

“Dissecting the mechanisms of steroid resistant leukotriene synthesis in macrophages from Aspirin-exacerbated respiratory disease (AERD) patients”

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Type of Fellowship: Short term Research Fellowship

Host supervisor: Dr. Sven-Erik Dahlen and Dr. Craig Wheelock

Location: Center for Allergy Research Karolinska Institutet, Stockholm (Sweden)

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1. Introduction

Aspirin-exacerbated respiratory disease (AERD) is characterized by bronchial asthma, chronic rhinosinusitis with nasal polyps, and hypersensitivity to aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs).^{1,2} This syndrome affects 7% of adult-onset asthmatics and 14% of adult asthmatics with severe asthma.³ AERD is characterized by intolerance to aspirin and other non-selective cyclooxygenase (COX) inhibitors.^{4,5} Other hallmarks include elevated synthesis of bronchoconstrictive cysteinyl-leukotrienes (cysLTs), associated with increased expression of leukotriene C4 synthase (LTC₄S), Th2 cytokines and marked eosinophilic inflammation.⁶ In our recently published work, we have shown that **lipid mediator pathways are particularly resistant to steroid treatment** in nasal polyps from AERD patients.⁷ However, the potential mechanisms involved remain unclear. Granulocytes and mast cells are thought to be the major source of pro-inflammatory leukotrienes in AERD and therefore they are two cell types that have important roles in mediating many of the effects observed in this disease.⁸ We have also shown that airway epithelial cells interact with macrophages to increase the production of LTs in settings of chronic inflammation,⁷ suggesting a **key role of macrophages in the pathophysiology of AERD** as they express both the LTs and COX biosynthetic pathway enzymes. However, the contribution of macrophages to the development of this disease remains unexplored.

2. Aim of the project

The **aim of this project** is to **identify new pathological pathways in steroid resistance** by comparing the eicosanoid profile of macrophages from AERD patients to those from healthy individuals by **LC-MS/MS analysis**. In particular, we have explored the **impact of the type 2 cytokine IL-4**, which is responsible for the upregulation of LTC₄S by mast cells in AERD⁹ as well as the response to **allergens like house dust mite (HDM)**. We have investigated the macrophages **responsiveness to Prostaglandin E2 (PGE₂)**, because PGE₂ concentrations are reduced in AERD¹⁰ and one previous study reported that PGE₂ resistant granulocytes promote the severe respiratory tract inflammation and LT overproduction in AERD.¹¹ In addition, the proposed project was aimed at exploring the potential of **alternative**

immunomodulatory compounds, identified by our own group, to limit type 2 inflammation by reprogramming lipid mediator pathways in macrophages (patent application pending).

3. Work program and results

Monocytes were isolated from the blood of healthy human donors or AERD patients and cultured in the presence of human GM-CSF and human TGF- β 1 to induce a phenotype similar to airway macrophages. On day 6, cells were harvested by scraping and stimulated with PGE₂, IL-4, HDM or immunomodulatory compounds overnight. Macrophage culture supernatant was collected and mixed with equal volumes of methanol for LC-MS/MS analysis (Fig 1).

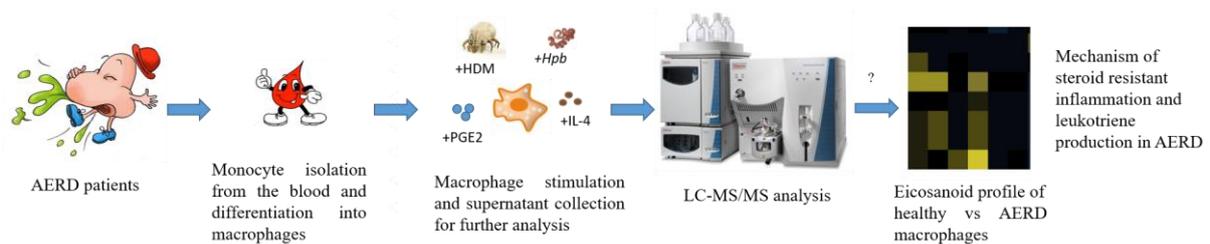


Figure 1: Overview of the work program and approach

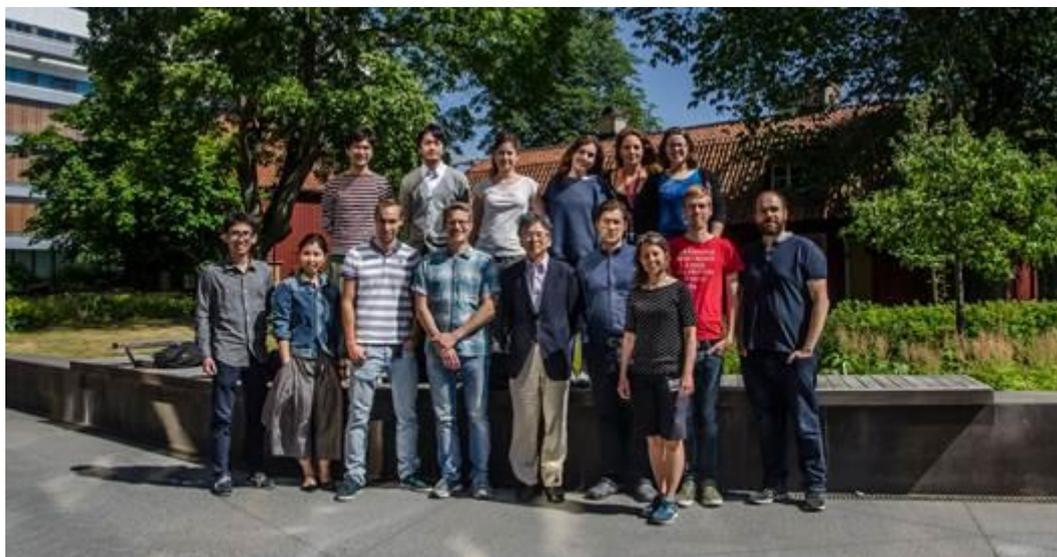
The development of this project has been possible thanks to this EAACI Research Fellowship. Being part of the **department of Medical Biochemistry and Biophysics at Karolinska Institute** gave me the great opportunity of learning **LC-MS/MS analysis** from experts in the eicosanoid field. During the first month of my fellowship, I was introduced into the fundamentals of liquid chromatography and mass spectrometry, understanding the working flow of the LC-MS/MS. Next, I learnt how to extract and process the samples before injection into the LC-MS/MS for analysis. In the meanwhile, we had some troubles with the performance of the equipment, and this allowed me to get briefly introduced in how to optimise instrument settings for high data quality. The next steps were focused on the analysis of the complex data set including statistical comparisons.

The extraction protocol developed in this laboratory is suitable for the extraction of lipid mediators derived from different polyunsaturated fatty acids (PUFAs) such as: Arachidonic Acid (AA), docosahexaenoic acid (DHA), Linoleic acid (LA) and eicosapentaenoic acid (EPA). The LC-MS method is suitable for the quantification of 135 lipid mediators using 42 internal standards. Screening so many different compounds is crucial to compare the eicosanoid profile of macrophages from AERD patients to those from healthy individuals and will provide new insights into the mechanisms involved in this disease, and the development of new treatment strategies for AERD patients. Due to reasons of confidentiality the unpublished results from this project cannot be shown in this report.

4. Acknowledgement

I would like to thank to Dr. Craig Wheelock and Dr. Sven-Erik Dahlen for supporting my application. It was my honour to join the department of Medical Biochemistry and Biophysics at the Karolinska Institute. This research institute has a worldwide outstanding reputation in the field of eicosanoids, and I have enjoyed the group meetings and discussions with the experts. I would also like to warmly thank all the members of the group, who made me feel part of the team from the first day. Special thanks to David Fuchs, who has spent a lot of his time introducing me into the complex world of mass spectrometry. I really appreciate his good mood and all the effort he put on answering all my questions.

During my time at the Karolinska Institutet, I had the pleasure of attending the lecture of Prof. Dr. Charles Serhan from Harvard University, learning more about bioactive small molecules during the resolution of acute inflammation. I also attended the lecture of Prof. Dr. Shinya Yamanaka, who won the Nobel prize in Physiology or Medicine 2012 for discovering that mature cells can be reprogrammed to become pluripotent. Furthermore, I had the honour of meeting Prof. Dr. Takao Shimizu and his medical research students from University of Tokyo, during their visit at the Karolinska Institutet. They gave us a deep insight into their research projects on the role of polyunsaturated fatty acids in the cell membrane.



Group picture taken during the visit of Prof. Dr. Takao Shimizu at the group of Prof. Dr. Craig Wheelock at Karolinska Institutet.

This EAACI Fellowship has improved my professional skills by learning a new laboratory technique in an international scientific research environment. This is a valuable knowledge exchange as we are currently establishing the LC-MS/MS analysis of eicosanoids in my home research institute. Furthermore, I am sure this will be the beginning of future collaborations with this group.

Last but not least, I would like to thank to the EACCI Headquarters for this opportunity and for all the advantages of being a junior member. This short research fellowship has fulfilled my expectations and I would encourage other researchers to apply for the next openings.

5. References

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