Fracture Healing is Accelerated in the Absence of the Adaptive Immune System

INTRODUCTION:
During physiologic homeostasis and in case of infection, the immune system and the skeleton are deeply interdependent on each other. Both systems closely interact, due to shared anatomical compartments, cell precursors and molecular mediators. However, the precise role of components of the immune system in fracture healing remains largely unknown. This project was aimed at studying the functional role of B- and T-cells as components of the adaptive immune system during fracture healing. The hypothesis was that fracture healing would be delayed in the absence of the adaptive immune system.

METHODS:
Complete absence of the adaptive immune system was modelled by using RAG1 (recombination activating gene 1) knockout mice lacking mature B and T lymphocytes. A standard closed femoral fracture was created in 8-10 weeks old wildtype (WT) and RAG1 knockout (RAG1−/−) mice. For μCT analysis and biomechanical testing, animals were sacrificed after 14, 21 and 28 days (N=8/group and time point). For histological analysis, sections of the callus region were stained with Movat Pentachrome and the callus tissue differentiation was evaluated by histomorphometry after 3, 7, 14, 21 and 28 days (N=8/group and time point) with an image analysis system (KS400, Zeiss, Eching, Germany). 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 higher torsional moment (Fig. 1) in the RAG1−/− in comparison to the WT group at day 14 (77 (69/84) % vs. 60 (50/63) %; [median (25/75 percentile)], p=0.005) and at day 21 (105 (95/122) % vs. 87 (77/88) %, p=0.009).

μCT evaluation of RAG1−/− specimens showed a decrease in the total volume of the callus (TV) from day 14 to day 21. In contrast, in the WT specimens the decrease of TV was seen one week later from day 21 to day 28. At day 21, TV was significantly lower in the RAG1−/− compared to the WT group (21 (16/33) mm³ vs. 36 (25/46) mm³, p=0.01). Histologically, the process of endochondral bone formation and the final remodeling of the bony callus appeared to be accelerated or advanced in the RAG1−/− compared to the WT mice. At day 3, hematoma was present in the fracture gap and proliferation of mesenchymal cells in the periosteal region could be observed in both groups. At day 7, a cartilaginous callus was seen in both groups, while woven bone formation and mineralization of the cartilaginous matrix was only detected in the RAG1−/− group. In the WT specimens, woven bone formation and mineralization of the cartilaginous callus started between day 7 and day 14, whereas in the RAG1−/− only a small number of chondrocytes remained at day 14. At day 21, the callus in both groups was mainly composed of newly formed bone filled with bone marrow. Bridging of the fracture was completed in the RAG1−/− at day 21 and in the WT at day 28. Histomorphometric analysis at day 7 showed a significantly higher fraction of bone (9 (5/16) % vs. 5 (2/7) %, p=0.021) in the callus of the RAG1−/− in comparison to the WT mice. In contrast, the fraction of cartilage in the RAG1−/− compared to the WT mice was significantly lower at days 7 and 14 (day 7: 36 (31/44) % vs. 48 (41/57) %, p=0.021; day 14: 5 (3/9) % vs. 13 (10/18) %, p=0.007).