

Final Report for the Short-term EAACI Fellowship 2017

Title of the fellowship: Identification of response biomarkers to allergen immunotherapy in local allergic rhinitis patients

Type of fellowship: short-term research fellowship

Duration: 01/10/2018-31/12/2018 (3 months)

Location: Immunomodulation & Tolerance Group, Faculty of Medicine-National Heart & Lung Institute, Imperial College (London, UK).

Host supervisor and group leader: Dr Mohamed H Shamji

Acknowledgements

First of all, I would like to express my deepest gratitude to my host supervisor, Dr Mohamed Shamji for welcoming me in his outstanding research group, and for his patience, time and commitment to science and teaching. I also want to thank all my colleagues at Imperial College for their help during my stay, which greatly facilitated the accomplishment of the fellowship goals. A special thanks goes to Prof Stephen Durham who welcome me at his clinic at Royal Brompton Hospital and with whom I had very inspiring discussions about airway immunology. Finally, I want to thank EAACI for giving me the opportunity to perform this fellowship and many other previous educational activities which have been crucial in my professional growth as a physician-researcher allergist.

What questions were addressed and why?

The main objective of the fellowship was to establish a collaboration between the home institution (Allergy Unit, IBIMA-Hospital Regional Universitario de Malaga-UMA) and the host group led by Dr Mohamed Shamji. This collaboration is intended to last for long time and to produce significant results in the field of the mechanisms of allergen immunotherapy (AIT).

The research questions addressed during the stay focuses on the mechanisms of AIT in the specific case of local allergic rhinitis (LAR). Several publications in the last three years have demonstrated that patients suffering from this new rhinitis phenotype can benefit from AIT, in a similar way than allergic rhinitis patients. The clinical evidence from double-blind placebo-controlled clinical trials shows that up to 70% of LAR individuals experience significant improvements in their symptoms and medication scores, and their quality of life together with an increase in the amount of allergen tolerated in the nasal challenge. Nevertheless, little is known about the mechanisms explaining this clinical improvement while the AIT is being administered. In this regard, one of the main goals of the fellowship was to investigate specific markers of (temporary) desensitization versus (permanent) tolerance. Additionally, the work developed during the research stay aimed to identify biomarkers of good response to AIT by analyzing several

immunological parameters in both responder and non-responder LAR patients who received a three-year course of the treatment.

These research questions are relevant because the clarification of AIT mechanisms in LAR individuals will help develop more effective and safe modalities of the treatment, and choose the best moment for therapy discontinuation. Moreover, the identification of response biomarkers will help select the best candidate patients to receive AIT, and to avoid long, expensive and inefficient therapies.

What was the nature of the research?

The research developed during the fellowship was translational in nature. The home institution (Malaga) had performed two clinical trials of AIT in LAR individuals due to local sensitization to two different allergens (house dust mites and grass pollen). During those trials, a nasal allergen challenge was performed during the follow-up visits, and blood (serum and PBMCs) and nasal lavage samples were collected before and after the provocations. All these samples were used for the objectives disclosed in the application for the fellowship.

Nasal Lavage and serum: in these samples the IgE-FAB assay was performed. This assay was described by the host institution and it quantifies the capacity of a biological fluid to inhibit the binding of the allergen to the complex IgE/CD23 expressed on the surface of a cell line. This inhibitory capacity relies on the presence of blocking antibodies in the biological fluid. Blocking antibodies are defined as antibodies with the same specificity than IgE but of different isotype (IgG4 or IgA). The experiments developed during the fellowship specifically investigated differences in peripheral and local (nasal) blocking antibodies.

PBMCs: in these samples different mechanisms of AIT were studied by means of flow cytometry. The investigations focused on the most recently pathways described by the host group: IL-35-producing B regulatory (Breg) cells, and T follicular helper (Tfh) cells. In allergic rhinitis patients, AIT has the capacity to increase IL-35 and Breg cells, which in turn modulate and control many effector pathways of type 2 responses. Tfh cells interact with naïve B cells to promote a differentiation to IgE-producing B cells, which is a crucial mechanisms of airway allergy. Recently, AIT was shown to decrease the number and activity of Tfh cells in subjects with allergic rhinitis. The experiments developed during the fellowship investigated if these two mechanisms also occur in LAR individuals showing a good response to AIT.

What was the result?

The research stay at the host institution set the basis for an ongoing and fruitful collaboration between the two groups involved. A significant harmonization of research experimental protocols was achieved, and this aspect will likely facilitate future activities and speed up the generation of results.

The analysis of the nasal lavage and serum samples from the clinical trial with grass-AIT was completed during the period of the fellowship, and preliminary results show a capacity of the treatment to induce both peripheral and local blocking antibodies in treated LAR patients, in line with the previous evidence for allergic rhinitis individuals. The experiments with the nasal lavage and serum samples from the house dust mite-AIT trial were started during the fellowship, and will be concluded in the next few months.

The flow cytometry analysis of the PBMCs was also started during the research stay. Relevant optimization efforts were done to establish an adequate panel of markers for flow cytometry. These experiments will be concluded during the next few months.

The research activities during the stay already produced one publication (*Layhadi JA, Eguiluz-Gracia I, Shamji MH. Role of IL-35 in sublingual allergen immunotherapy. Curr Opin Allergy Clin Immunol. 2019 Feb;19(1):12-17*), where the EAACI fellowship was properly acknowledged. Several other publications are currently in preparation and will be submitted in the next few months.

How will the findings impact future research?

The capacity of AIT in LAR patients to induce blocking antibodies in several biological fluids is crucial to understand the mechanisms of the therapy in this new rhinitis phenotype. Moreover, this aspect helps clarify the immunopathology of LAR, and paves the way to novel therapeutic approaches for the disorder. The generation of blocking antibodies could serve as response biomarker to AIT in LAR patients, similarly to previous evidences for allergic rhinitis individuals. Further studies should corroborate this aspect in larger cohorts of LAR subjects.

Similarly to blocking antibodies, the clarification of other mechanisms of AIT in LAR, such as the generation of IL-35-producing Breg cells or the inhibition of Tfh cells, could also help identify early response biomarkers. These advances might facilitate future studies further investigating the performance of the described biomarkers as predictors of response to AIT in LAR individuals.

Adaptation of the research from the original plan

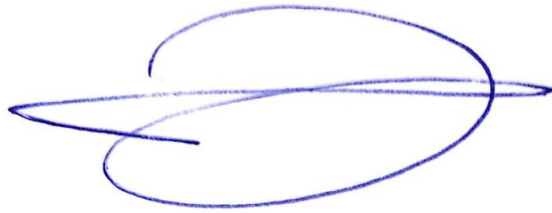
The original application included the study of several additional immunological parameters such as T cell subpopulations, dendritic cells and plasma cells. Because blocking antibodies are currently a hot topic in the research on AIT biomarkers, and IL-35-producing Breg cells and Tfh cells are among the most recently described cells involved in AIT mechanisms, we decided to focus first in these aspects. Because the collaboration between the host and the home groups will be ongoing in the next years, we plan to investigate these and other aspects in the upcoming months.

Personal reflection

This fellowship was an important step in my training as a physician-researcher specialized in allergic respiratory diseases. The experiments developed during the stay

allowed me to gain insight into cutting-edge research in translational immunology, and especially, in the identification of biomarkers for immunological treatments. Moreover, I had the opportunity to spend three months in a top-level university and to attend numerous seminars and research meetings, which helped me to understand the current research trends. I had also the chance to present my own research in one of those meetings. The interaction with my colleagues and my supervisor from Imperial College was very satisfying, and there were many opportunities to discuss research questions in a friendly and open atmosphere. To complete my training during the fellowship and to get an overview of the activities of the host group, I had the opportunity to attend the annual congress of the British Society for Allergy and Clinical Immunology in Telford (UK) in October 2018, and the Symposium on Experimental Rhinology and Immunology of the Nose in November 2018 in Ghent (Belgium). These two meetings were of the foremost interest for my training and research activities. Finally, there were also many opportunities for networking and we enjoyed various social activities including a lovely Christmas lunch with the basic and clinical research staff and a farewell visit to the beautiful city of Brighton.

Malaga, 15th January 2019

A handwritten signature in blue ink, consisting of several overlapping loops and a long horizontal stroke extending to the left.

Ibon Eguiluz-Gracia