Insulin-like Growth Factor-1 Binding Proteins Alter Osteoclastogenesis

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Introduction:
Aging is accompanied by a shift in bone marrow (BM) cell fate and greater osteoclastogenesis [1]. The production of IGF-1 and the binding proteins (BP) that comprise the IGF-1 complexes are also affected by aging [2,3]. To determine the auto/paracrine relationship between these constituents and osteoclastogenesis in BM we evaluated the osteoclastogenic potential of marrow-derived mesenchymal stromal cells (MSCs) from mice with IGF-1 deficiency due to knockdown of IGF-1 production by the liver or knockout of the binding proteins (BP) that stabilize IGF-1.

Methods:
We employed 10-16 week old, male, liver-specific IGF-1 deficient (LID), IGFBP-3 knockout (BP3KO) and acid-labile subunit knockout (ALSKO) mice and assessed BM cell differentiation in vitro and BM gene expression in vivo (n=5 mice per strain). All mouse strains were backcrossed to a C57BL/6J background. IACUC approval was obtained.

BM cells were flushed from femurs immediately after sacrifice and collected in α-MEM culture. Cells were then either cultured or sorted (FACS). For sorting cells were preincubated with rat anti-mouse CD16/CD32 (BD Biosciences) at 4°C to block Fc receptors. Cells were then incubated with fluorescently labeled antibodies: R-Phycoerythrin (PE)-c-Fms/CSF-1R (Santa Cruz Biotechnology) and PE-Cy7-conjugated rat anti-mouse CD45R/B220 (BD Biosciences). The cells were washed and then resuspended in staining buffer (Streptavidin-FITC, BD Biosciences). The cells were incubated for 20 min, washed, and resuspended in PBS containing 1% paraformaldehyde. Cell acquisition was performed in a Becton Dickinson FACScan, and a minimum of 10,000 events was acquired for each test. Data was analyzed with FlowJo software (v7.2).

Osteoclastogenesis was performed with MSCs cultured in αMEM supplemented with 10% heat-inactivated FBS with β-glycerolphosphate and ascorbic acid. Cellular alkaline phosphatase activity was determined using a para-nitrophenol phosphate based colorimetric assay with α-MEM culture. Cells were then either cultured or sorted (FACS). For co-culture experiments, primary osteoblast (OB) cultures were established as above from each mouse strain. Cells were harvested after 14 days and re-seeded in αMEM. Non-adherent cells from Control mice only were co-cultured with each OB culture for 7 days. Co-cultures were then fixed, stained and TRAP+ cells counted.

BM cells were isolated and cultured as above for 18 h. Non-adherent cells were re-plated in αMEM medium (10% FBS with RANKL and M-CSF) in an incubator for 4 - 7 days. Cells were then fixed and stained for tartrate-resistant acid phosphatase (TRAP) activity, and TRAP+ cells containing ≥3 nuclei were counted as mature osteoclasts (OC). IGF-1, ALS, or IGFBP-3 were added in separate rescue experiments.

For co-culture experiments, osteoclasts were cultured in α-MEM medium supplemented with 10% heat-inactivated FBS with β-glycerolphosphate and ascorbic acid. Cellular alkaline phosphatase activity was determined using a para-nitrophenol phosphate based colorimetric assay with α-MEM culture. Cells were then either cultured or sorted (FACS). For co-culture experiments, primary osteoblast (OB) cultures were established as above from each mouse strain. Cells were harvested after 14 days and re-seeded in α-MEM. Non-adherent cells from Control mice only were co-cultured with each OB culture for 7 days. Co-cultures were then fixed, stained and TRAP+ cells counted.

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Discussion:
Our results indicate that impairment of IGF-1 complex formation in BM alters cell fate by modifying osteoclastogenesis. Importantly, the determination of BM cellular populations that express the mouse colony stimulating factor-1 (M-CSF1) receptor (i.e., c-fms), which is a critical factor for osteoclastogenesis, and B220+ B cells, which express membrane-bound and secreted RANKL, suggest an in vivo role for both ALS and IGFBP-3.

The inability of IGF-1 to rescue osteoclastogenesis in ALSKO combined with the data indicating that the als gene need not be active for OC formation to be rescued with ALS protein suggest a direct role for ALS in determining cell fate in the BM micro-environment.

Our study further reinforces the tenet that there is a strong interaction among hematopoietic and mesenchymal stem cells in BM, and raises the possibility that the IGF-1 complex constituents IGFBP-3 and ALS play a more active role in the assignment of cell fate in BM than previously thought.

References:

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