Dipl. Biol. Anja Köhler: Abstract der Diss. zum Thema: Intravital visualization of hematopoietic stem cell and neutrophil behavior in long bones of mice

Hematopoietic stem cells (HSC) are precursors of all blood cells and primarily located in the bone marrow (BM) in adults. They interact closely with a special BM microenvironment, referred to as stem cell niche, which regulates cell proliferation, self-renewal, differentiation and migration/mobilization. As a current hypothesis it is postulated that the impaired hematopoiesis of aged stem cells is a consequence of an altered interaction with their niche. With a newly developed experimental setup for intravital 2-photon microscopy the dynamics and spatial arrangement of young and aged primitive hematopoietic cells in the long bones of mice were investigated to reveal possible changes in cell-niche contact. Until now all microscopic observations of BM-resident hematopoietic cells were obtained from cells located in a small bone marrow patch of the calvarium, although this compartment has known limitations with regard to HSC-biology.
Transplanted hematopoietic progenitor cells (HPCs) and early hematopoietic progenitor cells (eHPCs) were found to be completely immobile in murine long bones but displayed a permanent protrusion movement of the cell surface. Moreover, eHPCs from aged animals were located more distant from the inner bone surface and this correlated with an increase of the protrusion movement compared to eHPCs from young animals. This suggest a more quiescent microenvironment provided from the niche in areas close to the endosteum whereas cells that are located farther away from the inner bone surface are not longer in a extreme silent status.

In the second part the new intravital 2-photon microscopy method was used to analyze the cell behavior of neutrophils as an example for differentiated blood cells in the murine bone marrow. Neutrophils are the most abundant and, arguably, most important leukocyte type of the vertebrate immune system. Their lack or dysfunction is always associated with severe consequences to health. In “danger situations” such as peripheral infections, the release of neutrophils from bone marrow can be dramatically increased within hours, a process termed danger mobilization. The granulocyte colony-stimulating factor G-CSF is known to play an important role in this process and already a single injection of G-CSF leads to a massive neutrophil mobilization into the peripheral blood. However, although recombinant G-CSF has been used in clinical hematology for more than 20 years to overcome the severe problems of neutropenic patients the underlying molecular mechanisms by which danger mobilization is mediated on single cell level in the BM still remains unknown.
Neutrophils show a rapid and strong increase of motility in murine long bones after G-CSF treatment although it is known, that they can not respond directly to this trigger. The chemokines KC and MIP-2 were found to play a key role in recruiting neutrophils into the circulation by forming a gradient that neutrophils are able to follow after detection by their specific surface receptor CXCR2. Megakaryocytes were identified as a source of KC and MIP-2 production in the bone marrow. Moreover it could be demonstrated that both chemokines were released in response to thrombopoietin (TPO), the major trigger of megakaryocytes. It might be delivered by a F4/80 positive subpopulation of macrophages that was identified to be positive for the G-CSF receptor and was also able to produce TPO. However, if these cells are really involved in the process needs to be further investigated.