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COMMENTARIES

Osteoblasts May Take a Road Well-Traveled

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Commentary on: Eghbali-Fatourechi GZ, Lamsam J, Fraser D, Nagel D, Riggs BL, Khosla S. Circulating osteoblast-lineage cells in humans. *N Engl J Med.* 2005 May 12;352(19):1959-66.

Despite the tightly-controlled processes that link the activity of bone-resorbing osteoclasts to bone-forming osteoblasts, osteoclasts and osteoblasts have different origins. As early as the 1970s, particular forms of osteopetrosis were found to be cured by circulating osteoclast precursors in peripheral blood (1), and evidence that osteoclasts originate from hematopoietic stem cells is now undisputed (2). In contrast, osteoblasts originate from stromal stem cells, now usually called mesenchymal stem cells, in bone marrow (3). The idea that osteoblasts or their precursors might traffic via the same blood route as osteoclast precursors has appeared more recently and has met with some skepticism. A recent paper by Eghbali-Fatourechi *et al.* (4), suggesting not only that a physiologically significant number of osteoblastic cells circulate, but also that the number increases at times when increased bone remodeling is required, such as during growth spurts in adolescence and bone repair after injury, will undoubtedly add to the debate.

Previous support for the existence of circulating mesenchymal precursor cells with capacity to contribute to bone remodeling comes from several groups and models. For example, Zvaifler *et al.* (5), reported evidence of cells in human peripheral blood, with multipotentiality for mesenchymal lineages, including osteogenic cells, *in vitro*. This is consistent with data from Kuznetsov *et al.* (6) who found that a small population of cells within the peripheral blood of humans, mice, rabbits and guinea pigs adhered to tissue culture plates *in vitro*, a property of stromal elements. These cells also formed colonies with osteogenic capacity in ceramic-based implants placed subcutis in immunocompromised mice. Notably, the frequency of circulating osteogenic precursors measured by Kuznetsov and colleagues was extremely low, raising questions about their physiological significance. Thus, the recent findings of Eghbali-Fatourechi *et al*., in which as many as 5% of circulating cells are designated as potential osteoprogenitors, are striking (4). The higher frequency may reflect a key experimental difference: Eghbali-Fatourechi *et al*. assayed nonadherent cells directly from the peripheral blood, rather than the subpopulation of cells that are tissue culture plastic-adherent. Osteoprogenitors within a nonadherent fraction of bone marrow were proposed previously by Long and colleagues (7) who found cells with capacity to form colonies in semi-solid medium, express osteoblastic markers and deposit mineralized matrix within a population of low-density, nonadherent, marrow cells. These cells expressed little or no My10 antigen, an antigen found on most hematopoietic progenitors. Scutt's group also found that with time some initially nonadherent bone marrow cells adhere and form fibroblast colony-forming units *in vitro* (8). More recently, Horwitz and colleagues showed that plastic-nonadherent bone marrow cells have more than 10 times the bone-repopulating activity of plastic-adherent bone marrow cells in lethally irradiated mice (9).

Nevertheless, there remains a puzzling discrepancy between estimates of osteogenic precursors in different studies. Specifically, very high precursor frequencies have been reported in human peripheral blood by Eghbali-Fatourechi and colleagues

(4), while much lower frequencies have been measured by others in peripheral blood of multiple species (6) and in bone marrow of mouse and rat. Estimates of precursor frequencies in total unfractionated (adherent and nonadherent fractions) mouse and rat bone marrow populations are ~0.0005% (0.005% after 5-fluorouracil treatment) (10) and ~0.001-0.003% (11) respectively. Medium supplements (serum source [12]; hormones such as glucocorticoids [11]) and the presence of accessory cell lineages (11,13,14) are known to affect the frequency of osteoprogenitors detected in culture assays. Eghbali-Fatourechi *et al*. used positive immunoselection (FACS sorting) for cell-surface expression of osteocalcin (OCN) to obtain a population of cells enriched for osteoblast-associated marker expression and for ability to form a mineralizing matrix *in vitro* and after subcutaneous injection in immunocompromised mice. The authors did not, however, determine what proportion of the entire OCN-positive population contributed to the osteogenic endpoints measured or measure the frequency of specific clonogenic osteogenic cells. Such studies will be required not only to place these observations more fully into the context of the field but also to dissect the basis of some unexpected results. For example, although percentage of OCN-positive cells correlated with bone marker expression and bone formation, the percentage of alkaline phosphatase (ALP)-positive cells did not correlate in all cases, even though the ALP-positive population was also reported to be enriched for expression of osteoblast-associated genes and capacity for bone nodule formation (data not shown). As the authors recognize, this suggests that cells positive for OCN and ALP may represent different subpopulations, which will require further characterization of the OCN-positive cells to understand their relationship to other clonogenic osteogenic cells and to accessory populations. In this regard, Thiede and colleagues reported in the early 1990's that OCN is also expressed by bone marrow megakaryocytes and peripheral blood platelets (15). However, the FACS gates set by Eghbali-Fatourechi *et al*. presumably excluded both enucleated OCN-positive platelets and polyploidal megakaryocytes, accessory cells that can influence bone formation by osteogenic precursors in marrow (13). Osteoblasts have been reported to express a G-protein-coupled OCN receptor (16), but an OCN fragment was also shown recently to stimulate maturation of osteoclast precursors present in the Mac1-positive cFms-positive population of marrow cells (17). If this latter response is mediated by a cell surface receptor, an OCN-positive population could conceivably include preosteoclasts or other cells with capacity to bind secreted full-length or proteolytically-derived fragments of OCN (17). Such cells would be expected to be increased in serum under the same high bone remodeling conditions that correlate with the increased frequency of OCN-positive osteogenic precursors, as seen by the authors. Conversely, we will need to establish how OCN-positive osteogenic cells relate to a total osteoprogenitor pool when the fractionation is based on the presence of a marker usually thought to indicate a mature post-proliferative osteoblast state, but also heterogeneously expressed by mature osteoblasts (18,19).

If a sizeable pool of circulating osteoblastic cells does exist, where do they come from and what are their functions? The authors hypothesize that the frequency and changes in frequency of circulating osteoblasts with age and in states of high bone remodeling reflect their regulation by particular bone-responsive hormones and growth factors, e.g., IGF-I and IGFBP3, and their active participation in bone formation. In other words, they propose that circulating OCN-positive cells are part of a previously unrecognized mechanism whereby osteoprogenitors from the bone marrow, or possibly other sources, are transported to sites of remodeling as needed. Much more work will be required to address whether and how the birth, life (differentiation/maturation) and/or death of putative circulating osteoblasts are regulated by a variety of factors. However, Hauge *et al*. (20) reported a structure of flat lining cells that separate the bone marrow compartment, the presumed major source of osteoprogenitors, from the bone remodeling compartment. Based on this, Eghbali-

Fatourechi and colleagues propose that marrow osteoblastic cells use the sinusoids to reach remodeling compartments for bone formation and to reach the circulation for participation in bone formation at other skeletal sites, including at fracture healing sites distant from red marrow. Although interesting, as yet there is no evidence that the flattened lining cells described by Hauge *et al*. form an impenetrable barrier to bone marrow cells.

It is also worth considering that hematopoeitic stem cells from the bone marrow and bone marrow niche have access to the circulation, and the bloodstream has been found to be a viable route of delivering at least some marrow-derived cells to the bone (21,22). Thus, marrow mesenchymal stem cells or their progeny, using as yet molecularly and biochemically uncharacterized transmigration and homing strategies, may have access directly from marrow, especially if mobilized by specific recruitment signal(s). The relationship to a putative vascular pericyte pool of osteogenic cells (23) also needs to be addressed using additional immunological (e.g., cell surface markers) and functional tools. A corollary issue is whether the increase in number of circulating OCN-positive cells detected by the authors after injury to bone is a "passive" process, i.e., is due to their release from the marrow into the bloodstream following bone breakage or remodeling. Consistent with the authors' views, it may be difficult to reconcile the timeframe (analysis of blood samples acquired 20 days after bone trauma) for measurement of circulating osteoblasts with release from the bone marrow via a bone break. Yet, without a clear understanding of the relationship of OCN-positive to ALP-positive circulating cells and other potential progenitor pools, including their regulation and ability to mature in the circulation, the issue remains open.

The questions raised by the work of Eghbali-Fatourechi *et al*. will undoubtedly accelerate interest, debate and use of innovative genetic and molecular strategies to re-address the ontogeny of osteoblasts. One such recent study with retrovirally-marked transplantable marrow cells from the plastic-nonadherent population that were able to generate both functional osteoblasts/osteocytes and hematopoietic cells supports the idea that bone marrow contains a primitive cell able to generate both the hematopoietic and osteoblastic lineages (9). The latter result, together with work by Eghbali-Fatourechi *et al*. and others, is clearly provocative and begs for additional studies in this area as the implications for both basic biology and possible clinical applications are paradigm-shifting.

References

1. Walker DG. Osteopetrosis cured by temporary parabiosis. *Science.* 1973 May 25;180(88):875.

2. Teitelbaum SL, Ross FP. Genetic regulation of osteoclast development and function. *Nat Rev Genet.* 2003 Aug;4(8):638-49.

3. Aubin JE, Triffitt J. Mesenchymal stem cells and the osteoblast lineage. 2002. In: *Principles of Bone Biology*, ed. J.P. Bilezikian, L.G. Raisz,G.A. Rodan, 59-81. New York, NY: Academic Press; 2002.

4. Eghbali-Fatourechi GZ, Lamsam J, Fraser D, Nagel D, Riggs BL, Khosla S. Circulating osteoblast-lineage cells in humans. *N Engl J Med.* 2005 May 12;352(19):1959-66.

5. Zvaifler NJ, Marinova-Mutafchieva L, Adams G, Edwards CJ, Moss J, Burger JA, Maini RN. Mesenchymal precursor cells in the blood of normal individuals. *Arthritis Res.* 2000;2(6):477-88.

6. Kuznetsov SA, Mankani MH, Gronthos S, Satomura K, Bianco P, Robey PG. Circulating skeletal stem cells. *J Cell Biol.* 2001 May 28;153(5):1133-40.

7. Long MW, Williams JL, Mann KG. Expression of human bone-related proteins in the hematopoietic microenvironment. *J Clin Invest.* 1990 Nov;86(5):1387-95.

8. Scutt A, Bertram P. Bone marrow cells are targets for the anabolic actions of prostaglandin E2 on bone: induction of a transition from nonadherent to adherent osteoblast precursors. *J Bone Miner Res.* 1995 Mar;10(3):474-87.

9. Dominici M, Pritchard C, Garlits JE, Hofmann TJ, Persons DA, Horwitz EM. Hematopoietic cells and osteoblasts are derived from a common marrow progenitor after bone marrow transplantation. *Proc Natl Acad Sci U S A.* 2004 Aug 10;101(32):11761-6.

10. Falla N, Van Vlasselaer, Bierkens J, Borremans B, Schoeters G, Van Gorp U. Characterization of a 5-fluorouracil-enriched osteoprogenitor population of the murine bone marrow. *Blood.* 1993 Dec 15;82(12):3580-91.

11. Aubin JE. Osteoprogenitor cell frequency in rat bone marrow stromal populations: role for heterotypic cell-cell interactions in osteoblast differentiation. *J Cell Biochem.* 1999 Mar 1;72(3):396-410.

12. Kuznetsov SA, Mankani MH, Robey PG. Effect of serum on human bone marrow stromal cells: ex vivo expansion and in vivo bone formation. *Transplantation.* 2000 Dec 27;70(12):1780-7.

13. Friedenstein AJ, Latzinik NV, Gorskaya YuF, Luria EA, Moskvina IL. Bone marrow stromal colony formation requires stimulation by haemopoietic cells. *Bone Miner.* 1992 Sep;18(3):199-213.

14. Eipers PG, Kale S, Taichman RS, Pipia GG, Swords NA, Mann KG, Long MW. Bone marrow accessory cells regulate human bone precursor cell development. *Exp Hematol.* 2000 Jul;28(7):815-25.

15. Thiede MA, Smock SL, Petersen DN, Grasser WA, Thompson DD, Nishimoto SK. Presence of messenger ribonucleic acid encoding osteocalcin, a marker of bone turnover, in bone marrow megakaryocytes and peripheral blood platelets. *Endocrinology.* 1994 Sep;135(3):929-37.

16. Bodine PV, Komm BS. Evidence that conditionally immortalized human osteoblasts express an osteocalcin receptor. *Bone.* 1999 Nov;25(5):535-43.

17. Ishida M, Amano S. Osteocalcin fragment in bone matrix enhances osteoclast maturation at a late stage of osteoclast differentiation. *J Bone Miner Metab.* 2004;22(5):415-29.

18. Liu F, Malaval L, Aubin JE. The mature osteoblast phenotype is characterized by extensive plasticity. *Exp Cell Res.* 1997 Apr 10;232(1):97-105.

19. Candeliere GA, Liu F, Aubin JE. Individual osteoblasts in the developing calvaria express different gene repertoires. *Bone.* 2001 Apr;28(4):351-61.

20. Hauge EM, Qvesel D, Eriksen EF, Mosekilde L, Melsen F. Cancellous bone remodeling occurs in specialized compartments lined by cells expressing osteoblastic markers. *J Bone Miner Res.* 2001 Sep;16(9):1575-82.

21. Horwitz EM, Prockop DJ, Fitzpatrick LA, Koo WW, Gordon PL, Neel M, Sussman M, Orchard P, Marx JC, Pyeritz RE, Brenner MK. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. *Nat Med.* 1999 Mar;5(3):309-13.

22. Horwitz EM, Prockop DJ, Gordon PL, Koo WW, Fitzpatrick LA, Neel MD, McCarville ME, Orchard PJ, Pyeritz RE, Brenner MK. Clinical responses to bone marrow transplantation in children with severe osteogenesis imperfecta. *Blood.* 2001 Mar 1;97(5):1227-31.

23. Farrington-Rock C, Crofts NJ, Doherty MJ, Ashton BA, Griffin-Jones C, Canfield AE. Chondrogenic and adipogenic potential of microvascular pericytes. *Circulation.* 2004 Oct 12;110(15):2226-32.