Autogenic Mesenchymal Stem/Stromal Cells (MSC) are Superior to Allogeneic MSC in Regeneration of Large Bone Defects

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Introduction: Mesenchymal stem/stromal cells (MSC) represent a promising tool for the treatment of large bone defects due to their high proliferation potential and low immunogenicity. While the beneficial effect of autogenic stem cells on bone regeneration is well accepted (Kon et al. 2000; Hernigou et al. 2005), the efficacy of allogeneic cells has been inadequately investigated; there are only a few experimental studies on sheep (Berner 2013, Niemeyer et al. 2010, Coathup et al. 2013) and they report inhomogeneous results. As the efficient use of allogeneic stem cells would overcome the major drawbacks of autologous cells, namely their limited availability and protracted expansion, further investigations on the usability of allogeneic MSC are necessary. For the first time, this study investigated the potential of locally implanted autogenic and allogeneic human MSC for regeneration of a large bone defect in an animal model that mimics the human immune system.

Methods: To generate mice with a humanized immune system, immunodeficient NOD/scid-IL2Rγc⁻/⁻ mice were irradiated with 250 cGy and treated with 1x10⁶ human hematopoietic stem cells within 24 h after irradiation (according to Shultz et al. 2005). After reconstitution of a human immune system, a 1 mm diaphyseal defect was created in the right femur and stabilized by an external fixator. The defect was filled with either hMSC from the HSC donor (“autogenic”) or from a different donor (“allogeneic”) in a type-1 collagen gel, cell-free collagen matrix or left empty. The animals were sacrificed at 3, 10, and 35 days after surgery. The healing outcome was assessed by micro-computed tomography (μCT) and histomorphometry. The presence of human cells was studied by staining for human β2-microglobulin. To further analyze the healing process, sections were stained for human CD8⁺ T cells, PECAM (CD31), Runx2, and Osteocalcin. To test for statistical significance, ANOVA with post-hoc Bonferroni correction was applied; p≤0.05 was assumed as significant.

The experimental procedures were performed according to national and international regulations for the care and use of laboratory animals and were approved by the local ethics committee (Regierungspräsidium Tübingen, Germany, Registration No. 1000).

Results: Staining for human β2-microglobulin confirmed the presence of transplanted autogenic and allogeneic hMSC in the defect region. The number of transplanted cells in the bone defects were similar in both groups at all investigated time-points. Newly formed bone did not stain positive for human β2-
microglobulin. μCT analysis performed 35 days after surgery revealed a significant increase in bone formation in mice treated with autogenic hMSC by 132 % compared to mice that received allogeneic hMSC, by 131 % compared to cell-free collagen, and by 205 % compared to mice with untreated defects (Fig. 1). Histomorphometric analysis corroborated these findings; a significant higher bone fraction was evident in the autogenic treated group compared to the other treatments. Staining for hCD8 three days after surgery revealed no positive cells in mice that received autogenic hMSC; in contrast, hCD8⁺ cells were found next to the collagen implant in mice treated with allogeneic hMSC. The same was true for day 10 after surgery (Fig. 2). Staining for PECAM revealed positively stained blood vessels in the surroundings of the collagen gel on day 10 in both mice treated with autogenic and allogeneic hMSC. There were no obvious differences evident. After 35 days however, more stained structures were evident in mice treated with autogenic hMSC compared to mice that were treated with allogeneic cells, thus indicating increased angiogenesis. Furthermore, the distribution of the vessel-like structures differed between the groups; in autogenic treated animals, they were found throughout the regenerate while they were mainly located in the surrounding of the collagen gel in mice treated with allogeneic cells. Staining for the early osteogenic marker Runx2 on day 10 displayed more positively stained cells in the defect region of mice that received autogenic hMSC. After 35 days, staining for osteocalcin revealed more stained regions in mice treated with autogenic hMSC. In mice treated with allogeneic hMSC, osteocalcin-positive stained cells were also found in the residues of the collagen gel.

**Discussion:** Our results indicate a higher efficiency of autogenic hMSC for bone regeneration compared to allogeneic hMSC. Treatment with autogenic hMSC led to a significantly higher bone formation in the defect region compared to empty defects or defects in mice that received allogeneic hMSC or cell-free collagen implants. We found no signs of inflammation or strong T cell invasion in animals that received hMSC; though, there were more CD8⁺ T cells evident in mice treated with allogeneic hMSC. There are hints that T cells and interferon-gamma might be associated with inhibition of bone formation in allogeneic settings (Dighe et al. 2013); however this must be investigated further. It is still unclear, how the implanted cells contribute to bone regeneration. We found more cells positive for PECAM and Runx2 and more regions stained positive for osteocalcin in animals treated with autogenic hMSC compared to animals that received allogeneic cells. Together with the absence of bone stained positive for human β2-microglobulin, this might indicate an indirect action of the implanted cells via trophic factors rather than a direct contribution.

In conclusion, our results demonstrate a superior efficacy of autogenic hMSC treatment compared to allogeneic hMSC in supporting bone healing.

**Significance:** Application of allogeneic MSC for bone defect repair would overcome the limited availability of autologous materials for bone defect repair. However, our study gives evidence, that application of allogeneic MSC for bone defect repair results in an inferior outcome to the application of autogenic cells.
Fig. 1: μCT analysis of the healing outcome 35 days after surgery

Fig. 2: Staining for human CD8 on day 3 (A, B) and 10 (C, D) after implantation of allogeneic (A, C) and autogenic (B, D) MSC.