QUANTITATIVE 3D-ANALYSIS OF PLASMA CELL LOCALIZATION AND THEIR RELATION TO VASCULATURE IN MOUSE BONE MARROW

Zoltán Cseresnyés[1,#], Sandra Zehentmeier[2,6#], David Reismann[3,4], Marc Thilo Figge[1,5,*], Anja E. Hauser[3,6*]

[1]Applied Systems Biology, Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Jena, Germany; [2]Yale University, Yale School of Medicine, New Haven, USA; [3]Charité – Universitätsmedizin Berlin; [4]LaVision/Biotec, Bielefeld, Germany; [5]Faculty of Biological Sciences, Friedrich Schiller University, Jena, Germany; [6]Deutsches Rheuma-Forschungszentrum (DRFZ), a Leibniz Institute, Berlin, Germany; #Equal contributors *Shared senior authors

E-mail: zoltan.cseresnyes@hki-jena.de, thilo.figge@hki-jena.de, hauser@drfz.de

Plasma cells (PCs) are mature, antibody-secreting immune cells which are maintained as long-lived cells in specialized microenvironments (so-called niches) of the bone marrow providing long-term stable protective antibody titers. Long-lived PCs thus represent the cornerstone of humoral immune memory. However, many details of how the survival of long-lived PCs in bone marrow is guaranteed still remain enigmatic. Although several bone marrow cell types have been described to interact with long-lived PCs, the anatomic localization of PCs within the complex structure of the bone marrow vasculature has not been studied in detail. While arterioles provide the parenchyma with oxygen and nutrients, the fenestrated endothelial layers of the sinusoidal network allow for the easy entry of immune cells into the marrow, including e.g. PCs formed in the periphery, as well as for exit of large amounts of newly formed blood cells from the parenchyma into the circulation. Consequently, a detailed knowledge of the spatial distribution of PCs in relation to arterioles and sinusoids will contribute to our understanding of PC survival mechanisms, metabolism and function.

We examined the 3-dimensional (3D) structure of the bone marrow via 2-photon microscopy in whole-mounts of excised long bones of the mouse, using second harmonic generation and fluorescence labeling. The 3D image stacks were recorded on a LaVision/Biotec TriM Scope II microscope equipped with a Ti:Sa pulsed laser. Image resolution in x, y and z was improved by deconvolution based on measured point spread functions for our optical system using Huygens software. All bone components were reconstructed as 3D surfaces using Imaris and exported in the Virtual Reality Modeling Language format, which were placed into a virtual 3D environment built in the open-source animation software Blender via a custom written Python script. The surfaces were first used to measure the shortest experimental distances between each PC and three stationary bone marrow components (the bone cortex, arterioles and sinusoids), as well as between PCs. In computer simulations, the PC surfaces were redistributed randomly in the 3D bone marrow model, and the resulting distances of PCs to the stationary compartments were compared with the measured distances. By this approach, we received information on whether the PC distribution is guided, e.g. by a need to remain near the vasculature, or if they distribute randomly. We found that the measured minimum distances for PCs to sinusoids and arterioles were significantly shorter than the distances of simulated PCs to these vessels, pointing towards a guided distribution of PCs. PCs were found closer to sinusoids (25 µm average distance) than to arterioles (80 µm average distance). However, we identified a subpopulation of PCs which is found within 30 µm proximity to arterioles. The biological reason for this association needs to be investigated in the future.