



TGF- β 1-LIKE PEPTIDES FOR IMMUNOMODULATION IN ALLERGY

Final Report - Long Term Research Fellowship (01/06/2017 to 31/05/2018)
European Academy of Allergy and Clinical Immunology (EAACI)

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INITIAL CONSIDERATIONS

This report aims to present the results achieved during the Long Term Research Fellowship from EAACI granted in the period from 01/06/2017 to 31/05/2018. 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.

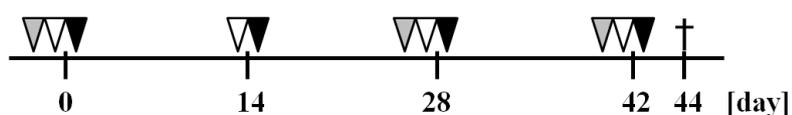
Central Hypothesis: TGF β 1-like peptide that was previously selected in the home institution by phage display technology and shown to mimic the native TGF β 1 immunomodulatory function with the potential to modulate immune responses to environmental allergens. In addition, such a peptide might represent a promising approach for allergy prophylaxis.

Major goal described in the original plan: Since transforming growth factor- β 1 (TGF β 1) has been shown to exert anti-inflammatory and immunosuppressive functions, the aim of the study was to evaluate the immunoregulatory capacity of two TGF β 1-like peptides to inhibit inflammatory and allergic processes in a therapeutic mouse model using Bet v 1 and its hypoallergenic derivative BM4.

Adaptations from the original plan: Based on preliminary results we decided to test, *in vivo*, only one TGF β 1-like peptide, since it presented best activity *in vitro*. The original plan aimed to investigate the action of the mimetic peptide in a therapeutic mouse model using Bet v 1 and its hypoallergenic derivative BM4. Although we still aim to investigate this approach in future studies, we have opted to investigate the role of the mimetic peptide during the process of allergic sensitization, in a prophylactic manner. We have chosen this model based on preliminary results achieved in a mouse model of inflammation. Furthermore, based on results from other groups in our department, we have opted to use a recombinant Phl p 5 (a major allergen of timothy grass) to induce sensitization in mice. Group 5 contains some of the most important grass pollen allergens which are recognized by IgE antibodies of almost all grass pollen allergic patients and induce strong IgE antibody responses, which justifies the choice of this allergen for this study.

Mice immunizations

To induce allergen-specific IgE response, 5 female BALB/c mice (6–10 week-old) were sensitized intradermally (i.d.) with 25 µg of recombinant Phl p 5 diluted in PBS. Another group composed of 5 mice were pre-treated subcutaneously (s.c.) with 100 µl of TGFβ1-mim peptide diluted in PBS at 1 µM, followed by i.d. immunization with 25 µg of Phl p 5 diluted in PBS. Three mice constituted the naïve group. Immunizations were performed on days 0, 14, 28 and 42, and mice sacrificed on day 44. To investigate the induction of Phl p 5-specific IgE responses, blood samples were drawn from the saphenous vein on days 14, 28 and 42 after first immunization. The immunization schedule is presented below.



▽ Blood drawing

▽ 1h pre-treatment with 1 µM TGFβ1-mim peptide (s.c.)

▼ Immunization with 25 µg rPhl p 5 (i.d.)

† Sacrifice, blood and spleen collection

Results

TGFβ1-mim peptide modulates antibody response *in vivo*

The immunization with the recombinant Phl p 5 allergen for 44 days significantly induced the allergen-specific total IgE response, proving that mice were successfully sensitized. ELISA analysis shows that, in serum from Phl p 5-sensitized mice, the TGFβ1-mim peptide was able to suppress the levels of IgE, the main antibody involved in the allergic response, as well as IgG2a and IgG1, markers for Th1 and Th2 lymphocytes respectively. Furthermore, mice treated with the peptide rendered substantially higher levels of IgA, an antibody that is known for its capacity to inhibit IgE-mediated allergic reaction.

TGFβ1-mim peptide modulates cytokines production *in vivo*

To determine the number of IFN-γ, IL-4 and IL-10 producing spleen cells, an ELISpot assay was carried out. Immunization with Phl p 5 resulted in high induction of IFN-γ and IL-4 release upon allergen stimulation. In mice treated with the TGFβ1-mim peptide, levels of IFN-γ and IL-4 secreted by Phl p 5-restimulated splenocytes were significantly lower while levels of IL-10 were significantly higher. Since IFN-γ is a cytokine that contributes directly and indirectly to Th1 differentiation and IL-4 is a cytokine that induces Th2 differentiation, the downregulation of these two cytokines indicates a modulation of either Th1 or Th2 polarization, essential event to control an inflammatory/allergic response. On the other hand, the upregulation of IL-10 indicates that the peptide

played an anti-inflammatory action since this cytokine plays a crucial, and often essential, role in preventing inflammatory pathologies.

TGF β 1-mim induces Treg cell production *in vivo*

It has long been known that the production of Treg cells is a key mechanism in allergen-specific immunotherapy. Hence, molecules that are able to induce the production of Treg cells could potentially act in providing a regulatory action against environmental allergens. To investigate the influence of the TGF β 1-mim in inducing Phl p 5-specific Treg cells during the process of allergic sensitization, flow cytometry analysis of splenocytes from BALB/c mice was carried out. Naïve and Phl p 5-sensitized mice rendered comparable levels of CD4⁺/CD25⁺/Foxp3⁺ T cells, a total of 16.1% and 16.2%, respectively, whereas mice treated with the TGF β 1-mim peptide rendered a total of 43,9%. Therefore, consistent with the notion that TGF β cytokine can induce Treg cell, the treatment of Phl p 5-sensitized mice with the TGF β 1-mim peptide was also able to modulate Th differentiation.

Relevance of our findings

In this study, we investigated the immunoregulatory action of a TGF β 1 mimetic peptide in mice immunized with Phl p 5, a major allergen from timothy grass extract. It has long been known that activated Th2 lymphocytes produce IL-4, subsequently inducing B cell production of IgE, a key event in the development of allergic symptoms. In contrast, differentiated Th1 cells secrete IFN- γ , and are responsible for delayed type hypersensitivity. Since failure in the Th1/Th2 balance can lead to inflammation and allergy, the relative suppression of Th2 cells by the relative increase of Th1 activities, or vice versa, was thought to be a mechanism in which to restore the balance in an immune disorder. However, another mechanism involving the induction of Treg cells is shown to be responsible for the induction of immune homeostasis. Thus, the modulation of cytokines, and consequently T and B cells, may impact approaches for the treatment of allergy and inflammation.

In summary, in this study we have shown a regulatory role of a TGF β 1 mimetic peptide in the modulation of an inflammatory allergic response. The mimetic peptide was able to modulate Th1 and Th2 responses via the regulation of cytokines and antibodies, to induce Treg cell differentiation, as well as other important cellular events that promote the exacerbation of the allergic or inflammatory microenvironment. These findings strongly imply a potential use of the TGF β 1-mim peptide as an immunomodulatory compound to enhance immunity 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 2017 in Helsinki, and at the International Symposium on Molecular Allergology (ISMA) 2017 in Luxembourg. All presentations of the re-

sults regarding this project acknowledged the EAACI financial support. In addition, the results described in this report were recently 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

First of all, 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 and for having shared with me her great knowledge during several meetings to discuss the results obtained in the project. Also, I would like to thank Dr. Lorenz Aglas for the great help with the animal experimentation and many different techniques that were used to enrich this project. 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, and I would strongly recommend Dr. Ferreira's group for applicants that aim to have an internship in the fields of allergy.