INTRODUCTION:
Fractures have the potential to heal through two distinct mechanisms: intramembranous and endochondral ossification. When stabilized, murine tibial fractures heal through intramembranous ossification, and when left non-stabilized, murine tibial fractures heal through endochondral ossification. The inflammatory phase of fracture healing corresponds to the timing of cell fate decisions at the fracture site and may be partially responsible for directing modes of fracture healing. In this study, we explore the role of Interleukin-1β (IL1β), a pro-inflammatory cytokine, in the modulation of fracture repair in a murine tibial fracture model.

METHODS:

In vitro: MC3T3-E1 cells and primary murine mesenchymal stem cells were cultured in DMEM +/- IL1β (10ng/mL) for proliferation studies and in osteogenesis medium +/- IL1β (10ng/mL) for differentiation studies. Proliferation and differentiation of primary murine mesenchymal stem cells and MC3T3-E1 cells were assessed using BrDU and Alizarin Red staining, respectively. BrDU staining was quantified using stereochemistry.

In vivo: All experimental procedures were approved by the UCSF Institutional Animal Care and Use Committee. Tibial fractures were created by three point bending in 10-14 week old male mice (C57BL/6J or IL1R1−/−). Fractures in IL1R1-null mice were both stabilized and not stabilized depending on experimental arm. Animals were allowed to ambulate freely. All fractures in wild-type animals were not stabilized. Animals received either one injection of IL1β (40 ng) at the site of fracture at 24 hours after injury (1x study group) or this injection plus injections at 48 and 72 hours after injury (10 ng each) (3x study group). Fracture calluses were collected at 7, 10, 14, and 28 days after fracture, fixed in 4% paraformaldehyde, decalcified in 19% EDTA over 10-14 days, embedded in paraffin and sectioned at 10µm intervals. Bone and cartilage were visualized by Milligan’s Trichrome and Safranin O/Fast Green staining, respectively. The total volume of the fracture callus, new bone, and new cartilage was assessed using histomorphometry.

RESULTS:
IL1β promoted the proliferation of MC3T3-E1 cells as compared to control (treated n=4; control n=5; p<0.05, Fig. 1A) and inhibited the proliferation of primary murine mesenchymal stem cells as compared to control (treated n=5; control n=4; p<0.01, Fig. 1B). IL1β promoted the differentiation of MC3T3-E1 cells as compared to control (4 wells / 5 wells, Fig. 1C) and inhibited the differentiation of primary murine mesenchymal stem cells (Fig. 1E) as compared to control (5 wells / 5 wells; Fig. 1D). Fractures in IL1R1−/− mice exhibited no alteration in healing at days 7, 10, 14, and 28 post-injury. Histomorphometry at days 10 and 14 in non-stabilized fractures demonstrated no difference between IL1R1−/− mice and controls (data not shown). Histomorphometry at day 10 in stabilized fractures demonstrated no difference between IL1R1−/− mice and controls (data not shown). Mice receiving 1 or 3 doses of IL1β exhibited no difference in fracture repair as compared to controls. We detected no significant difference in total callus size, cartilage volume, or bone volume at days 10 and 14 post-injury in treated animals (Fig. 1F,G,H).

DISCUSSION:
IL1β significantly promotes the proliferation and differentiation of MC3T3-E1 cells and significantly inhibits the proliferation and differentiation of primary murine mesenchymal stem cells in vitro. However, through both loss- and gain-of-function experiments, we were unable to demonstrate a role for IL1β during the natural progression of fracture repair in vivo. This may be due to the compensatory actions of other cytokines or a decreased reliance on IL1β during bone healing. Further work is required to distinguish these possibilities.

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