Extracellular Matrix Deposition in Fracture Healing Appears Orchestrated by Immune Cells

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Introduction: Fracture healing is one of the few regenerative processes in which tissue is restored without scar formation. Healing is a multistage process which appears to be highly regulated: It starts with a pro-inflammatory phase which rapidly shifts to anti-inflammatory signaling. Healing involves different cell types in a complex cascade of events associated with specific molecular signaling cues. Beside its relevance to basic research it becomes a clinical concern, once healing is disturbed. The interdependency of the immune and skeletal system has recently been proven to be essential for bone healing. In order to understand the role of adaptive immunity, specifically, the B and T cells with their cytokine signaling, we analyzed fracture repair in WT mice and mice without adaptive immunity. B cells are an essential source for the RANKL antagonist OPG and contribute to the balance in the osteoblast / osteoclast ratio (1). We therefore hypothesized that bone healing would be inferior in the absence of the adaptive immune system.

Methods: Closed fractures were performed in the left femur of 8 week old male recombinant activating gene 1 knockout (RAG1-/-) mice lacking mature T and B lymphocytes (and as a control in wild type mice of the same age) according to Bonnarens and Einhorn (2). Mice were evaluated at 3, 7, 14, 21, and 28 days, n=20/ time point and underwent histological and immune histological analyses to characterize the biological cascade of healing and multi photon microscopic (second harmonic generation signal), and scanning acoustic microscopic (50 MHz) analysis to characterize the extracellular matrix deposition in early bone healing. Animal experiments were performed in accordance with the national welfare guidelines and were approved by the local legal representative (G206/08, G282/07).

Results: The amount and distribution of collagen in the fracture callus showed distinct differences in animals without B and T cells. At day 7, the areas positive for collagen II were similar in RAG-/- and WT animals while areas positive for collagen I were distinctly reduced in RAG-/- animals. 14 days post fracture, a lower density of fibrillar collagen and an altered organization was visible under multi photon microscopy in the fracture callus perimeter of the RAG-/- animals. While higher numbers of osteoblasts were located in the collagen I positive areas of the fracture callus perimeter in the WT mice, the RAG-/- animals showed an erratic osteoblast distribution, including osteoblasts in tissues adjacent to the fracture callus. Material characterization by means of scanning acoustic microscopy confirmed an overall reduced stiffness and a shift in elasticity pattern in RAG-/- compared to WT animals 14 days post fracture.

Discussion: In previous studies we demonstrated that bone healing was superior by means of mechanical torsional strength and stiffness in the animals lacking adaptive immunity (1). A more detailed analysis however, revealed that their extracellular matrix organization and the process of matrix deposition seems to be substantially altered with the lack of mature B and T cells. Specifically the collagen I deposition appears altered, which results from the interplay of osteoblasts and B- and T-cells during bone formation. The area where woven bone (Col I) is synthesized becomes revascularized following the hypoxic chondrogenic stage (Coll II) of fracture healing. The T and B cells and their cytokines reappear in the WT animals, while their signaling is missing in the RAG-/-animals. At this healing stage differences in the collagen pattern were observed in this study. Thus, we conclude that adaptive immunity could have a guiding function that directly influences extracellular matrix deposition and thus determines the quality of tissue regeneration.

Significance: In the last years, the role of immune cells in bone healing gained more and more in importance. However, more basic understanding is needed in order to develop specific therapeutic treatment strategies for patients. This project gives a better insight in the interaction between immune and bone cells.

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