volume of serum was slowly thawed at 4ºC, and a fixed concentration of a mix of internal standards was added to monitor analytical performance. Analytes were extracted using liquid-liquid or solid phase extraction, and the recovered volume was transferred to a liquid chromatography glass vial with insert for LC-QqQ-MS analysis.

Sample analysis

After preparation, samples were analyzed on a Waters Acquity UPLC system coupled to different mass spectrometers depending on the analytical platform:

- **Sphingolipids**: Xevo TQ-S.
- **Small polar metabolites** (positive and negative modes): Xevo TQ-S micro.
- **Oxylipins**: Xevo TQ-XS.

Data processing

Data processing and peak integration were performed using Waters MassLynx™ and TargetLynx™ software, respectively. Lastly, relative or absolute quantification of the analytes was performed depending on the analytical platform.

4. WORK PROGRAM AND RESULTS

During this three-month mobility period, the work program was scheduled as follows:

1. **Weeks 1-5** (March 25th – April 26th). The first month was dedicated to completing the training and safety courses required for independent work in Prof. Wheelock's laboratory. In addition, familiarity with the LC-QqQ-MS instrumentation and laboratory workflow was developed through supervised work on other ongoing projects within the research group.
2. **Weeks 6-10** (April 29th – May 31st). The second month was focused on analyzing the 68 serum samples from this project using the four previously mentioned targeted methodologies for the detection and quantification of sphingolipids, small polar metabolites, and oxylipins.
3. **Weeks 11-14** (June 3rd – June 28th). During the third and last month, the data treatment stage (e.g. data pre-processing, development of the processing methods, and peak integration) was initiated for all the metabolites included in the four targeted analytical platforms.

By the end of the mobility period, the integration of the metabolites included in the sphingolipids and small polar metabolites (positive polarity) methods was completed, and the data are now ready to be exported for interpretation and statistical analysis of the results in the coming weeks. Finally, data analysis for the oxylipins and small polar metabolites (negative polarity) methods is still ongoing and will be continued from the home institution while receiving remote support from the host institute to complete the study.

5. CONCLUSIONS

Although FA to LTPs is the main cause of allergic reactions in young adults, there are currently no treatments that are effective in all patients. Therefore, new clinical strategies are urgently needed. In this sense, based on previous evidence, the use of prebiotics such as pectins, which restore the gut microbiome, may be beneficial in FA to LTPs. For these reasons, this project was focused on evaluating the effects of a dietary intervention with two types of pectin on the lipidic systemic profile of LTP-allergic patients using targeted metabolomics.

The results of this project will be integrated with other omics data (e.g. proteomics and epigenetics) and matrices (e.g. metabolomics and metagenomics data from feces) with the ultimate goal of **developing new intervention and treatment approaches to improve the lives of patients allergic to LTPs**.

The results of this mobility will be included in at least one scientific article, which will foreseeably be published in a scientific journal with a high impact index. This is due both to the quality of the research performed, and to the international collaboration of two highly renowned centers, pioneers in the techniques used for the execution of the study.

6. ACKNOWLEDGEMENTS AND PERSONAL REFLECTIONS

I would like to start by thanking Prof. Craig E. Wheelock for supporting my application and hosting me during these three months in the Institute of Environmental Medicine at the Karolinska Institutet. It was a true honor to be part of his team, and I felt very welcome and like family from the first day. I would also like to thank Dr. Antonio Checa for his guidance, his teaching, and for sharing his knowledge with me, not only for this mobility project but for my scientific career in general. I really appreciate it. Finally, I can't forget about all the members of the group, my lab mates, from whom I have learnt a lot and who have been an essential support both inside and outside the laboratory.

During my time at Prof. Wheelock's lab, I had the opportunity of working with globally recognized leading researchers in the field of metabolomics and oxylipins. Furthermore, I was able to work with Waters LC-QqQ-MS instruments, which were new to me, and learn new methodologies for quantifying key lipid mediators in inflammatory diseases such as allergy and asthma. In addition to my laboratory work, I had the pleasure to participate in the weekly group meetings, in which we discussed the multiple ongoing projects of the group, and I could share ideas with experts, not only in the field of mass spectrometry, but also in other techniques that the group works with. Overall, I think this experience has allowed me to grow as a scientist, and to stablish a very valuable international collaboration between my home and host institutions.

Last but not least, I would like to thank EAACI for awarding me this Short Term Research Fellowship, and for giving me the opportunity to make this fulfilling mobility a reality. This fellowship allowed me not only to complete my project, but also to visit an exceptional institute and meet with great scientist in the field of allergy and exceptional people, for what I will always be grateful. Therefore, I highly encourage other young researchers and EAACI Junior Members to participate in the upcoming openings of the EAACI Clinical and Research Fellowships.

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