Scanning Microscopy

Volume 3 | Number 3 Article 27

10-26-1989

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Scott C. Miller
University of Utah

Louis de Saint-Georges
University of Utah

Beth M. Bowman
University of Utah

Webster S. S. Jee
University of Utah

Scanning Microscopy, Vol. 3, No. 3, 1989 (Pages 953-961) 0891-7035/89$3.00+.00
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

BONE LINING CELLS: STRUCTURE AND FUNCTION

Scott C. Miller*, Louis de Saint-Georges[1], Beth M. Bowman and Webster S. S. Jee

Division of Radiobiology, School of Medicine, University of Utah, Salt Lake City, Utah 84112. [1]Permanent address: CEN/SCK Radioprotection Department, Boeretang 200 B2400 MOL, Belgium.

(Received for publication May 25, 1989, and in revised form October 26, 1989)

Abstract

Bone lining cells (BLC's) cover inactive (nonremodeling) bone surfaces, particularly evident in the adult skeleton. BLC's are thinly extended over bone surfaces, have flat or slightly ovoid nuclei, connect to other BLC's via gap junctions, and send cell processes into surface canaliculi. BLC's can be induced to proliferate and differentiate into osteogenic cells and may represent a source of "determined" osteogenic precursors. BLC's and other cells of the endosteal tissues may be an integral part of the marrow stromal system and have important functions in hematopoiesis, perhaps by controlling the inductive microenvironment. Because activation of bone remodeling occurs on inactive bone surfaces, BLC's may be involved in the propagation of the activation signal that initiates bone resorption and bone remodeling. Evidence also suggests that BLC's are important in the maintenance of the bone fluids and the fluxes of ions between the bone fluid and interstitial fluid compartments for mineral homeostasis.

Key words: Bone lining cells, bone, osteogenesis, mineral metabolism, calcium, bone marrow.

Address for correspondence:
Dr. Scott C. Miller
Division of Radiobiology, Building 586
University of Utah
Salt Lake City, UT 84112
Phone number (801) 581-5638

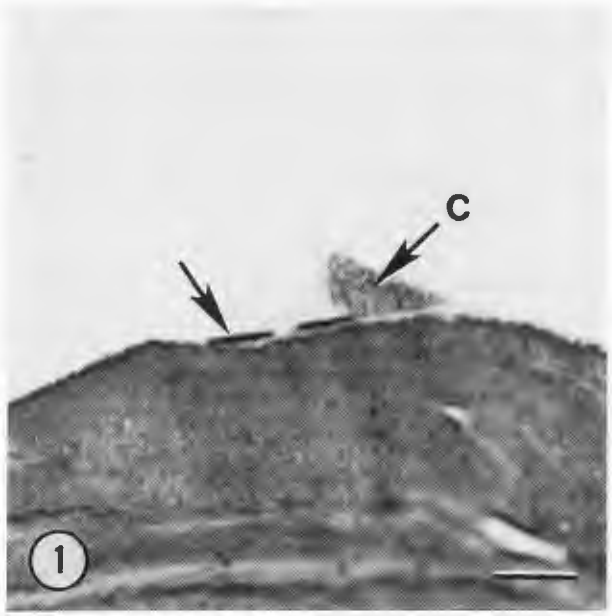
Introduction

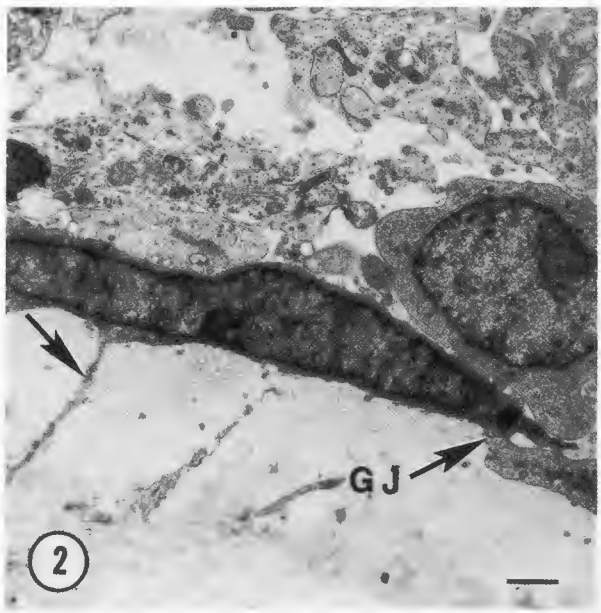
Bone lining cells (BLC's) are histologically inconspicuous cells that cover 'inactive' (nonremodeling) bone surfaces in the adult skeleton [31]. BLC's appear to be derived from osteoblasts that have ceased their osteogenic function and have spread over the bone surface. BLC's are best described on endosteal and endocortical surfaces [34], but they have been described on other bone surfaces, such as in osteons [9]. Because a relatively small fraction of the total skeletal surface is remodeling at any given time, the majority of skeletal surface is inactive and covered by BLC's, thus making this cell one of the most common bone cells in the skeleton. Although BLC's cover nonremodeling or "resting" bone surfaces, these surfaces, and the cells associated with them, may be physiologically functional in terms of calcium exchange and mineral homeostasis. Although BLC's might have a number of important functions in skeletal and mineral metabolism and homeostasis, they are not well characterized.

Morphology of bone lining cells

BLC's, as described on endosteal and endocortical surfaces, are a component of the endosteal tissues that interface the osseous and hematopoietic tissues of the bone marrow. As proposed in an editorial [34], BLC's have some distinct morphological features that allow them to be recognized and defined as a phenotype distinct form other cells. BLC's are flattened over the bone surface such that when cut perpendicular to the surface, they appear as very thin and elongated. Only nuclear profiles of BLC's are usually resolved by light microscopy (fig. 1). Nuclear profiles are usually less than 1.0 μm, sometimes less than 0.5 μm in thickness, while the cytoplasm is often as thin as 0.1 μm in thickness (figs. 2-4). By light microscopy it can be difficult to distinguish BLC's from other cells such as stromal cells, adventitial cells, adipose cells, marrow sac cells, and osteoprogenitor cells. The lineal surface density of BLC's is about 19 cells per mm bone surface perimeter in fatty marrow sites in the young adult beagle [31] and about 21 cells/mm surface on endosteal surfaces of adult male Japanese quail [2].

BLC's are, by definition [34], directly apposed to the bone surface, although they may appear to be a component of several layers of cells adjacent to the bone surface. As observed by transmission electron microscopy, the BLC cytoplasm can be very thin and attenuated over the bone surface, containing few organelles (figs. 3-5). Sometimes profiles of endoplasmic reticulum, mitochondria, and some free ribosomes were found,





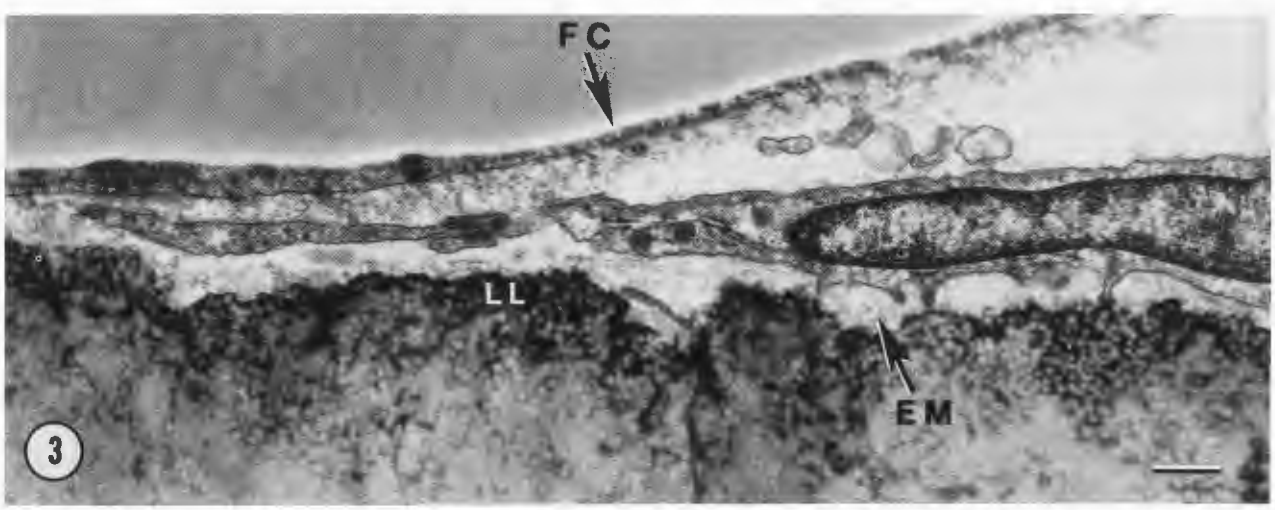


Fig. 1. Light micrograph of BLC nuclei (arrow) on a bone surface taken from a fatty bone marrow site in an adult beagle. Decalcified, epoxy embedded and stained with methylene blue and toluidine blue. C: India ink-perfused capillary. Bar = 10 µm.

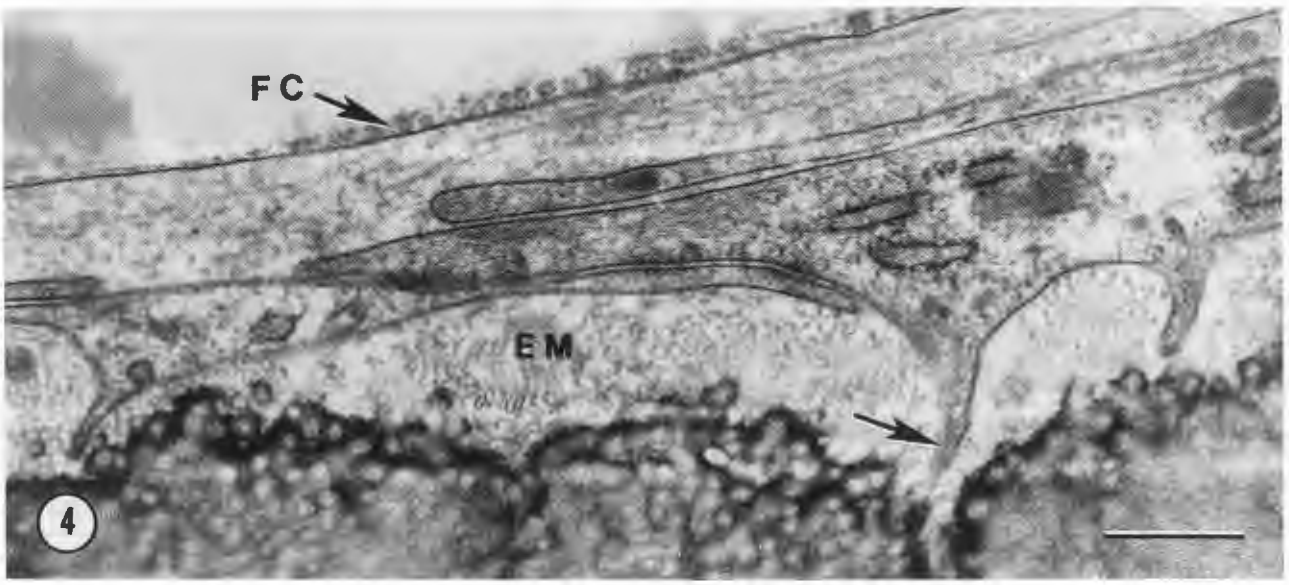
Fig. 2. TEM of a BLC on the endosteal surface from an adult beagle. The nucleus is elongated and the cell has processes extending into canaliculi (arrow). Decalcified. GJ: Gap junction. Bar = 1.0 µm.

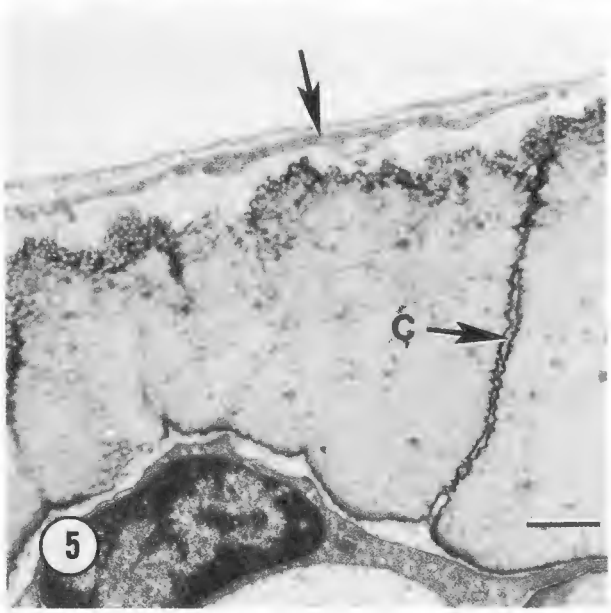
Fig. 3. TEM of a BLC on the endosteal surface from an adult beagle. Decalcified. LL: Lamina limitans; EM: Endosteal membrane; FC: Fat cell cytoplasm. Bar = 0.5 µm.

particularly near the nucleus. In canine fatty bone marrow, BLC nuclei were located usually near capillaries [32] while the cytoplasm extended for considerable distances over the surface.

On resting bone surfaces, as observed by electron microscopy of decalcified sections, there is an osmiophilic dense region that is commonly known as the "lamina limitans" [50], (figs. 3-5), although it is known by other names including "line of demarcation", "resting line", "dense line", "osmiophilic lamina" and "peripheral zone" [59]. During bone resorption, the lamina limitans is one of the first organic structures to disappear. Scherft [50] suggested that the lamina limitans forms from the adsorption of organic material onto the surface of the mineralized matrix and thus is presumed to represent the boundary of the mineralized surface in areas where active bone resorption or mineral accretion are not occurring.

Between the lamina limitans and the BLC's is a layer of unmineralized connective tissue, usually 100-500 nm in thickness (figs. 3-5). This layer may be present to some extent on all resting bone surfaces and has a different ultrastructural appearance than the osteoid that will normally mineralize. This layer, termed the "endosteal membrane" [40], contains amorphous material and some collagenous and reticular fibers that appear morphologically different from the collagenous fiber bundles seen in bone [31] (fig. 4). The collagenous fibers in the endosteal membrane also lack the orientation that is typical of bone matrix collagenous fiber bundles. While it





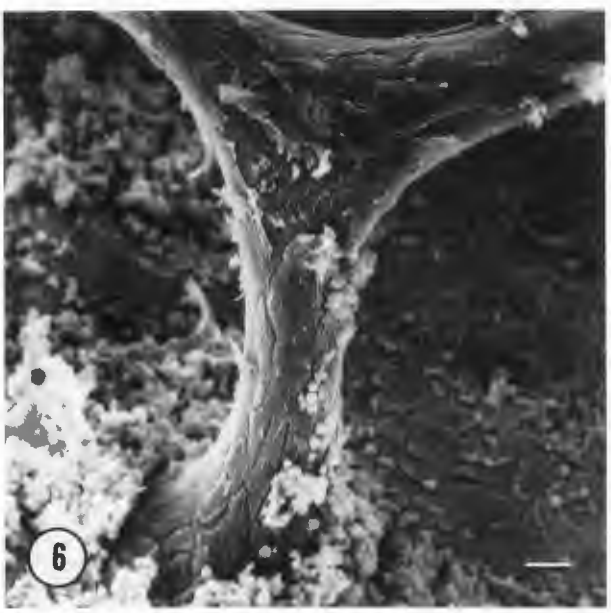


Fig. 4. Higher magnification TEM of BLC cytoplasm from an adult beagle. BLC cell processes extend into the bone (arrow). Decalcified. EM: Endosteal membrane; FC: Fat cell cytoplasm. Bar = 0.5 μm.

Fig. 5. TEM of endosteal surface of a fatty bone marrow site from an adult beagle. The BLC cytoplasm is thinly extended over the surface (arrow). A canaliculi (C) extends from an osteocyte lacunae to the bone surface. Decalcified. Bar = 1.0 μm.

Fig. 6. SEM of bone surface cells on a trabeculae of a mouse femur following partial removal of the bone marrow. Perfusion fixation with formalin, critical point dried and coated with gold. Bar = 20 μm.

has been a common practice to refer to this layer of unmineralized connective tissue as "osteoid", osteoid is a term that should be reserved specifically for unmineralized bone. The origin of this unmineralized tissue layer on the bone is not known, but may be the final secretion products of osteoblast as they cease their matrix synthesis function and become BLC's. Similar appearing connective tissues, although in lesser amounts, have also been described on the other side of the BLC, separating them from fat cells, reticular cells and marrow cells [31].

When BLC's from rodent tissues are observed by SEM, they appear as a continuous sheet, or pavement of cells, over endosteal surfaces (figs. 6-8). The surface of the cells exposed to the marrow is very smooth with few microvilli. This ultrastructural feature of these cells is also evident in transmission electron micrographs. In

specimens that are fixed by perfusion *in situ* and carefully prepared for SEM, the boundaries between the cells are almost indistinguishable (fig. 8). When observed by TEM, gap junctions are often seen joining adjacent BLC's (fig. 2).

There is however, one caveat concerning the identification of BLC's by SEM - the functional state of the cells is not obtainable using this technique. When the surface of the cells are observed, it is difficult to distinguish osteoblasts from BLC's. Osteoblasts can be readily recognized in light and electron microscope sections and their functional state can be assessed using fluorochrome labeling. This has led to some confusion on terminology as the term 'bone lining cell' has sometimes been used to generically describe all bone surface cells [10], regardless of their phenotype and function.

BLC's often have cell processes that extend into canaliculi, and contacts with osteocyte cell processes have been described [12]. In areas there the bone surface has been fractured, exposing the BLC-bone surface interface, small cell processes can be seen extending into canalicular openings (fig. 9). The presence of BLC processes extending into the bone likely anchor the cells to the surface such that when the overlying marrow cells are removed, the BLC's remain attached to the surface, allowing them to be visualized by SEM. When the marrow plugs that are separated from the bone are examined by SEM, there is a smooth layer of cells that appears to invest the bone marrow [30] (fig. 10). These marrow investing cells have been called "marrow sac cells" and are considered an integral part of the endosteal layer. The function of these cells is not known but they may have both osteogenic and hematopoietic potential.

While SEM images of BLC's in rodent bone would suggest that these cells form a continuous layer over the bone surface, it is not clear if they form a continuous layer over all bone surfaces in longer lived species. Early light microscope studies suggested that some surfaces, particularly from older or severely ill individuals, lacked a cellular lining [20,41]. Gaps between BLC's were observed in fatty marrow sites in both dogs [31] and bats [12] but not in red marrow sites in humans [59].

Functions of bone lining cells

Bone lining cells as a source of osteogenic precursors

Histological evidence suggests that BLC's are derived from osteoblasts that have become inactive, lost most of their cellular protein synthetic organelles, and spread over the bone surface. This view of the origin of these cells is based on morphological observations, but does not preclude the possibility that some may be derived from other endosteal cells or stromal cell components.

The residence time of BLC's on the bone surface is dependent on the bone remodeling rate and the lifespan of the animal. For example, in longer lived species and at bone sites with low remodeling rates, BLC's may be resident on bone surfaces for long periods of time, perhaps years [35]. The differences in residence time and estimated lifespan of these cells may account from some of the anatomical differences that have been described in BLC's among different species.

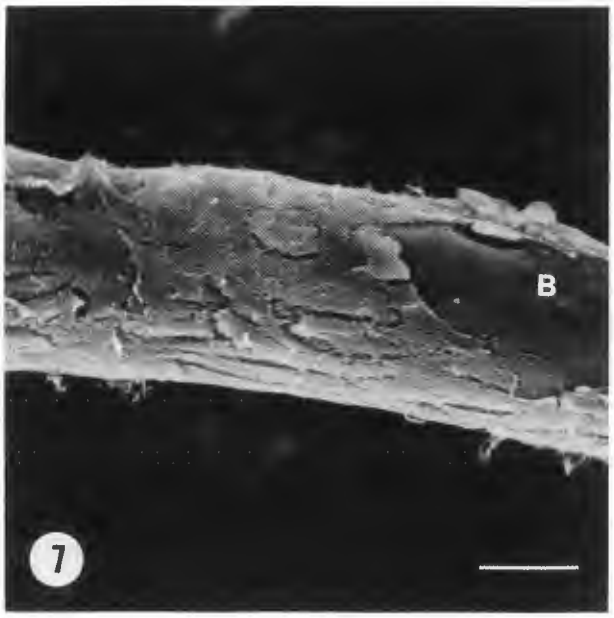
The proliferative capacity and differentiation potential of BLC's has been investigated in a model of estrogen stimulated bone formation in birds. Like mature osteoblasts, osteoclasts and osteocytes, BLC's rarely incorporate a ^{3}H-thymidine label, indicating that they are not actively dividing [2]. However, following estrogen administration to male birds, the proliferation of BLC's has been observed [2,21]. In this model, the BLC's also appear to differentiate into osteoblasts that form medullary bone deposits, although the contribution of other cells also appeared likely [33]. The BLC's were considered to first become preosteoblasts and then differentiate into osteoblasts [38]. From these studies it was concluded that the BLC is an 'inducible' osteogenic precursor cell because it differentiates into the osteoblast phenotype in response to an inductive signal. If these observations made in birds could be extrapolated to other species, then BLC's could represent a pool of osteogenic precursors and may be involved in induced states of osteogenesis such as fracture repair, osteogenesis following marrow extirpation and radiation exposure, and the direct stimulation of osteogenesis by fluoride or prostaglandins. Because of the evidence suggesting that BLC's retain osteogenic potential, BLC's are considered as 'target cells' for the induction of skeletal cancers from bone-seeking radionuclides [45] . It is also possible that the BLC's may be "determined" osteogenic precursors. Determined osteogenic precursor cells are those cells that are capable of spontaneous bone formation [39]. If the BLC's is a determined osteogenic precursor cell, then it may be considered as a preosteoblast or osteoprogenitor cell, but this remains to be demonstrated.

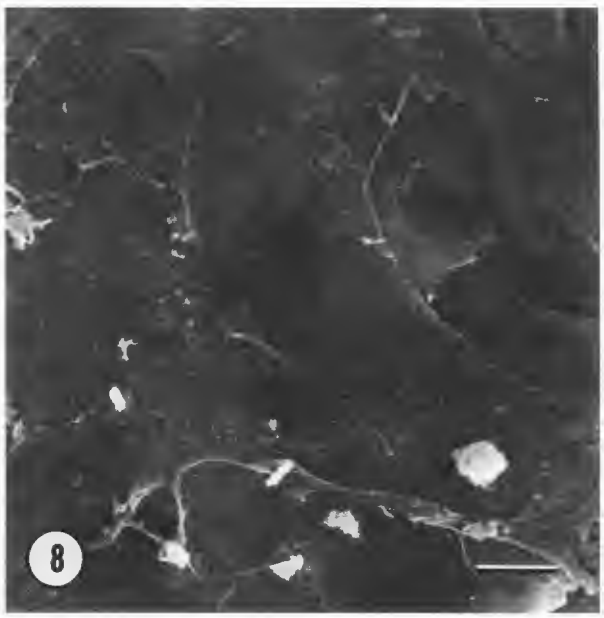
Bone lining cells and the regulation of hematopoiesis

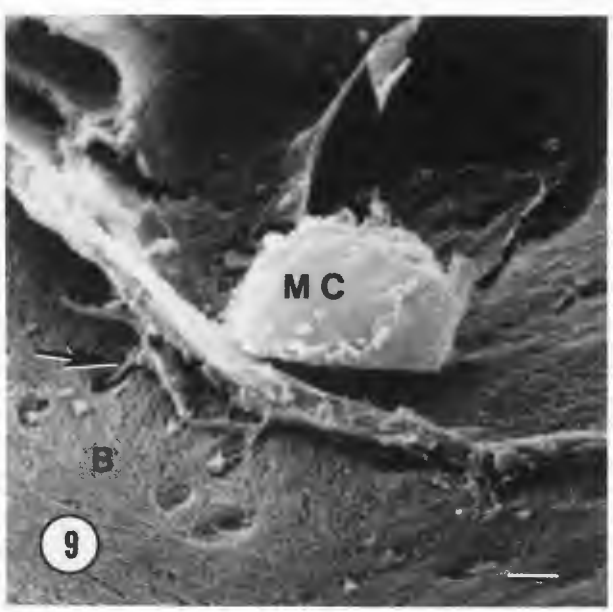
McLean and Urist [27] noted that endosteal cells served as both the covering for bone surfaces and the outer investment of bone marrow. They suggested that this endosteal tissue may have both osteogenic and hematopoietic capacities. BLC's, and other cells of the endosteal layer are thought to be continuous with the stromal system of the bone marrow [60] and may be involved in the regulation of hematopoietic functions [10]. The interrelationship between osteogenic cells and marrow stromal cells is further supported by the finding that cells isolated from the marrow stroma can express osteogenic potential [39].

Developmental and functional relationships between osseous and hematopoietic tissues may be mediated by components of the stromal system and cells of the endosteal surface, perhaps including BLC's. For example, it is well recognized that the development of the marrow cavity and the seeding of the bone marrow occur in an ordered sequence that involves endochondral osteogenesis. In experimental settings, the reconstitution of marrow tissues is preceded by the formation of new cancellous bone [44,52]. The cells responsible for the regeneration of the marrow stroma following marrow ablation appeared to arise from the endosteal surface of the bone [42,44]. In addition, cells on the endosteal surfaces may regulate vascular ingrowth necessary for reconstitution of the hematopoietic cells [1,3]. On the other hand, bone formation occurs prior to marrow reconstitution following ectopic implants of whole bone marrow [43]. The stromal cells of the marrow transplants appear to give rise to the osteogenic cells that form bone trabeculae in the implant [54,55]. These studies demonstrate an interdependence of osseous and hematopoietic tissues that may be mediated by the stromal system, perhaps including the bone lining cell.

It is becoming evident that stromal domains exist within the bone marrow, each of which commits the resident pluripotent hematopoietic cells to a particular differentiation pathway [22]. The proliferation and differentiation of the hematopoietic cells within these microenvironmental compartments appears to be regulated by the marrow stromal system [15]. It is also recognized that pluripotent stem cells in the bone marrow, particularly CFU-C, are concentrated along the bone surface [23]. The stem cells near to endosteal surfaces also







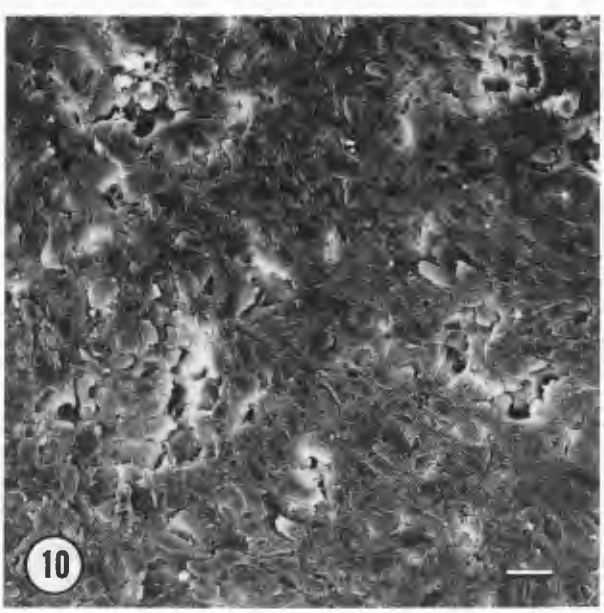


Fig. 7. SEM of bone surfaces cells spread on a trabecular surface of a mouse femur following removal of the bone marrow. Perfusion fixation with formalin, critical point dried and coated with gold. B: Bare bone surface. Bar = 20 μm.

Fig. 8. In well fixed specimens, the boundaries between the very flat and overlapping BLC's on endocortical surfaces are difficult to distinguish. Perfusion fixation with formalin, critical point dried and coated with gold. Bar = 10 μm.

Fig. 9. SEM of a fractured endocortical bone surface of a mouse femur illustrating the interface between the BLC and the bone surface (B). Processes from the BLC are extending into surface canaliculi (arrow). Perfusion fixation with formalin, critical point dried and coated with gold. MC: Marrow cell. Bar = 1 μm.

Fig. 10. SEM of marrow sac cells investing the bone marrow plug following separating of the marrow from the bone in an adult beagle. Perfusion fixation with formalin, critical point dried and coated with gold. Bar = 20 μm.

proliferate more rapidly than those away from bone [49]. The association of stem cells with endosteal surfaces has led to the suggestion that endosteal bone surface cells, perhaps including the bone lining cell, are totipotent stem cells capable of giving rise to proliferating progenitors of hematopoietic cells, stromal cells as well as osteogenic cells [18].

Detailed morphological descriptions of the

relationships of BLC's with hematopoietic cells [60] has led Deldar et al. [10] to suggest that BLC's contribute to, or perhaps even regulate the hematopoietic inductive microenvironment. This could involve direct cell to cell interactions or by the elaboration of paracrine factors that control hematopoiesis. There is recent evidence that hematopoiesis is regulated through direct cell contact with cells of the stromal system via growth factors bound to the cell surface [46].

Bone lining cells and the activation of bone remodeling

The activation of bone remodeling occurs on inactive bone surfaces, presumed to be covered by BLC's. The first histological evidence of bone remodeling is the recruitment of osteoclasts to the site and the initiation of bone resorption. Little is known about the mechanism of activation of bone remodeling, but there is increasing evidence that cells of the osteoblastic lineage play a role in the regulation and modulation of osteoclast activities, including bone resorption and osteoclast recruitment and differentiation. These interactions may provide the physiological and anatomical basis for the "coupling" of bone formation and resorption processes. There is some evidence that bone remodeling units may be activated to repair or prevent fatigue damage [24,40] and to allow the skeleton to adapt to mechanical usage [13,14]. It is presumed that a signal for the activation of bone remodeling must be transmitted to the bone surface where osteoclast progenitors can be recruited. Because of the anatomical location of bone lining cells, they are a possible candidate for the propagation or transduction of the activation signal that initiates the cellular cascade associated with bone remodeling [34].

BLC's, being apparently derived from osteogenic cells, could possibly be involved in the activation and initiation of new remodeling units by releasing factors that are chemotactic for osteoclasts or their progenitors and perhaps control their differentiation [reviewed in 4,19,47,58]. Osteoclasts have been shown to be derived from hematopoietic stem cells [51] and the mechanisms of osteoclast regulation may be similar to those involved in the regulation of hematopoietic cell proliferation and differentiation, discussed earlier.

Cells of osteogenic lineage, perhaps including BLC's, may regulate osteoclast function and differentiation. For example, bone resorption by isolated osteoclasts on devitalized bone slices was not affected by interleukin-1 [57], tumor necrosis factor-a and ß [56], parathyroid hormone [28] or 1,25-dihydroxyvitamin D_3 [29] unless the osteoclasts were co-cultured with osteoblasts. In these studies, there was a significant increase in the number of resorption cavities, suggesting increased cellular efficiency and/or increased osteoclast activation in the presence of osteoblastic cells. Prostaglandins PGE_1, PGE_2 and PGI_2 were also found to be inhibitors of isolated osteoclasts, but when co-cultured with osteoclasts these substances caused a considerable increase in cell spreading [6]. These observations have been interpreted as implicating a role of osteogenic cells in the regulation of osteoclastic activities.

Bone lining cells may also regulate resorption by restricting access to the bone surface. BLC's may retract and expose the "bare" bone surface and this may initiate the signal for the chemoattraction of osteoclasts and/or their progenitors. There is evidence that osteoclasts are activated upon exposure to mineralized bone surfaces, but not the intact endosteal surface [7]. Mineralized bone surfaces are covered by the endosteal membrane (fig. 4) and this may function as a protective barrier over the mineralized tissue. When this membrane is removed, the mineralized matrix may facilitate the recruitment and attachment of osteoclast progenitors [8]. BLC's, or other cells that come in contact with the bone surface, may secrete enzymes to remove the unmineralized endosteal membrane on the bone surface. In this regard, osteogenic cells have been demonstrated to synthesize collagenase [17,48] as well as plasminogen activator [16]. Plasminogen activator is considered to be a potentially potent activator of procollagenase [58]. It should be noted, however, that most of the evidence that demonstrates interactions between osteogenic and osteoclastic cell populations has been gathered in culture systems that use fetal or neonatal tissues or transformed cell lines. It is not clear if the "signals" that might regulate these rapidly developing and transformed systems would be the same as those encountered in the mature skeleton.

As discussed earlier, osteogenesis appears to be a necessary prerequisite for the development of bone marrow, suggesting that the formation of osseous tissue establishes a suitable microenvironment for hematopoiesis. The opposite may also be true - that is, a normal hematopoietic stromal system is necessary for normal bone remodeling. In studies using the op/op osteopetrotic murine mutant, Wiktor-Jedrezejczak et al., [61,62] found that the resorption defect appears to be caused by an abnormal bone marrow microenvironment that impairs the differentiation of osteoclasts from hematopoietic stem cells. The defect may be caused by reduced production of colony stimulating factors (CSF's) from the marrow stromal system. In vitro, CSF's will promote the differentiation of hematopoietic stem cells and restore bone resorption by osteoclasts. These data provide evidence that the stromal system, of which the bone lining may be an integral part, plays an important role in the activation of bone remodeling by producing a paracrine factor that promotes the differentiation of the osteoclast.

Bone lining cells and the functional bone membrane

It was recognized many years ago that the electrolyte composition of the bone fluid compartment was different from that of the interstitial fluids [36]. From these observations it was suggested that there was a functional "membrane" on the bone surface that separated and perhaps regulated the exchange of electrolytes and molecules between these compartments [37]. Because BLC's cover most of the bone surface in the adult skeleton, these cells, as well as other bone surface cells, are considered the cellular components of the "functional bone membrane" [25]. The presence of gap junctions between BLC's [31,34] (fig. 2), as well as between BLC's and osteocytes [11], suggests that regions of bone volume might act as a functional syncytium. The metabolic and electrical coupling of cells within bone might facilitate a number of physiological functions including the conversion of mechanical signals into remodeling activity and/or the movement of mineral in and out of bone.

Further experimental evidence supports a role of BLC's in the regulation of mineral homeostasis, independent of any role in bone remodeling [5,26,53]. BLC's may be the most important skeletal cells in the minute-to-minute regulation of mineral homeostasis [34]. Mineral exchange between the fluid compartments might occur via intercellular channels and involve membrane pumps [26].

Acknowledgements

Portions of work cited here were supported by Public Health Service Grant DE-06007 from the National Institutes of Health and U. S. Department of Energy Grant DEFG60289ER60764.

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Discussion with Reviewers

A. M. Parfitt: Observations in my laboratory, confirmed by Ericksen, relating surface cell morphology to distance from the cement line as a marker of this passage of time, strongly suggest that lining cells represent one morphological expression of the terminal differentiation of osteoblasts. Is there any more direct evidence for this conclusion?

Reviewer I: The underlying theme throughout the manuscript is that BLC are preosteoblasts or osteoblast precursors, but this lacks scientific proof and should be considered one possibility.

Authors: The evidence that BLC's are derived from osteoblasts is similar to that presented by Dr. Parfitt - it is from morphological observations. Light and electron microscope pictures can be generated which appear to represent a transition from osteoblast to BLC, but we agree that firm evidence of their origin from osteoblasts is lacking. It is entirely possible that some BLC, particularly in the adult skeleton of long lived mammals, may be derived from other tissues such as the marrow stromal system.

A. M. Parfitt: The evidence that lining cells have proliferative capacity in birds is strong, but the formation of medullary bone has no counterpart in mammals, and lining cells may not have the same function. Is there any direct evidence that lining cells in mammals are capable of giving rise to osteoblasts?

Authors: The data in birds are from studies where medullary bone formation was induced with estrogen (text refs. 2,33,38). In this model the BLC appears to be an inducible osteogenic cell. We are not aware of any studies that have addressed this important issue in mammals, perhaps because a suitable mammalian model is presently lacking.

S. C. Marks, Jr: Would it be possible to separate marrow from bone lining cells (Figs. 7-9), culture them separately to examine production of and/or response to cytokines, etc., and recombine them to evaluate the independence or interdependence of the skeletal homeostatic and hemopoietic functions of these cells?

Authors: Such studies will eventually have to be done to finally determine the functions and capabilities of these cells. BLC are very adherent to bone surfaces, likely because they have cell processes extending into canaliculi and we are not sure if they could be successfully isolated from adult tissues.

S. C. Marks, Jr: Is the endosteal membrane a precursor of osteoid or is it a separate layer secreted when bone formation at a site is completed? Are there any data as to its composition, i.e. collagen type or proteoglycan content which might prevent formation of the typical banding pattern of type I collagen?

Authors: The observations made on the endosteal membrane are usually incidental to other findings and no studies that we are aware of have focused exclusively on this tissue. As noted, the structure of the endosteal membrane does appear to be different from osteoid and, as you suggest, there may be some differences in the proteoglycan content. Considering the speculations on the possible physiological functions of the endosteal membrane, further studies are clearly needed.

T. J. Chambers: My only substantial disagreement is with the conclusion in the last paragraph, concerning the role of BLC's in mineral homeostasis - this role is difficult to

square with the absent PTH-hypercalcaemic response in osteopetrotics that are curable with hemopoietic transfusions. This suggests that osteoclasts are required for PTH-hypercalcaemia. Similarly, only osteoclasts in bone are thought to have CT receptors.
Authors: We agree that the possible role of BLC's in mineral homeostasis is far from resolved and additional examples could be presented arguing for osteoclasts as having a substantive role in mineral homeostasis (including some published data from our own laboratory, Miller, J Cell Biol. 76:615-618, 1978). The role of the BLC in mineral homeostasis as suggested by others (text ref. 53) and reviewed in the present manuscript, was that of minute-to-minute regulation of small mineral fluxes rather than the larger changes involved in pathological and certain experimental situations. We must also consider the possible role of osteoblasts and osteocytes in the fine regulation of mineral homeostasis.

S.B. Doty: If BLC's behave as a functional bone membrane and regulate ion movement into or out of bone, then the presence of large gaps between cells would be counterproductive to this function. I notice in the SEM's (Figs. 6 and 7) the presence of bare bone matrix. It is possible to explain this morphology with the suggested bone membrane function?
Reviewer I: Do BLC's cover most of the bone surface?
Authors: The continuity and presence of BLCs on all bone surfaces is not resolved. We think that the bare bone surfaces seen in the figures are due to mechanical separation of the cells from the surface. Bare bone surfaces have been described and even in areas where there appears to be a continuity of bone surface cells, tracer molecules can penetrate to the bone surface, suggesting that the lining cells do not have barrier functions similar to that found in some epithelial linings, for example. In our own studies of canine fatty marrow, we have noted "bare" bone surfaces, but these were usually distant to the marrow capillaries, where the physical separation of the bone fluid and interstitial fluid compartments might be most important (text ref. 31).

S.B. Doty: The authors state that another possible function of the BLC would be to "regulate the hemaopoietic inductive microenvironment" or the "elaboration of paracrine factors". However, the morphology of the BLC indicates that the cell is undifferentiated. Does this not suggest that whatever the function of the BLC as an undifferentiated cell, it must differentiate into something else (ie., not a BLC) before it can carry out these suggested functions? Another way to ask the same question is that if the BLC is a preosteoblast, as the authors suggest, then does this mean that the well differentiated osteoblast is the cell which carries out all these functions?
Authors: The general morphology of the cell, in terms of its location on the cell surface and extension of cytoplasmic processes over the bone and into canaliculi suggests to us that this cell may be quite differentiated rather than undifferentiated. The presence of gap junctions also suggests a differentiated phenotype. However, the cell does not contain extensive amounts of some organelles that are typically associated with differentiated functions, such as rough endoplasmic reticulum seen in protein secreting cells, although some are seen. This could suggest that the cell has very limited, but directed functions, typical of some differentiated cells. The BLC has a morphology and ultrastructure not unlike some types of stromal and reticular cells and it is becoming apparent that these cells have highly specific and selective functions in certain organs, such as bone marrow and lymphatic tissues.

Reviewer I: Using your figures and data, specifically 20 cells/mm plus small nuclei plus small cytoplasmic profiles might suggest that a small relative surface is covered by BLC's.
Authors: These cellular density data quoted in this paper could be misinterpreted if the morphometric assumptions and methods involved in the collection of these data are not considered (see text refs. 2,31,33,34). The lineal density data are collected based on the frequency of nuclear profiles in sections. Thus the density figures that are cited are based on the number of BLC nuclei intersected, and as inferred in the question, the nuclei have smaller dimensions that the entire cellular profiles. From these lineal density numbers, areal density estimates of cell size may be generated and this would be a more accurate estimator of actual cell size. The statement that small cytoplasmic profiles might suggest that a small surface area is covered is not necessarily correct. The profiles, as described and illustrated in this paper and others cited in the text, are thin but extended over the bone surfaces.

Reviewer I: The authors should consider that signals for bone remodeling may come from the matrix itself?
Authors: Various matrix molecules have been identified that have biological activities and we agree that some may have roles in bone physiology, but how these putative regulators might act via the various bone cells, including BLC's *in situ* is unknown at this time.

S.B. Doty: In order to initiate the osteoclastic attraction to bone, the authors suggest that the bone lining cell may retract to expose the underlying bone surface. Do BLCs contain a cytoskeletal system which would permit them to expand and/or contract over the bone surface?
Authors: That BLCs might contract to permit other cells access to the bone surface has not been demonstrated, although it has been presented as a possible cellular mechanism (see text ref. 47). We do not know if the BLC has the necessary cytoskeletal components for this.

Reviewer I: Is there any direct evidence that bone lining cells might regulate osteoclast activities in vivo?
Authors: No experiments have been done to address this important issue. The suggestions that BLCs might be involved with osteoclast activities is based on several assumptions. The first is that BLCs have functional properties similar to osteogenic cells. The second is that osteogenic cells can regulate osteoclast activities, as suggested from some in vitro studies, as discussed in the text. Because of the anatomic location of BLCs on resting bone surfaces relative to resorption domains, it may be more likely that BLCs are involved with osteoclast recruitment during modeling and remodeling activation rather than regulation of osteoclast activities and functions after their recruitment to a skeletal site.