Femoral Marrow Ablation Decreases Remote Osteogenic Potential of Bone Marrow in a Rat Model
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INTRODUCTION:
Cells with osteogenic potential in the circulation have the ability to home to the site of fracture healing. However, the site of origin of these circulating cells is unclear. A recent study found that bone marrow progenitor cells can migrate into the circulation and improve fracture healing, implying that one source of migrating osteogenic cells is intact marrow remote from the site of injury. Colony forming unit-fibroblasts (CFU-F) are derived from osteoprogenitor cells and are used to assess the osteogenic potential of bone marrow stromal cells to form bone. Ablation of bone marrow is a common model to study intramembranous bone regeneration. In the present study, we evaluated the osteogenic potential of bone marrow at a remote site, the contralateral femur, after performing a marrow ablation in the index femur. We hypothesized that if progenitor cells are mobilized from intact, remote marrow in animals with a localized challenge known to induce bone regeneration, then the osteogenic potential of the intact, remote site will be depressed.

METHODS:
Animal Model: In an IACUC approved protocol, 4 Sprague-Dawley male rats (300-325g) received unilateral marrow ablation of the left femoral canal. After exposing the knee joint, the surface of the intracondylar notch was breached and the marrow contents were disrupted with a 1.5 mm diameter drill bit and the femoral cavity was flushed with sterile saline. Soft tissues were closed with sutures. Another 4 rats (300-325g) remained intact with no surgery and served as the control.
Cell Culture: 6 weeks after surgery, all the animals were sacrificed. Both femurs of the ablation group and the left femur of the group not receiving surgery were harvested. The proximal and distal ends of the bones were cut off at the level of epiphysis. The bone marrow was flushed out and suspended into 10ml αMEM containing 10% FBS. After counting the nucleated cell number in the suspension, the cells were seeded in triplicate at a density of 10^5/cm² in 6-well plates. The cells were cultured for 5 days, and then changed into αMEM media with 10% FBS, 50 μg/ml ascorbic acid and 1mM dexamethasone. Media was changed twice weekly thereafter. The cultures were stopped and fixed with alcohol at the end of 14 days.

CFU-F Assay: The fixed CFU-F cell cultures were stained with Fast BB Blue for alkaline phosphate (ALP) to identify ALP positive (+) CFU-F colonies. The plates were scanned, and the digital images were analyzed by NIH Image J software. The area of ALP (+) CFU-F colonies was measured at a tested grayscale threshold 80. Then, the same plates were stained with Toluidine Blue and scanned to determine the total CFU-F area. Osteogenic efficiency was calculated by dividing the area of ALP (+) CFU-F by the area of total CFU-F.

Statistical Analysis: Group comparisons were made with the Mann-Whitney test (p<0.05 as significant).

RESULTS:
The control site had the most ALP (+) CFU-F colonies, followed by remote site and ablation site (Fig. 1). Compared to the control femurs, the remote site femurs in the ablation group had a 57.5% decreased ALP (+) CFU-F area (Fig. 2, p = 0.043). In the ablation group, the femurs receiving the ablation had an even lower ALP (+) CFU-F area compared to the control, a 74.9% decrease (Fig. 2, p = 0.021). The osteogenic efficiency followed the same pattern, with an 19.6% depression in the remote site femurs compared to the control femurs (p = 0.021) (Fig. 3). Total CFU-F areas were not different in any of the femurs (data not shown).

DISCUSSION AND CONCLUSION:
The present study demonstrates that the area of ALP (+) CFU-F and the osteogenic efficiency of the remote site bone marrow were depressed 6 weeks after marrow ablation in the femur. Since the same number of nucleated cells were seeded and total CFU-F was equivalent in the groups, these findings indicate that the ablation injury reduces the ability of CFU-F from the remote site to become osteogenic (i.e. become ALP(+)). Because the bone marrow of the remote site was not directly disturbed, a possible explanation for this remote site reduction in osteogenic potential could be depletion of the osteoprogenitor pool at the remote marrow site. Thus, our data support the hypothesis that ablation of the femoral marrow cavity affects the osteogenic potential of bone marrow at a remote site (the contralateral femur in this case). The finding may help clarify the source of cells that migrate to sites of bone regeneration.

REFERENCES:

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