This report describes my research project, activities and achievements over the period of my fellowship (1st June 2017 – 31st May 2018) at the Swiss Institute of Allergy and Asthma Research (SIAF), Davos, Switzerland.

Background & Rationale

Development of allergen-specific immune tolerance that includes Treg cells and Breg cells has been suggested to be the basis of food allergen tolerance. The worldwide prevalence of cow’s milk allergy indicates that up to 8% of children in the U.S.A are food allergic to cow’s milk and the frequency of cow’s milk allergy estimated from 0.5 to 7.5% in westernized countries [1-3]. In Switzerland, the allergy report indicates that approximately 4-8% of the Swiss population has a food allergy (aha! Swiss Allergy Centre, 2018). The allergen-specific B cells may play a role in the induction of allergen tolerance. Therefore, we aim to perform the in-depth study on the aspects of B cell properties during food-immune tolerance induction and food AIT.

Hypothesis

Development of a long-term food allergen-specific immune tolerance is decisive for the success of food-AIT, its molecular mechanisms particularly the involvement of B regulatory cells remain to be elucidated.

Objectives

This study examines the involving role of B cells in food allergy, particularly, cow’s milk allergens (αS1-casein), from allergic and healthy individuals.
Research methodology

Subject

Peripheral blood mononuclear cells (PBMC) will be collected from healthy and allergic individuals who follow the clinical food-AIT in Allergy Unit, Department of Dermatology, University of Zurich. In our case, we first set up and examined the specificity of allergen-specific B cell sorting using isolated PBMC from 8 highly sensitized αS1-casein donors in the Swiss Institute of Allergy and Asthma Research (SIAF), Davos, Switzerland.

Labeling the allergens

The purified major allergen αS1-casein from cow’s milk was labeled with biotin (Sigma-Aldrich) and followed by the conjugation of streptavidin-PE (BioLegend) and streptavidin 635 (Thermo Fisher Scientific). This double-labeled αS1-casein was optimized in the antigen titration experiments and the suitable concentration was then used for the identification of food allergen-specific B cells.

Food allergen-specific B cells

For the isolation of food allergen-specific B cell, PMBC was stained with viability dye and antibodies against surface markers (CD19, CD27, IgG, IgA, and IgM). αS1-casein specific B cells were identified and purified using double-labeled allergen by flow cytometry.

Immortalization of food allergen-specific B cells

The αS1-casein specific B cells were transduced with a retroviral vector containing GFP, BCL6, and Bcl-xL and activated with CD40L-L cells in the presence of IL-21 for 36 hours. The combination of BCL-6 and BCL-XL overexpression and the CD40L/IL-21 culture system help to identify B cells that have several properties and enable the isolation of allergen-specific antibodies.

Food allergen-specific antibody detection

To detect αS1-casein specific antibodies, the ELISA specific for human IgE, IgG, IgG1, IgG4 and total IgG were performed with the in-house donor’s serum. The ELISA was used to measure the allergen-specific immunoglobulins in the serum. Briefly, ELISA 96 well plates were coated with purified major allergens αS1-casein overnight at 4⁰C. Serum was diluted and incubated in the plates. After, the antigen-antibody complex was detected and incubated with TMB substrate. The reaction was stopped by 2M H2SO4 solution and read at 450 nm by using the microplate reader.

Next generation transcriptome of specific B cells

Functional suppressor of B regulatory cells will be identified. Surface molecules of food-antigens specific B cells will be demonstrated. Frequency analyzes between allergen and tolerance profiling in primary cells. The whole immune system profiling will be conducted by multicolor flow cytometry (Table 1).
Table 1. Marker panel for cell analysis by flow cytometry

<table>
<thead>
<tr>
<th>Cells</th>
<th>Marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>B cells</td>
<td>CD19, CD27, CD38, CD24, CD10, IgM, IgD, CD71, CD1d, CD5, IgG, IgE, IgA, IL-5, IL-10, IL-13, IL-17, IL-22, IFNγ</td>
</tr>
</tbody>
</table>

Results

We firstly screened the αS₁-casein specific Immunoglobulin E and G in 26 healthy donors from the Swiss Institute of Allergy and Asthma Research (SIAF), Davos, Switzerland. Based on the ELISA results (Figure 1), 8 highly sensitized αS₁-casein donors were selected for PBMC isolation and followed by the sorting of αS₁-casein specific B cells.

The αS₁-casein specific B cells were purified using double-labeled allergen by flow cytometry (Figure 2). The live cells were identified with viability dye and antibodies against surface markers (CD19, CD27, IgG, IgA, and IgM). Gating strategy was explained in Figure 2.
The αS₁-casein specific B cells were immortalized with the special technique developed by Spits H [4, 5]. The immortalized B cell clones were stimulated with αS₁-casein for 5 days and the supernatants were collected for Immunoglobulins and secreted cytokines detection (Figure 3).

To achieve the highest purity of αS₁-casein specific B cells, the pool of immortalized B cell clones were re-purified with the same B cell sorting strategy. We successfully purified 64% of αS₁-casein specific B cells (Figure 4A). Total IgG and specific IgG1, IgG1 and IgG4 to αS₁-casein production from culture supernatants were analyzed in ELISA. The results showed the level of specific IgG1, IgG1 and IgG4 to αS₁-casein positive B cells increased when compared to αS₁-casein negative cells (Figure 4B).

Future experiments

αS₁-casein specific B cell clones will be single sorted and further analyzed in transcriptome with the next generation sequencing. Functional suppressor of B regulatory cells will be identified as well as the surface molecules. As this work was successfully performed in 8 highly sensitized αS₁-casein donors, we are now recruiting samples from the allergic donors for the research project.
Conclusions

This study is mainly focused on the characterization of allergen-specific B cells in cow’s milk allergen. We successfully identified αS₁-casein specific B cells and produced the immortalized αS₁-casein specific B cells clones. Interestingly, αS₁-casein specific B cells showed the increased level of specific IgG, IgG1, and IgG4 from highly sensitized individuals. These interesting findings needed to be further investigated to understand the mechanisms of immune tolerance to food allergen in allergic patients.

Impacts and benefits of this study

We aim to develop the easy and ready-to-use detection method for food AIT patients. This detection method will be very accurate and cost-effective. It will help physicians to improve the treatments of food immunotherapy. Our study will lead to the great benefits of preserving the right treatments to food AIT patients. Moreover, the new findings our study can be used in other research areas such as autoimmunity, organ transplantation, cancer, and infertility.

Research publication (review article) during the fellowship


Additional Fellowship activities

- Attended 2 courses: “Proposal Writing & Funding” and “Research data management for young researcher” by the Graduate School Grabünden, University of Zurich, Switzerland.
- Attended “4th Davos-Zurich Research Day, Akdis Lab meets Boyman Lab” at the Department of Immunology, University Hospital Zurich, Switzerland.
- Organization committee (IT group) of the World Immune Regulatory Meeting XII (WIRM), 14-17 March 2018, Davos, Switzerland.
- Organization chair (poster session 13) of the World Immune Regulatory Meeting XII (WIRM), topic: Genetics of chronic diseases.
- Poster presentation in the World Immune Regulatory Meeting XII (WIRM), T and B cell memory and immune regulation I (poster session 3), topic: Mechanisms of immune tolerance to food allergens (P023), (attached on page 7).

Personal reflection and acknowledgments

Firstly, I would like to express my gratitude towards the EAACI for choosing me to be the winner of long-term research fellowship award 2017. This EAACI Research Fellowship
supported me financially to perform the research project in the Swiss Institute of Allergy and Asthma Research (SIAF), Davos, Switzerland.

Not only that, I would like to express my gratefulness to my host supervisor, Prof. Dr. med. Cezmi A. Akdis for allowing me to perform the research training and be a part of the SIAF team. My sincere thanks go to Prof. Dr. med. Mübeccel Akdis for stimulating scientific discussions and valuable comments, which were instrumental in the completion of my research project. I’m very thankful to Dr. Willem van de Veen for his encouraging research support, research guidance, and insightful comments which incented me to widen my research from various perspectives. Besides my host supervisors, I am grateful and thankful to all SIAF members for making my time spent in the laboratory very interesting and memorable.

During this research fellowship, I have the great chance to adapt myself to a new research environment and learn new laboratory techniques. I improved my knowledge of flow cytometry, immortalization of allergen-specific B cells, and analysis of allergen-specific Immunoglobulins subclass. Fortunately, Prof. Dr. med. Cezmi A. Akdis has given me the best opportunity to prolong my stay for the next following year in order to finish my research project and produce the good quality research publications.

Finally, I strongly encourage young researchers who have great interests in the field of allergy and clinical immunology to submit your research projects to the EAACI fellowship.
Mechanisms of immune tolerance relevant to food allergy

SIAF
University of Zurich™

Pattarapon Sotitsukasona, Willem van de Veen, Mülbeccel Akdis.
Swiss institute of Allergy and Asthma Research (SIAF), Obere strasse 22, Davos Platz, CH-7270, Switzerland

Background
The prevalence of food allergy is an increasing public health concern affecting millions of people worldwide.
Up to 8% of children in the U.S.A in food allergic and the frequency of cow’s milk allergy estimated from 0.5% to 7.5% in westernized countries. The allergen specific B cells may play a role in the induction of allergen tolerance.

This study examines the role of B cells in food allergy, particularly, cow’s milk allergens. The cow’s milk allergens, αS1-casein specific B cells will be purified from allergic and healthy individuals and their immunoglobulins will be measured.

Methods
• Peripheral blood mononuclear cells (PBMC) from highly sensitized αS1-casein donors were isolated by density gradient centrifugation.
• αS1-casein specific B cells were identified and purified using dual-color staining with fluorescently labeled αS1-casein allergen by flow cytometry. The immortalization of αS1-casein specific B cells were performed by the transduction with a retroviral vector containing GFP, IL16 and IL21, and co-cultured with CD40L cells and IL-21.
• Specific and total IgE, IgG1, IgG4 antibodies from supernatants of immortalized αS1-casein-specific B cells were measured by ELISA.

Results

Figure 1 Screening of αS1-casein specific immunoglobulins in highly sensitized donors.
(a) Specific IgE, (b) specific IgG. Donor 1 & 2 were selected for αS1-casein specific B cells sorting.

Figure 2 Sorting of αS1-casein specific B cells from PBMC in highly sensitized αS1-casein donors.
Gating strategy: lymphocytes were first gated from the forward and side scatter plot. After the double discrimination, viability gated, αS1-casein biotin conjugated and CD4+CD21- were identified as αS1-casein specific cells.

Figure 3 Immortalization of αS1-casein specific B cells.

Figure 4 Production of αS1-casein specific B cells and immunoglobulin production.
(a) αS1-casein specific B cells at day 10
(b) Immunoglobulin detection from 2 donors: IgE, IgG1, IgG4, and IgG4

Conclusions
• This study is focused on the characterization of allergen-specific B cells in cow’s milk allergens.
• Interestingly, αS1-casein specific B cells showed the increased level of total IgG, IgG1, IgG1, and IgG4.
• These interesting findings need to be further investigated to understand the mechanisms of immune tolerance to food allergens in allergic patients.
References