A21 - Extracellular vesicles from activated platelets induce a shift towards proinflammatory monocyte subsets

B Fendl¹; R Weiss¹; T Eichhorn¹; A Spittler²; V Weber¹

¹Danube University Krems, Department for Biomedical Research, Krems, Austria, ²Medical University of Vienna, Core Facility Flow Cytometry & Surgical Research Laboratories, Vienna, Austria

Introduction:
Circulating monocytes comprise classical (CM, CD14++CD16⁻), intermediate (IM, CD14++CD16⁺), and non-classical (NCM, CD14⁺CD16+++) subsets. Changes in subset distribution, specifically a shift towards proinflammatory CD16⁺ monocytes, have been described in various pathologies including sepsis. We analyzed the distribution of monocyte subsets following monocyte isolation from whole blood and the potential influence of platelets and platelet-derived extracellular vesicles (EVs) on monocyte subset distribution.

Methods:
Monocyte subsets were characterized by flow cytometry after monocyte isolation by density gradient centrifugation and negative depletion of non-monocytes using two different monocyte isolation protocols (both from Miltenyi Biotec). The association of monocytes with platelets (CD41⁺) and platelet-derived EVs (CD41⁺lactadherin⁺) was assessed by flow cytometry.

Results:
Monocyte subset distribution was comparable for both isolation protocols (87.5±4.9% vs. 83.7±3.3% CM, 4.8±2.2% vs. 5.4±2.8% IM, 7.7±5.8% vs. 10.9±5.0% NCM for protocol I vs. protocol II; n=4) and did not differ from the distribution in whole blood. Isolated monocytes contained residual platelets and platelet-derived EVs. Overnight storage of isolated monocytes, but not of whole blood, led to a significant increase in IM (86.4±6.2% vs. 50.5±11.8% CM, 5.4±2.6% vs. 47.1±13.4% IM, and 8.2±4.2% vs. 2.4±2.0% NCM at 0h vs. 15h). Flow cytometry confirmed the association of monocytes with platelets and platelet-derived EVs as well as the uptake of EVs by monocytes.

Conclusion:
Storage of isolated monocytes induces a shift towards CD16 expressing proinflammatory monocytes, which seems to be mediated by residual platelets and platelet-derived EVs. It remains to be clarified whether EVs released from activated platelets can also trigger a shift towards proinflammatory, intermediate monocytes in vivo.