Selective Depletion of Macrophages Delays Fracture Healing

INTRODUCTION:
The immune system and the skeleton closely interact, due to shared anatomical compartments, cell precursors and molecular mediators. Fracture repair is characterized by an initial inflammatory reaction followed by a proliferative and remodeling phase which leads to restoration of skeletal integrity. The inflammatory phase following fracture is characterized by a rapid accumulation of various cellular and humoral components. A major factor in the inflammatory process is the migration of macrophages into the fracture area, as they stimulate repair by releasing different growth factors. But the impact of macrophage activation may shift from beneficial to detrimental as previously demonstrated in a rat model with impaired bone healing due to local macrophage activation (1).

This project was aimed at further elucidating the functional role of macrophages as effector cells of the innate immune system in the process of bone regeneration following fracture. The hypothesis of this study was that fracture healing would be delayed in the absence of macrophages.

METHODS:
A standard closed femoral fracture was created in 8-10 weeks old C57BL/6N mice. Fractures were created with a 3-point bending apparatus and stabilised with an intramedullary wire. Two groups were compared: a control group with standard fracture healing (WT) and a group that received selective macrophage depletion (MAC). 48 hours before fracture, depletion of the macrophages was induced by intravenous injections of 100 µl clodronate filled liposomes dissolved in 100 µl PBS. Injections were repeated every 5 days.

For biomechanical testing, animals were sacrificed after 21 (N=16/WT, N=8/MAC) and 28 days (N=8/group). Biomechanical testing was performed after 28 days (N=8/WT, N=4/MAC). For histological analysis, animals were sacrificed after 7 and 21 days (N=6/group and timepoint). Bones were decalcified in EDTA, embedded in paraffin, 4 µm-thick sections were stained with Movat Pentachrome and the callus tissue was evaluated by histomorphometry. Statistical comparisons between the groups were performed using the Mann-Whitney U-test. Significance was set at the p<0.05 level.

The study was approved by the local legal representative (LAGeSo: G 0206/08).

RESULTS:
Biomechanical testing demonstrated a significantly lower ultimate torque at failure (p<0.005) and torsional stiffness (p<0.003) in the macrophage depleted group (MAC) in comparison to the wildtype group (WT) at days 21 and 28 (FIG. 1).

µCT analysis showed that total callus volume (TV) and bone volume (BV) were significantly lower in the MAC at day 21 (14.5 (6.7/21.4) mm³ vs. (13.4 (4.3/25.6) mm³, p<0.001, [median (25/75 percentile)]) and 5.4 (3.8/8.4) mm³ vs. (10.4 (9.7/14.6) mm³, p<0.001). At day 28, no significant differences in TV and BV were found between both groups.

Bone mineral density (BMD) was significantly higher in the MAC group at day 21 (428 (383/558) vs. 365 (335/405), p=0.01) and day 28 (546 (470/596) vs. 321 (310/356), p=0.004). BV/TV was significantly higher in the MAC at day 28 (0.52 (0.44/0.56) vs. 0.29 (0.27/0.34), p<0.001).

Histomorphometrical analysis at day 7 showed a significantly lower fraction of cartilage (30 (26/38) % vs. 48 (41/57) %, p=0.003) in the MAC compared to the WT. At day 21, the differences were reversed with a significantly higher fraction of cartilage (18 (7/31) % vs. 2 (1.4) %, p=0.001) in the MAC (FIG. 2).

DISCUSSION:
In this study it was shown that selective depletion of macrophages results in a retarded fracture healing in mice characterized by the formation of a small, immature callus. The process of cartilage formation was not delayed, but there seemed to be a failure in endochondral ossification.

The results suggest that macrophages play a crucial role during the early phase of fracture healing. It is known that the initial fracture hematoma has osteogenic potential and contains macrophages (2). Macrophage function comprises the release of different growth factors and cytokines. On the one hand, a key function of macrophages might be the induction of osteoclasts via RANKL in combination with the release of other proinflammatory cytokines like IL-1 or TNFα. On the other hand, macrophages secrete TGFβ (anti-inflammatory cytokine). TGFβ also exerts anabolic functions on e.g. osteoblasts during fracture healing. In contrast to our depletion studies, experimental (over)activation of macrophages also resulted in reduced callus quality (1).

We therefore conclude that a balanced optimum of macrophage activation and function is needed to successfully initiate fracture healing. In this regard, both number and activity of macrophages might be essential factors for the fracture healing.

REFERENCES:

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