EAACI MEDIUM-TERM RESEARCH FELLOWSHIP, 2017

Kinetics and Effect of Type 2 Innate Lymphoid Cells on Nasal Tissue of Allergic Rhinitis after Repetitive Allergen Challenge and its Relation to TGF-Beta

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Duration: 6 months, July 2017 – December 2017
Introduction

In the past years, the molecular mechanism behind allergic diseases and allergen immunotherapy has been thoroughly investigated, however, many aspects of this inflammation pathway in nasal tissue remain scarcely defined. My previous observership opportunity with Prof Stephen Durham and his team in Allergy and Nose Clinics at Royal Brompton hospital cultivated my passion for Immunology research. With this research fellowship, my primary goal, to learn and gain skills of novel laboratory methods in the field of allergic disorders and allergen immunotherapy, was met exceedingly.

Project Background

Type 2 Innate lymphoid cells (ILC2s) are newly identified cells morphologically similar to lymphocytes; however, lacking lineage-specific receptors and markers. If stimulated with IL-33, IL-25 or thymic stromal lymphopoietin (TSLP), they have been shown to promote type 2 immunity by releasing T helper 2 cytokines such as IL-5 and IL-13.1 ILC2’s express several characteristic receptors such as the inducible T cell co-stimulator (ICOS), the co-inhibitory receptor killer cell lectin-like receptor G1 (KLRG1), and the cytokine receptors IL-25 (IL17RB) and IL-33 (T1/ST2), preferentially express TH2 cytokines, particularly IL-5 and IL-13 and have the potential to augment local TH2-driven allergic inflammation [1-3]. The counter-regulatory response that acts on ILC2’s is not clear yet, although its well established that T regulatory, IL10 and TGF-beta are an essential component in counter interacting and inhibiting allergic diseases.

Allergic rhinitis and nasal polyps are excellent models for studying inflammation in the nose and nasal mucosal tissue is easily accessible for analyzing immunopathological mechanisms. Nasal allergen challenge represents an in-vivo experimental model that has contributed tremendously to understanding the underlying mechanisms of allergic rhinitis.4

Project aim

Using novel Immunohistochemistry techniques, our aim was to look into the effects on the local tissue allergic inflammation in nasal mucosa of allergic rhinitis patients and identify the role of ILC2’s compared to healthy individuals.
Fellowship outcomes

I. The period I spent as a research fellow enabled me to;
   - develop a comprehensive understanding of key regulatory and epigenetic mechanisms driving cellular and humoral immune tolerance, specifically in the context of allergy and nasal polyps
   - develop skills in basic science research and laboratory techniques,
   - achieve a wider understanding of the management of difficult and uncontrolled cases involving the nose namely allergic rhinitis and chronic rhinosinusitis, nasal polyposis, sarcoidosis, cystic fibrosis and other immune disorders.

II. I learned the following laboratory techniques essential for carrying out my research project;
   - Immunohistochemistry staining for bright-field and immunofluorescence using triple & quadruple staining
   - Cutting and storing biopsy sections
   - PBMC isolation
   - B cell and T cell isolation
   - Intracellular and surface staining
   - Protein Labeling
   - Flow cytometry, FACS cytometry
   - Quantitative polymerase chain reaction (qPCR)
   - Basophil activation test (BAT)

III. In order to be able to conduct research at Imperial College, I received training and certificates in tissue governance and ethical considerations in scientific research, taking consent and good clinical practice. I gained knowledge in procedures for transferring, storing and using biological materials of human origin for use in research and reporting research incidents. I also attended a course on immunohistochemistry techniques.

IV. I had the chance to attend the weekly Inflammation Repair and Development Section research seminars, journal clubs and Clinical and Laboratory team conjoint weekly meetings which gave me a deeper understanding of the mechanisms of immune tolerance in allergy, including sublingual and subcutaneous immunotherapy.
V. I also had the enriching opportunity to attend the Nose Clinics at Royal Brompton Hospital which are held every Tuesday with an experienced multidisciplinary team of Allergists and ENT surgeons who have expertise covering a wide range of nasal disorders from allergic rhinitis and chronic rhinosinusitis with nasal polyps to difficult to treat conditions like sarcoidosis and cystic fibrosis and beyond. My time spent there, under the supervision of Prof Durham and Mr. Hesham Saleh, expanded my horizon on the importance and necessity of an interdisciplinary approach in developing effective diagnostic and therapeutic strategies for nasal disorders.

VI. With the guidance of Dr Aarif Eifan and Dr Mikila Jacobson sharing their immense knowledge and expertise in the field, I learned and developed skills of single, double and triple immunohistochemical and fluorescence staining in both paraffin-embedded and frozen nasal tissue. I gained skills in performing qPCR while briefly working with Dr Mohammed Shamji and his dedicated team at the Allergy and Clinical Immunology laboratory.

Preliminary results & Future plans

Due to the time-consuming nature of the training of complex laboratory methods, six months of my fellowship was mostly spent on learning the laboratory techniques and acquiring the necessary experience to conduct the research. Hence, to help me continue my ongoing project, Prof Durham kindly offered me to extend my fellowship for another six months and provided me a stipend from Imperial College. This period enabled to grasp the wider spectrum of both the theoretical and practical aspects of allergy research. Although standardization in immunofluorescence is quite challenging due to the number of pre-analytical, analytical, and post-analytical factors known to influence staining, we were able to validate our antibodies using immunofluorescence on both formalin-fixed paraffin embedded and frozen nasal tissue sections. We stained using double and triple combinations of panels including anti human lineage cocktail (CD3, CD14, CD16, CD19, CD20, CD56), anti ST2 (IL1RL1), anti KLRG1, anti CD11b, anti CD127, anti ICOS, anti IL-17RB (Figure 1). To omit incorrect interpretations our primary antibodies were compared with negative (Isotype control) and positive controls (anti AA1 and anti CD3). As planned previously during our validation experiments; in our main triple staining panel Lineage negative, ST2 and KLRG1 positive cells would be identified as ILC2 cells. One of the two main issues we faced during our experiments were the autofluorescence of eosinophils which we were able to control with various quenching methods to an extent but not completely. However, the morphologic distinction of eosinophils let us easily distinguish them from our target ILC2 cells. The other obstacle was the highly cross-reactive nature of polyclonal antibodies resulting with unspecific binding.
We were successful in staining ILC2’s using triple staining technique with bright field microscopy immunohistochemistry using DAB, fast red, and fast blue dyes in frozen nasal polyp tissues (Figure 2).

Figure 1: Nasal polyp tissue frozen section double immunofluorescence staining, mouse lineage cocktail with Alexa Fluor 594 as a secondary, and rabbit KLRG1 with Alexa Fluor 488 as a secondary, x40.

Figure 2: Nasal polyp tissue frozen section triple bright field immunohistochemistry staining, mouse lineage cocktail with alkaline phosphatase fast red, rabbit KLRG1 with DAB, and goat ST2 alkaline phosphatase fast blue x100. The arrow shows possible ILC2 cell stained blue and brown.

Acknowledgements
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My sincere thanks go to my EAACI mentor Dr Glenis Scadding for her never-ending support and guidance. I encourage everyone with an interest in allergy and clinical immunology to apply for an EAACI fellowship.

References: