Fellowship – support for stay 2021-2022

Thanks to the Fellowship of the Czech Society for Atherosclerosis (ČSAT) I was able to travel to the United States of America and enroll in a 6-month traineeship, between January and July, at the Liver Disease Immunobiology Laboratory at the Division of Gastroenterology and Hepatology – Mayo Clinic in Rochester, Minnesota.

With more than 100 years of history, Mayo Clinic was born officially in the small city of Rochester in the year 1889 with little more than two dozen beds. Following the devastation of the city of Rochester MN by the tornados in 1883, Mother Alfred, a Sister of St. Francis, approached Dr. William Worrall Mayo with the vision to build a hospital in Rochester. In this proposal, the Sisters of St. Francis agreed to build the hospital and supply the nursing staff, if the doctor provide the medical staff. And it so happened that Dr. William Worrall Mayo become the consulting physician and his two sons, Dr. Will and Dr. Charlie Mayo, now known as „the Mayo brothers“, served as attending surgeons. Ever since its creation, Mayo has been in the vanguard of innovation many times, a couple of examples of that are when in 1910 created an organizational structure known as the „integrated multispecialty group practice“ which is the creation of a team of specialists with diverse skills that collaborate to provide the optimal care to each patient; or when two members of the Mayo Clinic staff (Edward Kendall Ph.D. and Philip Hench M.D.) received the Nobel Prize for Medicine or Physiology in 1950 for the discovery of the hormone cortisone and its application for the treatment of rheumatoid arthritis.

Since its creation in Rochester, MN (the main campus), Mayo Clinic has expanded to other locations across the country into the states of Arizona and Florida. However, the fact that Mayo Clinic is a value-driven organization based on the memorable acronym RICH TIES (Respect, Integrity, Compassion, Healing, Teamwork, Innovation, Excellence, Stewardship) following the motto „The Needs of the Patient Come First“ never changed. Currently, it ranks number 1 as the best hospital in the nation and worldwide according to the U.S. News & World Report, and is a prestigious institution recognized worldwide for its reputation, technology, and advances in science.

This internship was developed at the Liver Disease Immunobiology Laboratory at the Division of Gastroenterology and Hepatology – Mayo Clinic (Rochester, MN). The overall goal of this laboratory, led by PharmD. Petra Hirsova, Ph.D., is to discover critical cellular and molecular processes underlying nonalcoholic steatohepatitis (NASH) pathogenesis, identify druggable targets, and develop novel therapeutic strategies. NASH, as the name suggests, affects individuals with low to no alcohol consumption and is a concerning progressive illness that affects approximately 5% of the USA population with a lifetime cost of 222.6 billion dollars for all NASH patients (as of 2017). NASH is an aggressive form of fatty liver disease defined by liver inflammation which may progress to advanced scarring (cirrhosis) and end-stage liver disease (i.e. liver failure). There is currently no pharmacologic treatment available for those patients and liver transplantation is the only available option for the management of NASH patients with end-stage liver disease, such as NASH patients with liver failure and/or hepatocellular carcinoma.

The high prevalence, the lack of treatment, and its role as a risk factor for other pathologies make the research of the mechanism underlying NASH development, as well as the identification of possible biomarkers a research topic in high demand. Indeed, the pro-atherogenic lipid profile in NASH patients is one of the responsible factors for the increased risk of life-threatening cardiovascular events.

For instance, the Liver Disease Immunobiology Laboratory at the Division of Gastroenterology and Hepatology has a long-lasting interest and significant expertise in NASH and utilizes biologically relevant mouse models, genetic and pharmacologic *in vivo* approaches, and state-of-the-art methodologies such as immunophenotyping by mass cytometry (CyTOF) and single-cell RNA sequencing (CITEseq). The laboratory is interested in the immunologic component of NASH, such as researching infiltrating leukocytes in the liver and monocyte-derived macrophage factors of liver disease progression and fibrosis development. They have developed *in vitro* models mimicking NASH to study cell-to-cell communication and interaction between various liver cell types and have extensive experience with animal experimentation and mouse models of NASH that displays high fidelity to human disease.

Thus, the goal of this 6-month stay at so prestigious institution was to deepen my knowledge of NASH pathophysiology and to understand the overall process of project development, from the critical thinking behind the writing of a grant project and how the project is developed, troubleshoot and through the various methods of sample analysis and data curation. For this, I had the opportunity to collaborate on the undergoing research projects in the host lab related to NASH pathophysiology.

Moreover, a big portion of the internship was devoted to learning innovative methods not currently available at my home institution in the Czech Republic, including isolation of primary mouse cells (e.g., intrahepatic leukocytes and splenocytes) and the methods of purification of cell types, for example, to select specific subpopulations of liver leukocytes (e.g. CD4 or CD6 T-cells) through positive or negative selection using magnetic beads. Isolation and purification of cell type population (or subpopulation) are especially useful for the investigation of cell type-specific effects in the phenotype or pathophysiology progression of the disease. For example, the detection of alterations in a specific receptor in small cell populations in the liver, i.e. the expression of receptors and cytokines expressed by liver intrahepatic leukocytes during NASH development, may be masked by the protein or mRNA expression of the remaining cell types present in the liver, thus not possible to be evaluated in whole tissue lysates. These isolated cells can be used for protein and mRNA extraction for classical molecular methods, such as Western blotting and qPCR. Another approach is to submit these isolated cells to single-cell analysis such as CyTOF or CITEseq and group them into clusters of differentiation and elucidate the cell type-specific effect or evolution during the disease, thus elucidating the role of the cellular population in the pathology.

CyTOF (Cytometry by the time of flight) is a method for immunophenotyping of hepatic leukocytes using mass cytometry to quantify labeled targets on the surface and interior of single cells through detection of immunolabeled targets with heavy metal tags by mass cytometry. This methodology provides valuable information about protein expression, immunophenotype, and functional characterization at a single cell level. This is a valuable tool mostly in immunology, where for example, a small population of cells has diverse properties affected by numerous markers in various combinations.

CITEseq analysis is a new method that captures not only a quantitative and qualitative “snapshot” of cell surface proteins but also the RNA sequencing of every single cell, which results in combining transcriptomics with immunophenotyping. This technique is based on de detection of targets immunolabeled with DNA-barcoded antibodies to convert the detection of proteins into a quantitative readout. The number of reads from each conjugated DNA barcode reflects the identity and abundance of the target proteins, which results in a big amount of data that needs to then be curated by bioinformatics analysis to provide multimodal information on the state of the cells and clusters.

During my internship, I had also the opportunity to collaborate “hands-on” with mouse colony maintenance and to learn different methods of crossbreeding for genetic modification of rodents, such as how to develop whole body genetic knock-out mouse or how to use special techniques for engineered generation of the cell type-specific and inducible genetic modifications, and how to backcross a mouse model to the uniform background. These techniques use for example Cre-Lox technology which enables the generation of tissue-specific and inducible knockouts and thereby has control over the location and timing of gene expression. This technique is highly valuable not only for the advantages of creating a cell type-specific deletion of the gene but also to bypass genotypes for which the all-body knockout would generate an embryonic lethal phenotype, thus deletion of the gene in adulthood would no longer be lethal. An example of the benefit of the generation of liver cell type-specific knock-out is, for example, selective deletion of the gene only in the hepatocytes, and thus possible to evaluate the role of this gene in hepatocytes (a major component of liver parenchyma), or on the other hand, we can knock-out the gene only in hepatic stellate cells (the cell type responsible for collagen fiber production), and elucidate in which cell type the gene of interest produces the disease, or whether the interest gene is important in a specific pathway during the progression of the disease.

Because no overseas journey would be complete solely with work-related skills and knowledge, besides the scientific activities of this traineeship, I had also the opportunity to grow at a personal level by interacting with a different cultural environment composed not only of USA citizens but also talk and share the intercultural

experience with people that come from all around the world for work, training or to be treated at so prestigious Mayo Clinic. Additionally, Minnesota, also known as “the land of the 10 000 lakes” is a very nice place for an adventure through the amazing nature in the summer, with green parks and sparkling lakes that resurge from under the winter snow when spring approaches, all quiet winter cities are slowly replaced by the noise of the people in the streets and the series of summer events, street markets, and concerts through downtown and riverside. Overall it was a wonderful and absolutely priceless experience, that would not have been possible without the support of the CSAT.

Thank you,

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