ImmunoTools *special* Award 2022

Anna Schmuckenschlager, PhD-student

Supervisor: Assoc. Prof. MMag. Dr. rer. nat. Alice Assinger

Vienna Platelet Lab, Institute of Vascular Biology and Thrombosis Research, Medical University of Vienna, Schwarzspanierstraße 17a, 1090 Vienna, Austria

Impact of Puumala Hantavirus infection on primary hemostasis: Investigation of the underlying mechanisms of thrombocytopenia

Platelets are blood cells that mediate haemostasis and thrombosis. They constantly patrol the vasculature for tissue damage. Upon sensing vascular injury platelets become quickly activated and together with the coagulation system they stop bleeding. Apart from their crucial function in haemostasis platelets also function as immune sentinels and modulate innate and adaptive immunity. Their high sensitivity, rapid responses and their huge number makes platelets perfect players in first line defence against invading pathogens.

Upon platelet activation, via e.g.: interaction of infected endothelium or direct interaction with pathogen, platelets upregulate surface receptor expression and secrete mediators including cytokines, chemokines and immunoglobulins. This allows for the interaction with and modulation of leukocytes and endothelial cell functions, which results in direct immune responses against invading pathogens.

The current COVID-19 pandemic, which is associated with thrombotic complications, clearly shows that viral infections can dysregulate the haemostatic system. But SARS-CoV-2 is not the only virus that affects haemostasis: Viral hemorrhagic fevers (VHF) caused by various RNA virus families are associated with impaired haemostasis, leading to a clinical picture of increased bleeding and decreased platelet counts (thrombocytopenia). VHF pose a global threat as cases are ever increasing and the distribution of individual virus strains is expanding. Even in Austria a milder form of VHF frequently occurs, which is caused by Puumala Hantavirus (PUUV). Such an infection normally causes milder symptoms but still half of reported patients require hospital care with symptoms including increased bleeding tendencies and thrombocytopenic events. Thrombocytopenia during PUUV infection may not only affect haemostasis but also platelet-mediated immunomodulatory

functions. Therefore, platelets are thought to play a pivotal role in disease manifestations of PUUV infections, but the underlying mechanism is yet unknown.

Thus, the aim of this project is to elucidate how PUUV infection modulates platelet counts and affects platelet functions. We speculate that PUUV infection may reduce the production of platelets by compromising differentiation and/or maturation of megakaryocytes. Further, we are interested in the complex interplay of cells residing in the bone marrow. PUUV infection might affect the microenvironment of the vascular niche and thereby interfering with platelet production, haematopoiesis and the migration of leukocytes.

We will investigate the impact of PUUV infection on platelet production *in vitro* and monitor effects of PUUV on megakaryocyte differentiation and maturation. Subsequently, effects of PUUV on megakaryocyte yield and pro-platelet formation of will be measured by flow cytometry. Additionally, platelets will be analysed by flow cytometry regarding surface expression of activation markers and hetero-aggregate formation with leukocytes.

Therefore, megakaryocytes will be stimulated with thrombopoietin (TPO), and platelet production will be investigated in the presence of PUUV. For analysing respective cell populations, we will employ flow cytometry antibodies for megakaryocytes and platelets (CD42b). Hematopoietic progenitor cells will be identified (CD34), and leukocytes (CD45) as well as lymphocytes, T- (CD3, CD4, CD8) and B-lymphocytes (CD19) will be analysed in regard of platelet interaction and leukocyte activation monitored (CD11b).

Together with ImmunoTools, this study will significantly contribute to a more comprehensive view of the pathogenesis of Hantavirus-induced haemorrhagic fevers and will help to better understand underlying causes of thrombocytopenia, platelet function and its associated complications. A better understanding could path the way for urgently needed treatment options in patients suffering VHF.