IN VIVO APPLICATION OF TGFβ1- AND IL-10-LIKE PEPTIDES FOR IMMUNOMODULATION OF ALLERGIC RESPONSES

Final Report - Medium Term Research Fellowship (01/06/2018 to 30/11/2018)

European Academy of Allergy and Clinical Immunology (EAACI)

Recipient:

Dr. Galber Rodrigues ARAUJO

Institute of Genetics and Biochemistry

Federal University of Uberlandia, Uberlandia, Brazil

E-mail: galber.araujo@gmail.com

Home Supervisor:

Prof. Dr. Luiz R. GOULART

Institute of Genetics and Biochemistry, Laboratory of Nanobiotechnology

Federal University of Uberlandia, Uberlandia, Brazil

E-mail: lrgoulart@ufu.br

Host Supervisor:

Prof. Dr. Fatima FERREIRA

Department of Molecular Biology

University of Salzburg, Salzburg, Austria

E-mail: Fatima.ferreira@sbg.ac.at

INITIAL CONSIDERATIONS

This report aims to present the results achieved during the Medium Term Research Fellowship from EAACI granted in the period from 01/06/2018 to 30/11/2018. The promising results obtained, as well as the intense activities carried out during the fellowship period, have created opportunities for a strong collaboration between the Laboratory of Nanobiotechnology of the Federal University of Uberlândia, Brazil, and the Department of Biosciences of the University of Salzburg, Austria, which will benefit both institutions.

Central Hypothesis: TGFβ1- and IL10-like peptides that were previously selected in the home institution by phage display technology can act as partners to modulate immune responses to birch pollen allergens. The combination of these two peptides might represent a promising approach for the modulation of the allergic response.

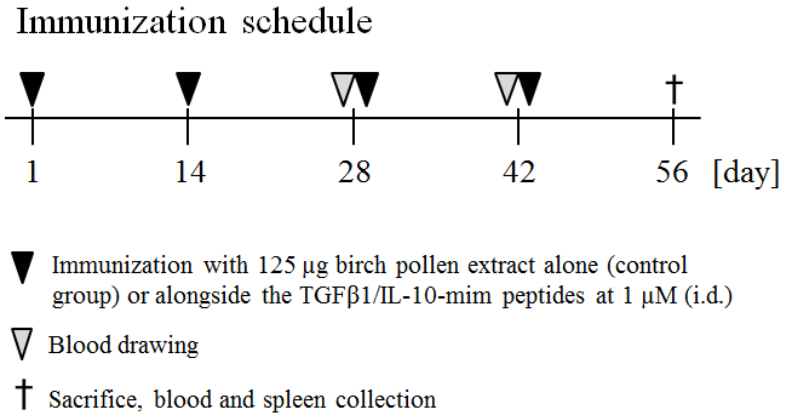
Major goal described in the original plan: Transforming growth factor-β1 (TGFβ1) and interleukin-10 (IL10) are cytokines defined by their ability to suppress pathological immune responses in the settings of allergy and other inflammatory diseases. Hence, the aim of the study was to evaluate the immunoregulatory capacity of the combination of TGFβ1- and IL10-like peptides to modulate Interferon-γ, IL-4 and Foxp3 expression in different murine reporter strains.

Adaptations from the original plan: The original plan aimed to investigate the action of the TGFβ1- and IL10-like peptides in different murine reporter strains. There were some unexpected challenges with the reporter strains that would consume more time than we planned for the medium term fellowship, for example, the optimization of the amount of allergens and time necessary to induce Interferon-γ, IL-4 or Foxp3 expression in these reporter strains. Although this is under investigation, for this study we opted to investigate the role of the mimetic peptides on modulating Interferon-γ, IL-4 and Foxp3 production in BALB/c mice during the process of sensitization to birch pollen allergens. The adopted mouse immunization protocol for birch pollen allergens is well standardized in the host institution.

METHODS

Mice immunizations and analysis

To induce allergic sensitization, 6 female BALB/c mice (6–10 week-old) were immunized intradermally (i.d.) with 125 µg of birch pollen extract diluted in PBS. Another group composed of 6 mice was immunized i.d. with 125 µg birch pollen extract in combination with TGFβ1- and IL-10-like peptides diluted at 1 µM in PBS. Three mice constituted the naïve group. Immunizations were performed on days 1, 14, 28, and 42, and mice sacrificed on day 56. To investigate the induction of Bet v 1-specific IgE responses (Bet v 1 is the major birch pollen allergen), blood samples were drawn from the saphenous vein on days 28 and 42 after first immunization. The immunization schedule is presented below. Flow cytometry, ELISA, ELISPOT and mediator release assays were conducted to evaluate the capacity of the peptides to modulate the *in vivo* immune response towards the birch pollen extract.



RESULTS

The combination of TGFβ1- and IL-10-like peptides modulates crucial events necessary to a successful immunotherapy

The immunization with birch pollen extract for 56 days significantly induced the allergen-specific total IgE response, as observed in the capacity of the serum from immunized mice to induce basophil degranulation, proving that mice were successfully sensitized. The combination of the TGFβ1- and IL-10-like peptides modulated the antibody immune response towards the birch pollen extract, as showed in the ELISA performed with sera from all groups of mice. Compared with the group of mice immunized with the birch pollen alone, mice treated with the –like peptides showed significantly less levels or IgE production, the major antibody involved in the allergic response. Furthermore, mice treated with the peptides rendered higher levels of IgA, an antibody that is known for its capacity to inhibit IgE-mediated allergic reaction and contribute to the modulation of the allergen-specific immune response.

The combination of the TGFβ1- and IL-10-like peptides was also able to modulate the cytokine immune response towards the birch pollen extract, as showed by ELISPOT analysis performed with sera from all groups of mice. Compared with the group of mice immunized with the birch pollen alone, mice treated with the –like peptides showed significantly less production of IFN-γ and IL-4 producing spleenocytes, the two major cytokines from Th1 and Th2 responses, respectively. This result indicates that the peptides were able to modulate the inflammatory and allergic responses towards the birch pollen extract. On the other hand, the treatment with the peptides induced IL-10 production. The observed upregulation of IL-10 indicates that the peptides played an anti-inflammatory action since the production of this cytokine is essential to prevent inflammatory pathologies.

The induction of allergen-specific regulatory T cells is a key mechanism for a successful immunotherapy. Hence, the induction of regulatory T cells production can potentially modulate the immune response against environmental allergens. Compared with the group of mice immunized with the birch pollen alone, FACS analysis using spleenocytes from mice treated with the –like peptides showed significantly higher levels of regulatory T cells ($CD4^+CD25^+Foxp3^+$). Furthermore, mice treated with the peptides showed statistically significant reduction in B cells ($CD19^+$) proliferation, confirming its modulatory action also in these cells.

Relevance of our findings

The combination of the TGFβ1- and IL-10-like peptides presented herein was able to modulate the antibody immune response, induce Treg cell differentiation, up- and down-regulate important cytokines involved in inflammatory/allergic processes triggered by Th1 and Th2 polarization, and to decrease basophil degranulation and B cell proliferation induced by birch pollen extract. In summary, our findings show that TGFβ1- and IL-10-like peptides act as great partners and can be potentially used in combination to modulate the immune response towards environmental allergens.

Dissemination of the findings

Results obtained during the period of the fellowship were presented at the European Academy of Allergy and Clinical Immunology (EAACI) congress 2018 in Munich, Germany, and at the Österreichische Gesellschaft für Allergologie und Immunologie (ÖGAI) 2018 in Vienna, Austria. All presentations of the results regarding this project acknowledged the EAACI financial support. In addition, the results described in this report will be soon submitted for publication in a high-impact journal within the field of allergy. All the publications deriving from this study will comply with the Open Access Policy of the University of Salzburg and will acknowledge the EAACI financial support.

Personal reflection and acknowledgements

I would like to thank the EAACI Headquarters team for all the support and brilliant work during all these years as an EAACI Junior Member, and for the opportunity to have this great experience, that has enriched me both professionally and personally. I would like to say a special thank you to my host supervisor, Prof. Dr. Fatima Ferreira, for having believed in the potential of this project, for her time and consideration to be always available to discuss the results obtained in the project, and for words of motivation. Also, I would like to thank the whole team from Dr. Ferreira's lab for all the help and contribution to this study. I was also able to benefit a lot from the other groups in the department of biosciences, especially regarding to cell culture and *in vivo* experiments. Concerning the infrastructure, the department of biosciences at the University of Salzburg is very well equipped and offered great conditions to perform every *in vitro* or *in vivo* experiment described in the project. Based on my extremely positive experience, I would strongly recommend Dr. Ferreira's group for applicants that aim to have an internship in the fields of allergy. Noteworthy, Salzburg is a very beautiful and cozy city that will certainly enhance your experience.