European Society for Immunodeficiencies
Mijnbouwstraat 23, Delft, the Netherlands
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ESID End of Fellowship report

1. Fellowship details

- First name: Víctor Andrés
- Last name: Sosa-Hernández
- Type of fellowship:

☐ **Short-term**

☒ **Medium-term**

☐ **Bridge grant**

- Fellowship's start and end date: November 4th 2022 – May 5th 2023
- Hosting institution: Center for Chronic Immunodeficiency of the Medical Center – University of Freiburg
- Supervisor: Prof. Dr. med. Klaus Warnatz and Dr. Bärbel Keller

2. Summary of the work done during the fellowship (max.400 words).

During my fellowship in the Center for Translational Cell Research (ZTZ) – Center for Chronic Immunodeficiency, I was working under the supervision of Prof. Dr. med. Klaus Warnatz and Dr. Bärbel Keller. I developed a project to characterize the origin and role of dysregulated tumor necrosis factor receptors (TNFR) expression in the $CD21^{Low}$ B-cell subpopulation, which is expanded in patients with common variable immunodeficiency (CVID). After studying the literature and testing of available antibody clones for flow cytometry, I focused on three members of the TNF receptor superfamily (TNFRSF) TNF receptor 1A (TNFR1A), TNF receptor 1B (TNFR1B) and lymphotoxin β receptor (LTβR). We recruited 14 CVID patients and 10 healthy individuals as controls. I established a 10-color flow cytometric staining panel to determine the expression density in different compartments of B cells from peripheral blood samples. Corroborating RNAseq data, TNFR1B and LTβ receptor protein expression were found to be increased on $CD21^{Low}$ B cells. TNFR1A was found without a noticeable increase between the $CD21^{Low}$ B cells and the other B-cell subsets.
In collaboration with Pascal Schneider (Lausanne, France) and Cristian Smulski (San Carlos de Bariloche, Argentine), we aimed to study the signaling pathways downstream of TNF-Ligand/TNF-receptor interaction by flow cytometry. This step was performed in healthy controls and later in CVID patients. In addition, to address the effects of TNFR signaling we evaluated the production of three cytokines (IL-10, TNF-α, IL-6), through a combination of stimuli with TNFR ligands and CpG ODNs (TLR9), in peripheral blood mononuclear cells (PBMCs) and isolated total B cells. Finally, to get insights into the regulation of TNF receptors expression on B cells, the density of TNF receptors was evaluated after stimulation with CD40L, IL-21, IgM, IFNγ, and CpG.

3. New skills acquired during the fellowship (max.200 words).

This collaboration allowed me to acquire different theoretical and practical skills. On the one hand, I acquired the perspective of how to approach the study of B cells in CVID patients and a new vision of the $CD21^{Low}$ B-cell subpopulation. I learned a new way of performing the analysis of the different compartments of B cells by flow cytometry. Also, I learned to evaluate signaling pathways by measuring phosphorylated proteins through the phosflow protocol. Likewise, I learned protocols for in-vitro cultivation of human B cells, to isolate total B cells from peripheral blood and culturing them for cytokine production. In addition, I deepened my experience in Multiparametric Flow Cytometry, with the bases and principles to perform a correct technique for this approach. Finally, I acquired basic knowledge and practice in spectral cytometry on the Sony SP6800 and ID7000 spectral cytometers, as well as a spectral cytometry seminar, within the Institute.

4. Your professional plan for the near future and how the fellowship impacted this plan (max 400 words).

Currently, I am now in my fourth year of PhD. During these years of preparation and study, my goal has been to analyze and understand the biology of B cells. My focus has been on autoimmune and infectious diseases. My short-term target is to finish my PhD this year. This fellowship allowed me to expand my vision in immunodeficiencies and with it to be able to enrich the data analysis and discussion of my research project in Mexico. Likewise, when I return to my laboratory in Mexico, I will be able to generate new experimental approaches to the diseases that we study. I will continue looking for and promoting research projects to collaborate. Finally, I plan to perform a medical specialty in the coming years, and this experience improved my practical and theoretical skills in immunology, which helped me to generate different research projects during its development.

5. Results obtained from your fellowship project. Please, mention any publications or meeting communications derived (if applicable, max 800 words).

The aim of my project was to elucidate the role of three members of the TNF receptor superfamily TNFR1A, TNFR1B and LTβ receptor in the $CD21^{Low}$ B-cell in the $CD21^{Low}$ B-cell subpopulation. Previously, a differential expression of several members of the TNF receptor superfamily, crucially involved in different biological processes, was found by transcriptomics in $CD21^{Low}$ B cells. By flow cytometry we confirmed that LTβR and TNFR1B are increased in the $CD21^{Low}$ B-cell subpopulation, both in controls and CVID patients. TNFR1A, as the classical TNF receptor binding soluble TNF-α was analyzed due to the crosstalk with TNFR1B and was found to be normal.

To evaluate the impact of increased TNF receptor expression, we stimulated mononuclear cells from controls and CVID patients with ligands for the LTβ receptor, the two bioactive forms of TNF (membrane-bound [mTNFx] and soluble [hTNF], reported to bind TNF receptors 1B and 1A, respectively) and as a negative control the ectodysplasin-A (EDA) receptor ligand. It is thought that soluble TNF can activate the canonical pathway of NFκB through p65 phosphorylation and IκBα degradation. While the stimulation of TNFR1B could activate MAPKs pathway.

In short periods of time (3,7,10,12,15 min) we evaluated pP38, pERK, mTOR, pP65 and in a period of 30 min we evaluated pERK and IκBα. As expected, we detected an activation in the canonical pathway of NFκB in T cells after stimulation with hTNF and interestingly also upon stimulation with the ligand mimicking mTNFx. Unfortunately, we could not detect TNFR1B signaling in B cells due to the limited time.

Since we could not visualize mTNFx signaling we aimed to detect the consequences of TNFR1B stimulation. We evaluated the secretion of IL-10, reported to be increased after combined stimulation with CpG and mTNFx. Additionally, IL-6 and TNF-α were evaluated. In a first approach, we stimulated PBMCs with different combinations of TNFR ligands with CpG for 48h. We did not detect changes in production of cytokines between B cells that were stimulated with TNFR ligands and those that were not. In a second experiment, we isolated total B cells and modified the concentrations of TNFR ligands. The results showed that the concentration of the ligands or isolation of the B cell compartment does not modify the secretion of the previously described cytokines.

Finally, to get insights into the regulation of TNF receptor expression we stimulated total B cells with different stimuli (IFNγ, CD40L, CpG, IgM, IL-21) for 48h. Interestingly, we observed an increase in LTβR, TNFR1A and TNFR1B receptors in the cells that were stimulated with CD40L+IL-21 and CpG.

6. Any other comments (max.200 words)

I would like to thank ESID for the great support, without you I could not have done it. I really appreciate and thank you very much for the opportunity to collaborate with the team of Dr. Warnatz. It was enriching for me the perspectives of Dr. Warnatz and Dr. Keller, as well as his amazing team.