**Fracture Healing in Immune Deficient Nod-Scid IL2Rγnull Mice**

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**ABSTRACT**

**INTRODUCTION:**
Fracture healing starts with an initial inflammatory phase, which is characterized by the invasion of the fracture hematoma by immune cells such as neutrophils, macrophages and lymphocytes. These cells act against endogenous and exogenous pathogens, and are considered to be important for the initiation of the regenerative process. Several studies have investigated the role of distinct immune cells in fracture healing. It has been demonstrated that the over-activation of neutrophils delayed fracture healing, whereas their depletion improved the healing outcome (1, 2). The depletion of macrophages impaired fracture healing (3). In contrast, defects in B- and T-cells in RAG– mice led to an acceleration of fracture healing (4). The aim of this study was to investigate the bone phenotype and fracture in a mouse model with severe defects in the innate as well as the adaptive immune response. Due to the general importance of the immune system, we hypothesized that fracture healing would be considerably disturbed in these mice.

**METHODS:**

Male Nod-Scid IL2Rγnull mice (12 weeks) were used. The mice exhibit severe defects in innate and adaptive immunity. Mice lack mature T- and B-cells, mature macrophages and natural killer cells, and have deficiencies in cytokine-signalling (5). As immune competent controls BALB/cByJ wild type mice were used. For the characterisation of the bone, 10 male (non-fractured) mice of each genotype were sacrificed at age 12 weeks. The stiffness of the femora was investigated by a three-point-bending test. The lumbar spine and the femora were scanned by µCT and histologically evaluated. From the remaining long bones the marrow was harvested for the in vitro cellular characterisation of osteoblasts and osteoclasts. Osteoclasts were stained by tartrate-resistant acid phosphatase and resorption activity was measured by pit assay. Osteogenic differentiation was analysed by von Kossa and alkaline phosphatase staining. Semi-quantitative PCR was used to determine the expression of osteogenic marker genes. For the fracture healing study, 24 mice from each genotype received a femur ostectomy, which was stabilized by an external fixator. The mice were sacrificed after 21, 28, and 35 days. For evaluation of fracture healing the callus was tested mechanically by a three point bending test, structurally by µCT measurements as well as by histomorphometry. The animal experiment was performed according to international regulations for the care and use of laboratory animals, and approved by the local ethical committee (Regierungspräsidium Tübingen, Germany). Statistics: Student’s t-test; level of significance: p<0.05.

**RESULTS SECTION:**

The Nod-Scid IL2Rγnull mice femora revealed a greater cortical thickness (Ct.Th, +5%, p<0.01) and an increased cortical bone mineral density (Ct.BMD, +2%, p<0.01) compared to the BALB/cByJ wild type mice. The bending stiffness of the femora displayed a tendencial increase (+28%, p=0.12) (Fig.1). The trabecular bone in lumbar spine of the immune deficient mice exhibited a significantly lower bone volume/total volume (BV/TV, -50%, p<0.01) and a reduced trabecular number (Tb.N, -57%, p<0.01), but a greater trabecular thickness (Tb.Th, +3%, p<0.01) and BMD (Tb.BMD, +17%, p<0.01). Osteoclasts derived from the Nod-Scid IL2Rγnull mice showed a significantly decreased resorption activity in vitro (Fig. 2). Osteoblasts did not display any differences in the in vitro osteogenic differentiation potential between both genotypes. The healed femora of the Nod-Scid IL2Rγnull mice showed a significantly decreased bending stiffness after 21 (p=0.01) and 35 (p=0.02) days in comparison to the wild type mice (Fig. 3). The immune deficient mice had a significantly larger callus area and an approximately 10-fold and 5-fold increased amount of cartilage after 21 days and 28 days, respectively (Fig. 4). The amount of newly formed bone (BV/TV) was reduced in Nod-Scid IL2Rγnull mice by 26% after 21 (p=0.052), 32% after 28 (p=0.02), and 41% after 35 days (p<0.01) as measured by µCT, which was confirmed by histological analysis.

**DISCUSSION:**

The Nod-Scid IL2Rγnull mice with a severe deficiency in the innate and adaptive immune systems exhibited an altered bone phenotype in comparison to wild type mice. The mechanical properties of the bone were slightly improved due to an increased cortical thickness and mineral density. BMD and trabecular thickness were also slightly increased in trabecular bone. This might be due to a reduced osteoclast activity as measured in vitro by the pit assay, whereas osteoblast activity was unchanged. Because osteoclasts derive from the same haematopoietic origin as immune cells, their poorer activity could be explained by defects in the precursor cells in the Nod-Scid IL2Rγnull mice. Our results suggested that fracture healing was delayed in immune deficient mice. This was demonstrated by a significantly reduced bending stiffness and a larger callus with inferior quality. However, bone could still completely regenerate in Nod-Scid IL2Rγnull mice, indicating that the immune system might be important but is not essential for successful fracture healing.

**SIGNIFICANCE:**

The present study provides a new insight into the interaction of bone and the immune system in fracture healing, leading to a better understanding of disorders in fracture healing in immunologically disturbed patients.

**AKNOWLEDGEMENTS:**

This study was funded by the European Union 7th framework program REBORNE (HEALTH-2009-1-42, EC-Caner 241879). None of the authors have any conflicts of interest.

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Paper No. 0149 • ORS 2012 Annual Meeting