

REPORT OF THE TUTOR ON DIPLOMA THESIS OF SILA AZAK, Bc.

Title of the work: **Characterization of the immune responses against SARS-CoV-2 virus**

Tutor: **MUDr. Zora Mělková, PhD**

Sila Azak, Bc., started to work on her diploma project in my Virology Lab at the Department of Immunology and Microbiology, First Faculty of Medicine, Charles University in the fall 2022. In her diploma project she characterized namely the changes of selected cytokines in a mouse model of COVID-19 after the treatment with heme arginate and remdesivir.

In our laboratory, we have described inhibitory effects of heme arginate on SARS-CoV-2 replication in tissue cultures. Consequently, we used transgenic mice expressing hACE2 under control of K18 promoter to determine the effects of heme arginate on SARS-CoV-2 titers and the course of infection in vivo. The assignment for Sila was to characterize changes in cytokine production in these mice at a specific time-point after the intranasal infection. To this end, she quantified selected cytokines at mRNA level in total spleen RNA using 1-step RT-qPCR, and at protein level in plasma using LegendPlex Cytokine Bead Assay (CBA). In addition, she performed lung histology to demonstrate inflammatory changes. During the work on the project, Sila had to face several challenges. Namely, she was really struggling with the online analysis SW for CBA provided by BioLegend – the SW kept changing in time, with each version yielding somewhat different results. Also, the mice and the material from infectious experiments was limited. Thus, despite the substantial effort, the results of Sila's work mostly did not yield statistically significant differences and only tendencies could be described. Nevertheless, Sila demonstrated ability to perform experiments independently and to analyze the obtained results.

During the work on the thesis project, Sila has got experience with several laboratory methods and approaches. She learned to handle tissue cultures and to use flow cytometer with the subsequent analysis using Diva SW. She isolated total RNA from tissue culture cells and from frozen mouse spleens, and consequently she performed 1-step RT-qPCR to quantify mRNA for selected cytokines. Further, she detected plasma cytokines using CBA and quantified them using the online analysis SW provided by BioLegend. Sila also used paraformaldehyde-fixed mouse lungs to prepare histological sections stained with hematoxylin-eosin and captured the microscopic pictures. Finally, Sila used GraphPad Prism for statistical analysis and graphical presentation of the results.

In conclusion, Sila Azak, Bc. proved the ability to perform experiments independently, to analyze and report the obtained results, as well as to critically comment on them. She has searched for the relevant scientific literature and references, and proved determination and goal orientation during the writing of the thesis. I recommend her diploma thesis to be accepted and to award Sila the degree of Magister.

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