EAACI Research Fellowship 2019
Final report

Awardee:	Dr. Chiara Tontini
Project title:	*"Immunologic effects of omalizumab on peripheral blood mononuclear cell subsets in patients with Chronic Spontaneous Urticaria"*
Fellowship type:	Medium Term Research Fellowship
Duration:	6 months
Location:	Department of Parasitology, Leiden University Medical Center (LUMC), Leiden (Netherlands)
Supervisor:	Prof. Hermelijn H. Smits

What questions were addressed and why?

Chronic Spontaneous Urticaria (CSU) is a disease that affects almost 1% of the population worldwide, and around 20% of affected individuals show the most severe anti-histamine-resistant form. To treat such, omalizumab, an anti-IgE monoclonal antibody, has proven effective in reducing urticaria disease activity scores and improving dramatically the quality of life of around 60-75% of treated patients. Given the success of omalizumab in treating the severe form of CSU, a paradigm shift towards an auto-allergic pathogenesis of CSU occurred. IgE and IgG auto-antibodies were identified in CSU patients, causing the release of vasoactive mediators by effector cells, like mast cells in the skin, and producing the signature symptoms (wheals and/or angioedema). While mast cells are the main actor in the generation of the symptoms, there is currently little to no literature on the contribution of other cell subsets, namely T cells and B cells, in the pathogenesis of urticaria, and no information on how treatment with anti-IgE modulates immune responses in CSU.

What was the nature of the research?

The aim of our study is to investigate peripheral blood mononuclear cell (PBMCs) subpopulations in patients with chronic spontaneous urticaria, before and after treatment with omalizumab. Our goal is to characterize different cell subsets among PBMCs (i.e. T and B cells, basophils, monocytes, dendritic cells) and assess T and B cells capacity to produce cytokines after in vitro stimulation, with either pro- and anti-inflammatory effect. Furthermore, we aim to analyze the impact of omalizumab therapy on the frequency of specific cell subsets, pro-/anti-inflammatory cytokine production capacity and correlate our findings to clinical outcomes (i.e. response/no response to treatment, use of medications, disease activity markers).

What was the result?

During my stay at the Department of Parasitology of the Leiden University Medical Center, under the supervision of Prof. Hermelijn H. Smits, I acquired substantial training on performing cell culture, how to optimize and perform flow cytometry assays and how to analyze cytometry data using unbiased gating and visualization tools for high-dimensional cytometry data (tSNE/HSNE). Furthermore, I received substantial training on different cytokine dosing methods, namely Cytometric Bead Array (CBA) and Enzyme-Linked Immunosorbent Assay (ELISA). Since June 2019 I generated and measured around 2000 samples, combining flow cytometry, ELISA and CBA analyses performed on PBMC and cell culture supernatants collected after in-vitro stimulation.

Data are currently being analyzed, but according to very preliminary results on cytokine assay on culture supernatants, we could observe an increased production of IL-4 by CSU patients compared to healthy controls that reduces upon treatment with omalizumab, in line with results obtained in asthma patients treated with

the anti-IgE monoclonal antibody (see Figure 1), and demonstrated an increased production of GM-CSF by B cells from patients during treatment with omalizumab (see Figure 2). Correlation with clinical parameters and serum biomarkers will also be performed, and results will be submitted to peer-reviewed journals this year.

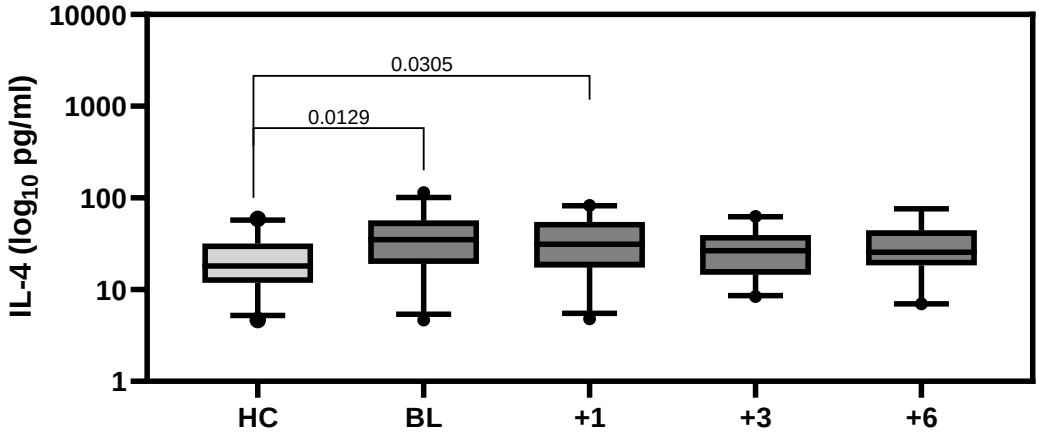

Figure 1.
Change in IL-4 production before (BL) and after 1 month (+1), 3 months (+3) and six months (+6) of treatment with omalizumab compared to age-matched healthy controls (HC). PBMC from CSU patients were stimulated for 48 hours using anti-CD3 plus anti-CD28 antibodies. IL-4 was measured on cell culture supernatants using Cytometric Bead Array. Mann-Whitney test was performed to compare HC versus patients in different timepoints.

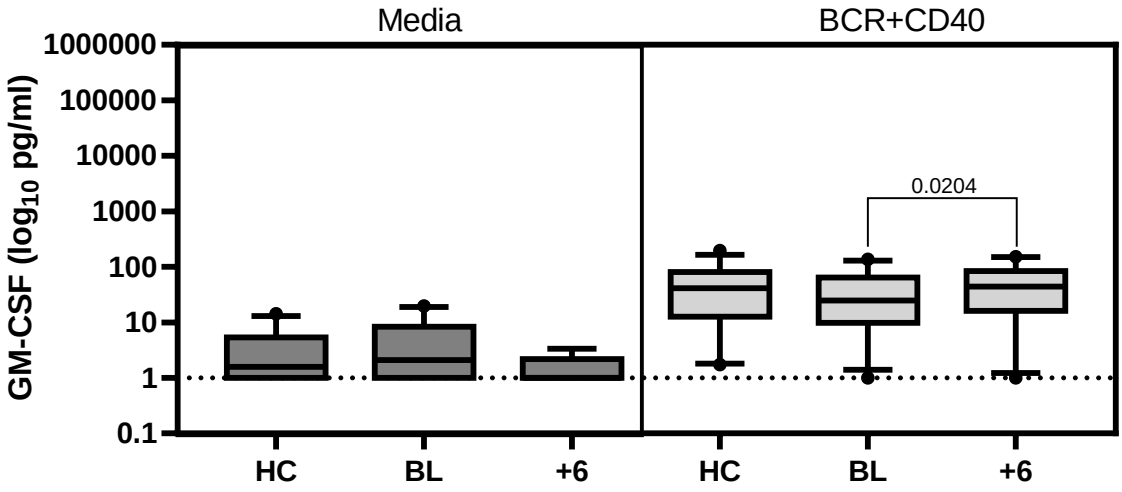

Figure 2.
Change in GM-CSF production before (BL) and after six months (+6) of treatment with omalizumab compared to age-matched healthy controls (HC). PBMC from CSU patients were stimulated for 48 hours using anti-IgG+IgM (BCR) plus anti-CD40 antibodies. GM-CSF was measured on cell culture supernatants using Cytometric Bead Array. Wilcoxon test was performed to compare HC versus patients in different timepoints.

How will the findings impact future research?

The results obtained on PBMC of urticaria patients treated with omalizumab will shed new light on both CSU pathogenesis and the immunomodulating potential of omalizumab, as observed in both asthma and atopic dermatitis patients. In addition, correlating both cytometric and cytokine production assays to patient-related outcomes and disease activity markers could also provide new biomarkers to, for example, guide omalizumab treatment by unveiling characteristics of subjects that do not respond to omalizumab treatment, or relapse upon discontinuation. Further information on the role of these cell subsets on skin's effector cells (i.e. mast cells) will complement these observations, and guide future research on the field.

Research course adapted from the original plan:

Due to partial sample loss, the final number of subjects studied was 28 instead of 30 as originally planned. From June to August 2019 we reworked and optimized the stimulation protocol and flow cytometry panels according to current literature and the results obtained from a pilot run we performed in 2018. Furthermore, we selected and optimized the multiplex cytokine assay for cytokine analysis of cell culture supernatant and performed test runs. Samples were processed and acquired in the month of October, while CBA and ELISA assays on cell culture supernatants were performed during the months of November and December. Due to time constraints, the unbiased gating and statistical analysis of collected results is still underway and will be carried out at the home institution in collaboration with the Department of Statistics of the Polytechnic University of Marche, with the remote assistance of collaborators from the group of Prof. Smits.

Personal reflection on what has been learned and how improvements can be made in the future:

During my stay in Leiden I had the wonderful opportunity to grow as an independent researcher, by receiving training and support in planning and performing the experiments required for the project. The knowledge and experience shared by the research group was of tremendous help for all the steps undertaken for this study, but I also had the chance to contribute to the host laboratory in return by optimizing standard operating procedures, flow cytometry panels and use of new reagents for human PBMCs analysis, that can be adopted and modified by the host institution for future use.

The work done on peripheral blood mononuclear cell analysis culminated in co-authoring a chapter for a laboratory methods book with Prof. Smits, recently submitted for peer-review. I also had the chance to attend advanced hands-on courses and symposia on flow and mass cytometry offered by the LUMC Flow Cytometry Core Facility, enriching my skillset and knowledge on flow and mass cytometry applied to research. I have also improved my scientific reporting skills by presenting data regarding my project to the group during the weekly meetings.

Being a medical doctor specialized in Allergy and Clinical Immunology, I strongly advise to pursue an EAACI Research Fellowship. Although basic research is considered especially daunting for medical doctors, it is a unique opportunity to step out of our comfort zone and adopt a new viewpoint on laboratory work, develop a new skillset and critical thinking applied to any aspect of our profession. My suggestion to colleagues that wish to go on this path is to train extensively on your laboratory skills beforehand, and don't give up on the hard tasks that laboratory work require to achieve your goal.

Acknowledgements

I wish to deeply thank my supervisor, Prof. Hermelijn Smits, for her relentless support in my ambition to become a translational allergist. By welcoming me to her group I made such an amazing experience abroad, made new friends and built memories that will last a lifetime.

This experience only fortified my will to become a full-fledged researcher, and I will continue my laboratory journey with a PhD starting from next year in another institution, wishing that this collaboration with Prof. Smits will continue to be fruitful and develop in further common projects in the future.

Last but not least, I wish to thank EAACI for their support in making this experience possible and feel so honored to be selected for such amazing and unique opportunity.

Dr. Chiara Tontini