

Final report to the Selection Panel of Serbian Clinical Immunology Fund

Project title: The effect of FFAR2 stimulation on human ILC3 and regulatory T cell proliferation and function

Principal investigator: Ivana Stojanović, PhD, Institute for Biological Research “Siniša Stanković“, National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia

The project has been completed successfully in accordance to the set aims. Human tonsillar cells were efficiently isolated using collagenase D-based enzymatic digestion technique. The *in vitro* culture of tonsillar cells resulted in high viability of cells after 48 h. Since the agonist for FFAR did not exhibit any effect on human ILC3 and Treg *in vitro*, our research switched to the evaluation of ILC3 and Treg after the treatment with newly synthesized aryl hydrocarbon receptor (AhR) agonist termed C43. Both ILC3 and Treg express AhR on their surfaces and their regulatory function can be enhanced through AhR stimulation. Therefore, we have evaluated the proliferation of ILC3 and Treg, determined their functional status and engaged in their freezing process for the future scRNA sequencing. For these experiments the following antibodies were purchased using the fund of the Serbian Clinical Immunology Fund: Human Hematopoietic Lineage Antibody Cocktail, eFluor™ 450, eBioscience™, CD4 Monoclonal Antibody (RPA-T4), FITC, eBioscience™, CD25 Monoclonal Antibody (BC96), PE, eBioscience™ and IL-2 Monoclonal Antibody (MQ1-17H12), PE, eBioscience™. In addition, for the cell freezing, we have purchased and used Corning® CoolCell™ LX Cell Freezing Container. These results have not been published so far and the paper is in preparation.

In addition to the proposed aims, we have achieved additional goals related to the connection of ILC3 and type 1 diabetes (T1D) in mice. We have used IL-2 Monoclonal Antibody that was purchased using funds from this project, for the evaluation of IL-2⁺ ILC3 proportions in lamina propria during T1D development. Results indicate the progression of T1D correlates with the reduced proportion of IL-2-producing ILC3 and Treg in lamina propria of streptozotocin-treated C57BL/6 mice and NOD mice that develop the disease spontaneously. The results of the study was published in *Molecules* journal. In this article, we have acknowledged the contribution through Serbian Clinical Immunology Fund.

Saksida T, Paunović V, Koprivica I, Mićanović D, Jevtić B, Jonić N, Stojanović I, Pejnović N. Development of Type 1 Diabetes in Mice Is Associated with a Decrease in IL-2-Producing ILC3 and FoxP3⁺ Treg in the Small Intestine. *Molecules*. 2023 Apr 11;28(8):3366. doi: 10.3390/molecules28083366.