IMPAIRED INTRAMEMBRANOUS BONE FORMATION FOLLOWING BONE MARROW ABLATION
IN THE ABSENCE OF TNF alpha SIGNALING

*Cho, T; **Einhorn, T; +**Gerstenfeld, L

*Musculoskeletal Research Laboratory, Department of Orthopaedic Surgery, Boston University School of Medicine/Department of Orthopaedic Surgery, Seoul National University College of Medicine, Seoul, Korea. +**Musculoskeletal Research Laboratory, Department of Orthopaedic Surgery, Boston University School of Medicine, Boston, MA. 715 Albany R205, Boston, MA 02118, 617-414-1660, Fax: 617-414-1661, lgersten@bu.edu

Introduction
The pro-inflammatory cytokine tumor necrosis factor-alpha (TNFα) is known to mediate osteoclast activity and bone resorption, however, its role in bone formation and osteogenesis has not been fully elucidated. Previous in-vitro data suggested that TNFα has inhibitory effects on osteoblastic lineage cells(1,2), and we have demonstrated an inhibition of subperiosteal new bone formation during fracture healing in TNFα receptor(TNFR)-deficient mice (p55−/−p75−/−)(3). However, both endochondral and intramembranous ossification are involved in fracture healing, it is not clear whether TNFα signals act directly upon the osteoblastic recruitment and differentiation, or indirectly by modulating chondrocyte differentiation and maturation. Bone marrow ablation is a unique model in which robust intramembranous bone formation is induced without an endochondral component(4). In order to investigate the direct in-vivo effect of TNFα signaling on the osteoblastic lineage, we have compared the response of TNFR deficient mice to bone marrow ablation to that of wild type mice.

Methods
All animal protocol received prior approval by the institutional animal care and use committee, and conformed to the federal guideline for the care and use of laboratory animals. Tibial bone marrow was ablated in the left tibiae of wild type C57BL/6J mice and TNFR-deficient mice. This was accomplished by inserting and rotating the inner and outer needles of the 25G and 23G spinal needles through a window just above the tibial tuberosity, followed by irrigation with saline. Animals were euthanized by cervical dislocation on days 0 (unoperated control), 1, 3, 5, 7, 14, 21 and 28 postoperatively. For the histologic examination, the tibiae were fixed, decalcified, embedded in paraffin, and stained with hematoxylin and eosin using standard techniques. Total RNA was isolated from tibial bone tissue excised from just distal to the proximal tibial epiphysial plate to the tibiofibular junction. Ribonuclease protection assay (RiboQuantTM, PharMingen, San Diego, CA) was performed demonstrating temporal pattern of induction of osseous matrix gene expression (wt: wild type C57 mice, ko: TNFR-deficient mice)

Results
Bone marrow ablation induced massive new bone formation within the first week of healing filling the marrow cavity with newly formed trabeculae. There was then a gradual restoration of normal hematopoietic elements and the marrow space architecture by day 28. At day 3, immature osteoblasts could be seen in the marrow cavities of wild type mice, while the knock-out mice had only granulation tissue along with residual hematoma in the marrow cavities. Type I collagen and osteocalcin mRNA expression was elevated 15 and 51 fold, respectively, above the unoperated control mice levels in the wild type mice, while those in the knock-out mice were only 7 and 20 fold, respectively, compared to unoperated knock-out mice. Expression of type II collagen and osteocalcin while those in the knock-out mice were only 7 and 20 fold, respectively, above the unoperated control mice levels in the wild type mice. The expression patterns of interleukin-1 receptor type 1 and macrophage inhibitory factor showed no significant differences between the wild type and knock-out mice.

Discussion
Comparison of bone marrow ablation healing in wild type vs. TNFR-deficient mice demonstrates that TNFα signalling is an important component of intramembranous bone formation. The new bone formation in TNFR-deficient mice was found to be delayed at day 3 by histologic examination, and the induction of osseous matrix gene expression was reduced compared to that of wild type mice. There also appeared to be a compensatory overexpression of TNFα and related cytokines during the healing process. These data suggest that the pro-inflammatory cytokine TNFα may have a role in the recruitment of mesenchymal cells into the osteoblastic lineage or on the differentiation of the osteoprogenitor cells into osteoblasts in response to injury. Further studies including in-vitro experiments using wild type and mutant cells may be necessary to delineate the direct effect of TNFα on the osteoblastic lineage cells.

References