IBMS BoneKEy. 2008 August;5(8):269-274
http://www.bonekey-ibms.org/cgi/content/full/ibmske;5/8/269
doi: 10.1138/20080328

PERSPECTIVES

Bone Cells, Osteoprogenitors, and Hematopoiesis

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Abstract

The role of bone cells in regulating hematopoiesis is increasingly being brought to general attention by recent studies. Data from murine models with mutations targeted to, or affecting the osteoblastic lineage have revealed a potential role of osteoblastic cells in regulating the hematopoietic stem cell niche. In addition, studies using transplantation systems in which the hematopoietic microenvironment can be transferred to heterotopic sites are revealing the specific role of individual subsets of osteogenic cells, defined by surface phenotype. In particular, the possibility is emerging that skeletal stem cells and hematopoietic stem cells may share a microanatomic location in the bone marrow, and interact with each other. While highlighting a somewhat neglected, and yet physiologically major, function of osteogenic cells, these data promise to reveal mechanisms underlying the "seed and soil" interaction between bone- and blood-borne hematopoietic progenitors, and also cancer cells. *IBMS BoneKEy*. 2008 August;5(8):269-274.

Hematopoiesis and Bone

Why postnatal hematopoiesis in humans is normally restricted to bone has remained an elusive question for a long time. The manner in which the question has been phrased or rephrased over time has largely reflected one or another dominant view, making specific terminologies popular. Originally put forward by Schofield in 1978 (1), to denote the unique site where hematopoietic stem cells would home and function in the bone marrow (BM), the term "niche" has been revived anew in the field of hematopoiesis. This largely reflects the resurging interest in the broader "niche" concept as related not just to the preferred site of hematopoiesis or hematopoietic stem cells, but to the specific microenvironment dictating stem cell location, retention, or self-renewal in different systems (2).

Seen from the "bone point of view," the issue of the hematopoietic stem cell "niche" hit center stage when two important studies demonstrated an increased number of assayable HSCs in two unrelated transgenic murine models sharing the feature of excess bone formation, and excess osteoblasts, in the bone-bone marrow organ (3;4). Through the evidence provided by these two studies, and others that followed, osteoblasts became the prime candidate cell type to which the unique role of establishing and maintaining the HSC "niche" would be ascribed. The concept of an "osteoblastic niche" would nicely fit the independent evidence that HSCs could be localized to the endosteal surface in murine long bones. By ascribing the regulation of HSCs to bone cells, the concept also effectively highlights hematopoietic regulation as a truly major physiological function of skeletal cells, to which bone biologists have traditionally devoted surprisingly little attention, in spite of multiple examples of cross-regulation and mutual adaptation of bone and hematopoietic tissues, both in physiology and in disease. However, as is often the case, concepts that become popular unavoidably lead to popular oversimplifications. Osteoblasts represent a defined maturational stage in the osteogenic cell lineage. Whereas all osteoblasts (cells in the process of depositing and mineralizing bone matrix on a growing bone surface) are part of the lineage, not all cells of osteogenic lineage are osteoblasts. Even at the bone surface, not all cells are osteoblasts. Bone-lining cells, while of osteogenic lineage, reside on bone surfaces, but do not actively deposit bone matrix, and are remarkably different, morphologically and functionally, from osteoblasts (to mention one of the most obvious phenotypic differences, most bone-lining cells do not express alkaline

phosphatase). Hence, physical association of HSCs with a bone surface does not necessarily mean association with an osteoblast. The concept of an "osteoblastic niche," when matched to a precise definition of what exactly an osteoblast is, may need to be further qualified, or rephrased, to more properly refer to cells of osteoblastic lineage.

The Hematopoietic Microenvironment – One or More Niches, One or More Cell Types

In addition to endosteal surfaces, HSCs can also be localized to other specific regions of the bone-bone marrow organ, such as the sinusoidal wall, or the hematopoietic space itself. To complicate the issue further, both sinusoidal walls (5), and hematopoietic space (6), are or can be directly contiguous with bone surfaces. Establishing physical contiguity of HSCs to one or the other of two contiguous structures such as sinusoids or the endosteum can be difficult, complicating the anatomical identification of the "niche." Further caution is warranted by consideration of the specific features of murine bone. For example, whereas the murine primary and secondary spongiosa, commonly taken as a model of human trabecular bone, is made of trabecular structures that are extensively covered with active osteoblasts, the vast majority of human trabecular bone is covered by resting surfaces. Each remodeling site, where true osteoblasts are found, only exists in human trabecular bone for a few weeks, which would imply, if true osteoblasts were the "niche" cells, that the "niche" is not a fixed structure in bone, and can even be dispensable for hematopoiesis to occur. As in many other areas, extrapolating results from murine models to a general concept of the HSC niche is difficult and requires caution.

Osteoblasts at the endosteal surface, endothelial cells at the sinusoidal wall, and "reticular" cells in the intervening hematopoietic space, all have been seen as cell types and microanatomical sites that establish or represent, respectively, the putative hematopoietic "niche" in bone marrow (7;8). It would be reasonable to assume, based on the sum of the evidence, that different specific microenvironments would dictate different functions of the HSCs. For example, they could be retained in one, self-renew (divide asymmetrically) in another, or undergo clonal expansion and maturation through symmetric cell division in yet another locale. Indeed, while popular, the term "niche" as applied to HSCs has been given multiple meanings, such as the site where HSCs would home and be localized, or the site where they would undergo self-renewal, or the site where they would simply remain quiescent and therefore be retained long-term. All of these sites would be comprised in a single conceptual space, best described by the term "hematopoietic microenvironment." Conceivably, different cell types in each locale would specify a different function and fate for HSCs.

What Exactly Establishes a Bone Marrow?

The availability of transplantation systems in which the formation of heterotopic bone and bone marrow *in vivo* can be observed allows for a unique experimental angle on the hematopoietic microenvironment. This line of work emanates from the pioneering studies of Tavassoli and Crosby (9), who showed that heterotopic transplantation of bone-less fragments of mammalian bone marrow leads to the formation of a heterotopic "ossicle," in which bone formation and establishment of hematopoiesis occurs sequentially. In these systems, the appearance of hematopoietic tissue at a heterotopic site is taken to imply the transfer, to that site, of the hematopoietic microenvironment. The work of Friedenstein and colleagues later established that this property could be assigned to a single cell, and that this cell would be found in the non-hematopoietic, stromal tissue of bone marrow. The phenotype of this cell, and above all its *in vivo* counterpart in the intact bone marrow, have both remained elusive for a long time. In fact, the stromal cell capable of generating heterotopic ossicles comprising heterotopic hematopoietic tissue is found in the wall of bone marrow sinusoids (Fig. 1), and is noted for a characteristic surface phenotype that allows

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Fig. 1. Cartoon depicting the reticular morphology and adventitial position of MCAM/CD146-expressing cells in the wall of human bone sinusoids, where hematopoietic stem cells can also be localized. MCAM/CD146-expressing stromal cells are osteoprogenitors. When transplanted heterotopically, they generate bone and a functional hematopoietic microenvironment, and self-renew into adventitial reticular cells in the heterotopic bone marrow sinusoids.

for its prospective isolation and enrichment. The Melanoma Cell-associated Adhesion Molecule (MCAM/CD146) is a key marker in that phenotype. Like several other markers, CD146 allows for the prospective isolation and enrichment of clonogenic stromal cells. Unlike other markers suited to this task, but not well suited to *in situ* studies, CD146 also allows for the direct correlation of the phenotype of the explanted clonogenic cells with their *in situ* identity prior to explantation, and their fate following transplantation. This allowed for the recognition that the clonogenic stromal cells, among which skeletal progenitors are found in bone marrow, physically coincide with CD146-

expressing subendothelial cells in the wall of bone marrow sinusoids, and actually reconstitute a compartment of identical cells in the heterotopic organs formed by transplantation (that is, they self-renew like *bona fide* stem cells) (10). Of note, MCAM/CD146 is expressed in subendothelial cells of microvascular walls in a variety of tissues (11). MCAM/CD146-expressing subendothelial cells isolated from a variety of tissues are clonogenic, like their bone marrow counterpart, but so far, the ability of CD146-expressing subendothelial cells to establish heterotopic bone and the hematopoietic microenvironment is restricted to those found in the bone marrow (unpublished data).

CD146-expressing, subendothelial cells in the human bone marrow are related, in one way or another, to each of the three cell types and anatomical sites putatively involved in making the "hematopoietic niche." While not an osteoblast, this cell type directly generates osteoblasts *in vivo*. While not an endothelial cell, this cell type physically associates with the abluminal surface of sinusoidal endothelial cells, both in the intact bone marrow and in the heterotopic organs generated by transplantation *in vivo*. Anatomically and functionally, this cell type appears to represent, in humans, the CXCL12-expressing "reticular" cell identified in the mouse bone marrow as a "niche" cell (6). Thus, not only can this cell type transfer and organize the hematopoietic microenvironment *in vivo*, but either coincides, or generates, or co-localizes, with all the cell types implicated so far in the HSC "niche" in bone marrow. It also appears to coincide with the long sought "stromal," "osteogenic," "skeletal" or "mesenchymal" stem cell found in bone marrow.

A Stem Cell-Maintained Stem Cell Niche

It is noteworthy that the abluminal surface of sinusoids, noted as at least one of the spatial specifications of the HSC niche (5), represents at the same time the "niche" for skeletal stem cells (10). Two stem cells would then be localized, and functionally interact with one another, at the same anatomical site. The only organ noted for the co-existence of two systems of stem cells, each directing the organization and function of a specific tissue, bone marrow may thus represent a unique example of functional interaction not just of two stem cell-dependent systems, but of two stem cells. One would make the "niche" for the other, and by modulating its own function and the fate of its own progeny, modulate hematopoietic function.

Can Hematopoiesis Occur in the Absence of Bone?

Bone formation can occur in the absence of hematopoiesis. Generation of functional osteoblasts is not invariably linked to the establishment of a hematopoietic microenvironment. For example, in secondary compact bone, bone formation proceeds unabated until Haversian systems are filled with bone, leaving no real marrow space between the central blood vessels and the bone surface. Experimentally, one can transplant osteogenic cells to the effect of establishing heterotopic bone that remains devoid of bone marrow (10;12). The two processes of bone formation and establishment of the hematopoietic microenvironment are in many cases dissociated from one another, and can be experimentally dissociated from one another. Strains of osteogenic cells otherwise capable of establishing heterotopic bone and bone marrow can be induced to form only bone if exposed to significant mitogenic stimuli (*e.g.*, FGF-2) *ex vivo* prior to transplantation (10). Conversely, the experimental establishment of a heterotopic hematopoietic tissue in the absence of bone and osteoblasts has never been obtained. In all current *in vivo* transplantation systems, bone formation invariably precedes the establishment of heterotopic hematopoietic "niches," a phenomenon that seems to recapitulate the sequence of events whereby during organogenesis, bone is established first, and then hematopoiesis colonizes the bone marrow cavity (13). Other important facts regularly precede the establishment of hematopoiesis in transplantation systems. One is the development of a system of sinusoids, which in transplantation systems

appears to be directed by, or at least involve, transplanted subendothelial cells (10). Of note, these cells produce angiopoietin-1 (10), a known regulator of vascular remodeling (14), which has been implied in maintaining the HSC niche (15). Another is the generation of cavities that become permeated by sinusoids, and then colonized by hematopoietic cells. Even though CD146-expressing subendothelial cells can generate and organize the entire hematopoietic microenvironment, it cannot be stated that they are necessary and sufficient for the job. As they generate osteoblasts and guide the formation of sinusoids, and do so in specific spatial contexts such as the excavation of microcavities, it will be necessary to dissect experimentally the role of each of these concurrent events in the establishment of the hematopoietic microenvironment. For example, it will be important to identify the specific experimental conditions, if any, under which cells that can establish either bone alone, or both bone and the hematopoietic microenvironment, only establish the hematopoietic microenvironment in the absence of bone. Likewise, it will be important to determine the conditions under which bone marrow subendothelial cells can direct the formation of sinusoids instead of capillaries, and whether this can happen in the absence of bone formation, or bone resorption.

From Physiology to Disease

Identification of one or more cell types that establish the unique hematopoietic microenvironment in bone will have important applicative fallouts. The common “seed and soil” paradigm underlying the establishment and maintenance in bone of normal hematopoiesis, of therapeutic hematopoietic progenitor/stem cells as in bone marrow transplantation, and of hematopoietic and non-hematopoietic cancer such as leukemia (16;17) or metastasis, promises to disclose important new functions of osteogenic cells, and hopefully new ways of manipulating them.

Conflict of Interest: None reported.

Peer Review: This article has been reviewed by Jane Aubin.

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