INTRODUCTION: Human skeletal aging begins in the third decade and is characterized as a gradual loss of bone mass due to excess osteoclastic bone resorption not balanced by bone formation. Osteoclasts are large, multinucleated cells derived from hematopoietic stem cells present in bone marrow. Osteoclast differentiation is regulated locally by marrow stromal cells (MSCs, a.k.a. mesenchymal stem cells) and osteoclasts through three major products: macrophage-colony stimulating factor (M-CSF), receptor activator of NF-kB ligand (RANKL), and osteoprotegerin (OPG). M-CSF supports proliferation of osteoclast progenitors by binding to its cell surface receptor c-fms on osteoclast progenitors; it also upregulates the receptor, RANK. RANKL promotes osteoclast differentiation by activating the cell surface receptor, RANK. OPG is a soluble decoy receptor of RANKL and thus inhibits osteoclastogenesis. We used human marrow to test the hypothesis that there are age-dependent changes in the expression of osteoclast differentiation factors and receptors in marrow cells.

METHODS: Bone marrow samples were obtained with IRB approval from tissue discarded during total hip replacement for advanced, non-inflammatory osteoarthritis. Low-density, undifferentiated bone marrow cells (BMCs) from young (<50 years) and older (>55) subjects were isolated by density centrifugation with Ficoll/Histopaque 1077. First, cells (BMCs) from young (<50 years) and older (>55) subjects were removed after 24 hrs for expansion of adherent MSCs. Second, aliquots of 3x10^7 BMCs were seeded into 60-mm tissue culture dishes with phenol red-free α-MEM medium, 10% Fetal Bovine Serum-heat inactivated, 100 U/ml penicillin, 0.1 μM streptomycin, and 0.5 μM fungizone. After 14 d, they were stained for tartrate-resistant acid phosphatase (TRAP)-positive multinucleated osteoclasts or prepared for gene expression analysis. Third, aliquots of BMCs were seeded and non-adherent cells were removed after 24 hrs for expansion of adherent MSCs.

RESULTS: First, constitutive levels of gene expression of the receptors c-fms and RANK were measured in freshly isolated BMCs from subjects with a wide range of age. Both c-fms (r=0.61, p=0.006) and RANK expression (Spearman r=0.59, p=0.008) were significantly increased with age (n=19). There was a strong positive correlation between c-fms and RANK expression in this series (r=0.85, p=0.0001). We also found an age-related increase in constitutive expression of PPARγ (r=0.550, p=0.015).

Second, BMCs were cultured to reveal the effect of age on spontaneous formation of osteoclasts. After 14 d, both the number of generated osteoclasts (r=0.89, p=0.007) and their expression of TRAP (r=0.75, p=0.037) were increased with age (n=8). In a larger series of n=15, expression was likewise correlated with age (r=0.60, p=0.019), with a 1.4-fold higher expression in cultures from older subjects (0.97 ± 0.27) than for younger subjects (0.69 ± 0.21, p=0.014).

Third, the direct role of MSCs in mediating the age-related increase in osteoclastogenesis was assessed by determining the effects of age on their expression of the regulatory factors RANKL, M-CSF, and OPG (Graphs). In cultures of adherent MSCs (36-87 years, n=23), the constitutive expression of RANKL was increased with age (r=0.41, p=0.049). Levels of OPG were inversely correlated with age (r=-0.43, p=0.039). There was an age-related increase in the ratio of RANKL/OPG (r=0.56, p=0.005). The levels of M-CSF did not appear to change with age.

Finally, the effects of in vitro addition of recombinant RANKL or OPG on osteoclast differentiation were determined. Addition of RANKL stimulated osteoclast differentiation in BMCs from young subjects by 20-29% (p<0.05); addition of 10 ng/mL OPG inhibited osteoclast differentiation in BMCs from elders (p=0.004).

DISCUSSION: The results indicate an age-dependent increase in osteoclast differentiation in vitro. The addition of exogenous osteoclastogenic factors was not required for osteoclast formation in the BMC cultures in this study because the high density seeding and presence of MSCs (and their products) facilitated in vitro osteoclast differentiation.

These data indicate an age-related increase in in vitro osteoclastogenesis that is associated with both an increase in expression of receptors c-fms and RANK in osteoclast progenitors, and, in the supporting MSCs, an increase in RANKL expression and a decrease in OPG. Age-related increases in the expression of c-fms and RANK may indicate an age-dependent increase in the number osteoclast progenitors or the number of receptors per cell, which would increase their response to M-CSF and RANKL. We also evaluated PPARγ because of a report that it and its agonists promote osteoclast differentiation in mice [1]; there was an age-related upregulation of PPARγ in human BMCs that may mediate signaling in osteoclast progenitors.

Human MSCs showed an age-related increase in RANKL gene expression and a decrease in OPG expression. The ratio of RANKL to OPG may be a key determinant for the levels of osteoclastogenesis and bone resorption. Consistent with this, we recently reported that addition of alendronate to BMCs from elders downregulated RANKL and upregulated OPG, with an average 50% decrease in RANKL/OPG ratio [2]. Further, BMCs from subjects being treated with alendronate had lower RANKL/OPG (0.65±0.35) than age-matched controls (1.28 ± 0.53, p=0.031) and generated 21% of the osteoclasts in control cultures (p=0.015) [2].

The inhibition of osteoclastogenesis by recombinant OPG in BMC cultures from older subjects to approximate the level of TRAP expression in younger subjects suggests in vitro rejuvenation of old marrow cells by this factor.

SIGNIFICANCE: There is an age-related increase in human osteoclast differentiation that may be due to intrinsic changes in osteoclast regulatory factors and their signaling; there was an age-dependent increase in expression of the cell surface receptors c-fms and RANK in osteoclast progenitor cells, as well as an increase in constitutive expression of RANKL and decrease in OPG by MSCs. The RANKL/OPG ratio in MSCs may be an important regulator of osteoclast differentiation and a target for rejuvenation.

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