Function of Neutrophil Granulocytes in Fracture Healing after Severe Trauma

Anna Kovtun¹, Stephanie Baur¹, Rebecca Wiegner¹, Markus Huber-Lang², Anita Ignatius¹.

¹Institute of Orthopaedic Research and Biomechanics, University of Ulm, Ulm, Germany, ²Department of Traumatology, Hand-, Plastic-, and Reconstructive Surgery, Center of Surgery, University of Ulm, Ulm, Germany.


Introduction: Fracture healing is a complex process, which is tightly regulated by the immune system (1). It is known that systemic inflammation can impair fracture healing, e.g. in case of multiple trauma (2, 3), the exact mechanisms being still poorly understood. Systemic posttraumatic inflammation is characterized by the rapid release of proinflammatory cytokines, and a strong activation of the complement system and inflammatory cells. Neutrophil granulocytes represent the first line of defense and are considered as one of the main triggers of systemic inflammation. Our group has previously observed increased numbers of neutrophils in the fracture callus of rats, which obtained an additional thoracic trauma (2). Therefore, we hypothesized that enhanced recruitment and activation of neutrophils are responsible for the disturbed fracture healing after severe trauma.

Methods: To study this hypothesis we used 12-week old C57BL/6J male mice, which obtained an osteotomy of the right femur stabilized by external fixator. Half of the mice additionally obtained a blunt chest trauma to achieve severe systemic inflammation (2). To study the role of neutrophils, mice received either anti-Gr1-antibody (Ly-6G Ab, clone 1A8) in order to deplete neutrophils, or control antibody (vehicle) i.p. 24h before surgery. Mice were sacrificed after 1, 3, 7, 14 and 21 days and fracture calli were analyzed biomechanically by a three point bending test and structurally by μCT measurements, as well as by histology and histomorphometry. The number of circulating neutrophils was assessed by counting of cells in blood smears, obtained before antibody injection, as well as immediately before surgery or organ harvesting. The animal experiment was performed according to international regulations for the care and use of laboratory animals and approved by the local ethical committee. Statistics: Student’s t-test. Level of significance: p<0.05.

Results: The Ly-6G antibody treatment resulted in a significant and robust decrease of circulating neutrophils down to 20% within 24h after injection, whereas control animals showed no changes of neutrophil numbers. The systemic depletion was stable up to 7 days. The surgery led to rapid recruitment of the neutrophils to the periosteal fracture area (Fig. 1) within first 24h, and elevated number of cells was observed up to day 3 after surgery. The antibody-treated group exhibited significantly less neutrophils in the fracture callus.

The thoracic trauma or Ly-6G antibody treatment led both to an impaired fracture healing, which was revealed by histomorphometry (Fig. 2) and biomechanical testing (Fig. 3). We observed a decreased bone content in fracture area and slightly, although not significantly, increased amount of cartilage and connective tissue. Moreover, the flexural rigidity of fractured bone was significantly lower in groups, which obtained anti-neutrophil antibody or thoracic trauma, or the combination of both. Comparing the biomechanical properties of fractured femur with an intact bone after application of thoracic trauma we observed significantly impaired bending stiffness: 51% and 26% respectively for vehicle-treated mice, 35% and 20% respectively for antibody-treated animals. μCT analysis showed predominantly unilateral callus formation in mice after thoracic trauma (Fig. 4), although bone mineral density, total callus volume or structural parameters did not vary strongly.
between all four groups.

Discussion: Firstly, our data verify the observations of Recknagel et al. demonstrating impaired fracture healing after severe trauma (2) and confirm the importance of inflammation in the regulation of fracture healing (3). Secondly, we found a decreased mechanical competence and bone quality of the callus after neutrophils depletion indicating impaired fracture healing. This is in contrast to previous studies in rats reporting improved bone healing after neutrophil depletion (4, 5). However, these authors performed depletion using an unspecific anti-neutrophil antiserum, which might induce additional inflammatory reactions. Our results suggest that a balanced number of neutrophils, which phagocytize destroyed tissue and secrete chemokines/cytokines, might be crucial to initiate downstream regenerative events. Thirdly, the depletion of neutrophils did not abolish the negative effects of the systemic inflammation induced by the thoracic trauma on fracture healing, possibly suggesting that neutrophils play a subordinate role in this scenario. However, a limitation of our study is, that fracture healing was severely affected in both the trauma and the trauma+depletion group hampering the detection of clear differences between the groups. Further studies with longer observation periods are ongoing in order to detect possible differences in the fracture healing outcome.

Significance: This study provides new insights into the pathophysiology of compromised bone healing in polytraumatic patients.

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