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Birth and Death of Bone Cells: Basic Regulatory Mechanisms and Implications for the Pathogenesis and Treatment of Osteoporosis

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ABSTRACT

The adult skeleton regenerates by temporary cellular structures that comprise teams of juxtaposed osteoclasts and osteoblasts and replace periodically old bone with new. A considerable body of evidence accumulated during the last decade has shown that the rate of genesis of these two highly specialized cell types, as well as the prevalence of their apoptosis, is essential for the maintenance of bone homeostasis; and that common metabolic bone disorders such as osteoporosis result largely from a derangement in the birth or death of these cells. The purpose of this article is 3-fold: 1) to review the role and the molecular mechanism of action of regulatory molecules, such as cytokines and hormones, in osteoclast and osteoblast birth and apoptosis; 2) to review the evidence for the contribution of changes in bone cell birth or death to the pathogenesis of the most common forms of osteoporosis; and 3) to highlight the implications of bone cell birth and death for a better understanding of the mechanism of action and efficacy of present and future pharmacotherapeutic agents for osteoporosis. (*Endocrine Reviews* **21:** 115–137, 2000)

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I. Introduction

And Athena lavished a marvelous splendor on the prince so that all the people gazed in wonder as he came forward. The elders making way as he took his father's seat. The first to speak was an old lord, Aegyptius, ***stooped with age****, who knew the world by heart.*

Homer, the Odyssey: translation by Robert Fagles

LOSS OF height (stooping), Dowager's hump, and kyphosis are some of the most visible signs of old age in humans. The primary reason for these involutional changes is a progressive loss of bone mass that affects the axial (primarily trabecular) as well as the appendicular (primarily cortical) skeleton. Loss of bone mass, along with microarchitectural deterioration of the skeleton, leads to enhanced bone fragility and increased fractures—the bone disease known as osteoporosis (1). Both men and women start losing bone in their 40s. However, women experience a rapid phase of loss during the first 5–10 yr after menopause, due to the loss of estrogen (2). In men this phase is obscure, since there is only a slow and progressive decline in sex steroid production; hence, the loss of bone in men is linear and slower (3). In addition to losing bone faster at the early postmenopausal years, women also accumulate less skeletal mass than men during growth, particularly in puberty, resulting in smaller bones with thinner cortices and smaller diameter. Consequently, the incidence of bone fractures is 2- to 3-fold higher in women as compared with men (4).

In addition to sex steroid deficiency and the aging process

itself, loss of bone mass is accentuated when several other conditions are present. The most common are chronic glucocorticoid excess (5), particularly its iatrogenic form, hyperthyroidism as well as inappropriately high T_4 replacement, alcoholism, prolonged immobilization, gastrointestinal disorders, hypercalciuria, some types of malignancy, and cigarette smoking (6).

Bone loss and eventually fractures are the hallmarks of osteoporosis, regardless of the underlying cause or causes. The bone loss associated with normal aging in women has been divided into two phases: one that is due to menopause and one that is due to aging and affects men as well (7, 8). In elderly women these two phases eventually overlap, making it difficult to distinguish the effect of sex steroid deficiency from the effect of the aging process itself. The effect of the aging process itself is also frequently obscured because of secondary hyperparathyroidism (9), resulting from impaired calcium absorption from the intestine with advancing age (>75 yr old). The bone loss that is due to glucocorticoid excess shares several features with the bone loss due to senescence, but also has unique features of its own. Nonetheless, as is the case with the other types of bone loss, the heterogeneity of the underlying conditions, some of which (*e.g.*, postmenopausal state, rheumatoid arthritis, etc.) independently contribute to skeletal deterioration, can distort the clinical and histological picture (10). Irrespective of the overlap, it is important to recognize that the pathogenetic mechanisms are quite distinct in the various forms of osteoporosis and that sex hormone deficiency and aging have independent effects.

During the last few years, there have been significant advances in our understanding of the pathogenetic mechanisms responsible for the bone loss associated with sex steroid deficiency, old age, and glucocorticoid excess. All these conditions do not cause loss of bone mass by turning on a completely new process. Instead, they cause a derangement in the normal process of bone regeneration. Therefore, to understand the pathogenesis of osteoporosis and rationalize its treatment, one must first appreciate the basic principles of physiological bone regeneration.

II. Physiological Bone Regeneration

The skeleton is a highly specialized and dynamic organ that undergoes continuous regeneration. It consists of highly specialized cells, mineralized and unmineralized connective tissue matrix, and spaces that include the bone marrow cavity, vascular canals, canaliculi, and lacunae. During development and growth, the skeleton is sculpted to achieve its shape and size by the removal of bone from one site and deposition at a different one; this process is called modeling. Once the skeleton has reached maturity, regeneration continues in the form of a periodic replacement of old bone with new at the same location (11). This process is called remodeling and is responsible for the complete regeneration of the adult skeleton every 10 yr. The purpose of remodeling in the adult skeleton is not entirely clear, although in bones that are load bearing, remodeling most likely serves to repair fatigue damage and to prevent excessive aging and its consequences. Hence, the most likely purpose of bone remodeling is to prevent accumulation of old bone. Remodeling, with positive balance, does occur in the growing skeleton as well. Its purpose, quite different from those proposed for the adult skeleton, is to expand the marrow cavity while increasing trabecular thickness (12).

A. Remodeling by the basic multicellular unit (BMU)

Removal of bone (resorption) is the task of osteoclasts. Formation of new bone is the task of osteoblasts. Bone resorption and bone formation, however, are not separate, independently regulated processes. In the uninjured adult skeleton, all osteoclasts and osteoblasts belong to a unique temporary structure, known as a basic multicellular unit or BMU (13). Although during modeling one cannot distinguish anatomical units analogous to BMU *per se*, sculpting of the growing skeleton requires spatial and temporal orchestration of the destination of osteoblasts and osteoclasts, albeit with different rules and coordinates to those operating in the BMU of the remodeling skeleton. The BMU, approximately 1–2 mm long and 0.2–0.4 mm wide, comprises a team of osteoclasts in the front, a team of osteoblasts in the rear, a central vascular capillary, a nerve supply, and associated connective tissue (13). In healthy human adults, 3–4 million BMUs are initiated per year and about 1 million are operating at any moment (Table 1). Each BMU begins at a particular place and time (origination) and advances toward a target, which is a region of bone in need of replacement, and for a variable distance beyond its target (progression) and eventually comes to rest (termination) (10). In cortical bone, the BMU travels through the bone, excavating and replacing a tunnel. In cancellous bone, the BMU moves across the trabecular surface, excavating and replacing a trench. In both situations, the cellular components of the BMUs maintain a well orchestrated spatial and temporal relationship with each other. Osteoclasts adhere to bone and subsequently remove it by acidification and proteolytic digestion. As the BMU advances, osteoclasts leave the resorption site and osteoblasts move in to cover the excavated area and begin the process of new bone formation by secreting osteoid, which is eventually mineralized into new bone.

The lifespan of the BMU is 6–9 months; much longer than the lifespan of its executive cells (Table 1). Therefore, continuous supply of new osteoclasts and osteoblasts from their respective progenitors in the bone marrow is essential for the origination of BMUs and their progression on the bone sur-

TABLE 1. Vital statistics of adult bone remodeling[a]

- Lifespan of BMU ~6–9 months
- Speed ~25 μm/day
- Bone volume replaced by a single BMU ~0.025 mm^3
- Lifespan of osteoclasts ~2 weeks
- Lifespan of osteoblasts (active) ~3 months
- Interval between successive remodeling events at the same location ~2–5 years.
- Rate of turnover of whole skeleton ~10% per year[b]

[a] From A. Michael Parfitt (13)

[b] The 10% per year approximation for the entire skeleton is based on an average 4% turnover per year in cortical bone, which represents roughly 75% of the entire skeleton; and an average 28% turnover per year in trabecular bone, which represents roughly 25% of the skeleton ($0.75 \times 4 = 3$ and $0.25 \times 28 = 7$; $3 + 7 = 10$).

face. Consequently, the balance between the supply of new cells and their lifespan are key determinants of the number of either cell type in the BMU and the work performed by each type of cells and are critical for the maintenance of bone homeostasis.

III. Osteoblastogenesis and Osteoclastogenesis

Both osteoblasts and osteoclasts are derived from precursors originating in the bone marrow. The precursors of osteoblasts are multipotent mesenchymal stem cells, which also give rise to bone marrow stromal cells, chondrocytes, muscle cells, and adipocytes (14–16), whereas the precursors of osteoclasts are hematopoietic cells of the monocyte/macrophage lineage (17, 18). Long before these cells could be cultured, the existence of multipotent mesenchymal stem cells was suspected (19), based on the evidence that fibroblastic colonies formed in cultures of adherent bone marrow cells can differentiate, under the appropriate stimuli, into each of the above mentioned cells; these progenitors were named colony forming unit fibroblasts (CFU-F). When CFU-F are cultured in the presence of β-glycerophosphate and ascorbic acid, the majority of the colonies form a mineralized bone nodule; these bone-forming colonies are known as CFU-osteoblast (CFU-OB) (16). Osteoblast progenitors may originate not only from stromal mesenchymal progenitors of the marrow, but also pericytes — mesenchymal cells adherent to the endothelial layer of vessels (20). Whereas osteoclast precursors reach bone from the circulation, osteoblast precursors most likely reach bone by migration of progenitors from neighboring connective tissues.

The development and differentiation of osteoblasts and osteoclasts are controlled by growth factors and cytokines produced in the bone marrow microenvironment as well as adhesion molecules that mediate cell-cell and cell-matrix interactions. Several systemic hormones as well as mechanical signals also exert potent effects on osteoclast and osteoblast development and differentiation. Although many details remain to be established concerning the operation of this network, a few themes have emerged (21). First, several of the growth factors and cytokines control each other's production in a cascade fashion and, in some instances, form positive and negative feedback loops. Second, there is extensive functional redundancy among them. Third, some of the same factors are capable of influencing the differentiation of both osteoblasts and osteoclasts. Fourth, systemic hormones influence the process of osteoblast and osteoclast formation via their ability to control the production and/or action of local mediators.

A. Growth factors and their antagonists

The only factors capable of initiating osteoblastogenesis from uncommitted progenitors are bone morphogenetic proteins (BMPs) (22). BMPs have been long implicated in skeletal development during embryonic life and fracture healing. More recently, it has become apparent that BMPs, and in particular BMP-2 and −4, also initiate the commitment of mesenchymal precursors of the adult bone marrow to the osteoblastic lineage (23). BMPs stimulate the transcription of the gene encoding an osteoblast-specific transcription factor, known as osteoblast specific factor 2 (Osf2) or core binding factor a1 (Cbfa1), hereafter referred to as Cbfa1 (24). In turn, Cbfa1 activates osteoblast-specific genes such as osteopontin, bone sialoprotein, type I collagen, and osteocalcin. The importance of Cbfa1 for osteoblasts has been highlighted by the evidence that knockout of the Cbfa1 gene in mice prevents osteoblast development (25, 26). In addition to Cbfa1, BMP-4 induces a homeobox-containing gene, distal-less 5(Dlx5), which also seems to act as a transcription factor, probably as a heterodimer with another homeobox-containing protein (Msx2). Like Cbfa1, Dlx5 regulates the expression of osteoblast-specific genes such as osteocalcin and alkaline phosphatase, as well as mineralization (27–29). Other factors such as transforming growth factor β (TGFβ), platelet-derived growth factor (PDGF), insulin-like growth factors (IGFs), and members of the fibroblast growth factor (FGF) family can all stimulate osteoblast differentiation (30, 31). However, whereas TGFβ, PDGF, FGF, and IGFs are able to influence the replication and differentiation of committed osteoblast progenitors toward the osteoblastic lineage, they cannot induce osteoblast differentiation from uncommitted progenitor cells.

In addition to growth factors, bone cells produce proteins that modulate the activity of growth factors either by binding to them and thereby preventing interaction with their receptors, by competing for the same receptors, or by promoting the activity of a particular factor. For example, osteoblasts produce several IGF-binding proteins (IGFBPs). Of these, IGFBP-4 binds to IGF and blocks its action, whereas IGFBP-5 promotes the stimulatory effects of IGF on osteoblasts (30). During the last few years, several proteins able to antagonize BMP action have also been discovered. Of them, noggin, chordin, and cerberus were initially found in the Spemann organizer of the *Xenopus* embryo and shown to be essential for neuronal or head development (32–35). Noggin and chordin inhibit the action of BMPs by binding directly and with high affinity with the latter proteins (36, 37). Such binding is highly specific for BMP-2 and 4, as noggin binds BMP-7 with very low affinity and does not bind TGFβ or IGF-I. Addition of human recombinant noggin to bone marrow cell cultures from normal adult mice inhibits not only osteoblast, but also osteoclast, formation, and these effects can be reversed by exogenous BMP-2 (23). Consistent with this evidence, BMP-2 and -4 and BMP-2/4 receptor transcripts and proteins are found in bone marrow cultures and in bone marrow-derived stromal/osteoblastic cell lines, as well as in murine adult whole bone. Noggin expression has also been documented in all these cell preparations. These findings indicate that BMP-2 and -4 are expressed in the bone marrow in postnatal life and serve to maintain the continuous supply of osteoblasts.

B. Cytokines

Since the early stages of hematopoiesis and osteoclastogenesis proceed along identical pathways, it is not surprising that a large group of cytokines and colony-stimulating factors that are involved in hematopoiesis also affect osteoclast development (38). This group includes the interleukins IL-1,

IL-3, IL-6, IL-11, leukemia inhibitory factor (LIF), oncostatin M (OSM), ciliary neurotropic factor (CNTF), tumor necrosis factor (TNF), granulocyte macrophage-colony stimulating factor (GM-CSF), M-CSF, and c-kit ligand. As opposed to the above mentioned cytokines that stimulate osteoclast development, IL-4, IL-10, IL-18, and interferon-γ inhibit osteoclast development. In the case of IL-18 the effect is mediated through GM-CSF (39).

IL-6 has attracted particular attention because of evidence that it plays a pathogenetic role in several disease states characterized by accelerated bone remodeling and excessive focal or systemic bone resorption (40). IL-6 is produced at high levels by cells of the stromal/osteoblastic lineage in response to stimulation by a variety of other cytokines and growth factors such as IL-1, TNF, TGFβ, PDGF, and IGF-II (41–43). Binding of IL-6 or other members of the same cytokine family (IL-11, LIF, OSM) to cytokine-specific cell surface receptors (in the case of IL-6, the IL-6Rα) causes recruitment and homo- or heterodimerization of the signal transducing protein gp130, which is then tyrosine phosphorylated by members of the Janus family of tyrosine kinases (JAKs) (44). This event results in tyrosine phosphorylation of several downstream signaling molecules, including members of the signal transducers and activators of transcription (STAT) family of transcription factors (45, 46). Phosphorylated STATs, in turn, undergo homo- and heterodimerization and translocate to the nucleus where they activate cytokine-responsive gene transcription (47). The α-subunit of the IL-6 receptor also exists in a soluble form (sIL-6R), but unlike most soluble cytokine receptors, it functions as an agonist by binding to IL-6 and then interacting with membrane-associated gp130 to stimulate JAK/STAT signaling (44). On the other hand, the soluble form of gp130 blocks IL-6 action (48).

Alone or in concert with other agents, IL-6 stimulates osteoclastogenesis and promotes bone resorption. The cells that mediate the actions of the IL-6 type cytokines on osteoclast formation appear to be the stromal/osteoblastic cells, as stimulation of IL-6Rα expression on these cells allows them to support osteoclast formation in response to IL-6 (49). These findings indicate that the osteoclastogenic property of IL-6 depends not only on its ability to act directly on hematopoietic osteoclast progenitors, but also on the activation of gp130 signaling in the stromal/osteoblastic cells that provide essential support for osteoclast formation. STAT3 activation in stromal/osteoblastic cells is essential for gp130-mediated osteoclast formation (50). Despite the effects of IL-6 on osteoclastogenesis in experimental *in vitro* systems, IL-6 is not required for osteoclastogenesis *in vivo* under normal physiological conditions. In fact, osteoclast formation is unaffected in sex steroid-replete mice treated with a neutralizing anti-IL-6 antibody, or in IL-6-deficient mice (51, 52). The most likely explanation for this is that the α-subunit of the IL-6 receptor in bone is a limiting factor, and that both a change in the receptor and the cytokine are required for the IL-6-mediated increased osteoclastogenesis, seen in pathological states.

IL-6 type cytokines are capable of influencing the differentiation of osteoblasts as well. Thus, receptors for these cytokines are expressed on a variety of stromal/osteoblastic cells, and ligand binding induces progression toward a more mature osteoblast phenotype, characterized by increased alkaline phosphatase and osteocalcin expression, and a concomitant decrease in proliferation (53, 54). Moreover, IL-6 type cytokines stimulate the development of osteoblasts from noncommitted embryonic fibroblasts obtained from 12-day-old murine fetuses (55). Consistent with the *in vitro* evidence, several *in vivo* studies have demonstrated increased bone formation in transgenic mice overexpressing OSM or LIF (56, 57).

TGFβ is another example of a factor affecting both bone formation and bone resorption (58). Thus, in addition to its ability to stimulate osteoblast differentiation, TGFβ increases bone resorption by stimulating osteoclast formation. Injection of TGFβ into the subcutaneous tissue that overlies the calvaria of adult mice causes increased bone resorption accompanied by the development of unusually large osteoclasts, as well as increased bone formation. The effects of TGFβ might be mediated by other cytokines involved in osteoclastogenesis as TGFβ can stimulate their production. Mice lacking the TGFβ1 gene due to targeted disruption exhibit excessive production of inflammatory cells, suggesting that this growth factor normally operates to suppress hematopoiesis (59).

C. Systemic hormones

The two principal hormones of the calcium homeostatic system, namely PTH and 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$], are potent stimulators of osteoclast formation (17, 60). The ability of these hormones to stimulate osteoclast development and to regulate calcium absorption and excretion from the intestine and kidney, respectively, are the key elements of extracellular calcium homeostasis. Calcitonin, the third of the classical bone-regulating hormones, inhibits osteoclast development and activity and promotes osteoclast apoptosis. Although the antiresorptive properties of calcitonin have been exploited in the management of bone diseases with increased resorption, the role of this hormone in bone physiology in humans, if any, remains questionable (61–63). PTH, PTH-related peptide, and 1,25-$(OH)_2D_3$ stimulate the production of IL-6 and IL-11 by stromal/osteoblastic cells (49, 64–66). Several other hormones, including estrogen, androgen, glucocorticoids, and T_4, exert potent regulatory influences on the development of osteoclasts and osteoblasts by regulating the production and/or action of several cytokines (21, 64, 67–69).

D. Adhesion molecules

In addition to autocrine, paracrine, and endocrine signals, cell-cell and cell-matrix interactions are also required for the development of osteoclasts and osteoblasts (70–72). Such interactions are mediated by proteins expressed on the surface of these cells and are responsible for contact between osteoclast precursors with stromal/osteoblastic cells and facilitation of the action of paracrine factors anchored to the surface of cells that are required for bone cell development. Adhesion molecules are also involved in the migration of osteoblast and osteoclast progenitors from the bone marrow to sites of bone remodeling as well as the cellular polarization

of osteoclasts and the initiation and cessation of osteoclastic bone resorption. More important, for the purpose of this review, adhesion molecules play a role in the control of osteoblast and osteoclast development and apoptosis (73–77).

The list of adhesion molecules that influence bone cell development and function includes the integrins, particularly $\alpha_v\beta_3$ and $\alpha_2\beta_1$, selectins, and cadherins, as well as a family of transmembrane proteins containing a disintegrin and metalloprotease domain (ADAMS). Each of these proteins recognizes distinct ligands. For example, some integrins recognize a specific amino acid sequence (RGD) present in collagen, fibronectin, osteopontin, thrombospondin, bone sialoprotein, and vitronectin (78).

IV. Reciprocal Relationship Between Osteoblastogenesis and Adipogenesis

The cells that comprise the bone marrow stroma can serve several diverse functions including support of hematopoiesis and osteoclastogenesis, fat accumulation, and bone formation (79). This functional adaptation is apparently accomplished by the plasticity of some of the stem cell progeny as exemplified by the ability of stromal cells to convert between the osteoblast and adipocyte phenotype. Thus, a stromal cell type known as the Westen-Bainton cell exhibits PTH receptors and high alkaline phosphatase activity and gives rise to osteoblasts during fetal development and in hyperparathyroidism. On the other hand, when marrow hematopoietic activity is reduced using chemotherapeutic agents, these cells convert into adipocytes and can support myeloid cell production (80–84). Further, adipocytes isolated by limiting dilution from cultures of rabbit bone marrow can form bone in diffusion chamber implants (85). Conversely, addition of certain fatty acids to cultures of osteoblastic cells causes them to differentiate into adipocyte-like cells (86).

It is likely that interconversion of stromal cells among phenotypes, as well as commitment to a particular lineage with suppression of alternative phenotypes, is governed by specific transcription factors. Indeed, Cbfa1 is required for commitment of mesenchymal progenitors to the osteoblast lineage. Mice that are deficient in this factor lack osteoblasts and mineralized bone matrix (26); and expression of Cbfa1 in fibroblastic cells induces transcription of osteoblast-specific genes (24). On the other hand, CCAAT/enhancer binding protein α (C/EBPα), C/EBPβ, and C/EBPδ, as well as peroxisome proliferator activated receptor γ1 (PPARγ1) and PPARγ2 orchestrate adipocyte differentiation (87–90). Introduction of C/EBPα in fibroblastic cells induces adipocyte differentiation (91, 92), and transfection of fibroblastic cells with PPARγ2 and subsequent activation with an appropriate ligand causes the development of adipocytes (93).

Using clonal cell lines isolated from the murine bone marrow, it has been demonstrated that PPARγ2 can convert stromal cells from a plastic osteoblastic phenotype that reversibly expresses adipocyte characteristics to terminally differentiated adipocytes. Moreover, PPARγ2 suppresses the expression of Cbfa1 and thereby osteoblast-specific genes (94). Similar to the inhibitory effect of PPARγ2 on the osteoblast phenotype, the combination of PPARγ and C/EBPα suppresses the muscle cell phenotype when transfected into G8 myoblastic cells (95). Taken together, these findings strongly suggest that PPARγ2 plays a hierarchically dominant role in the determination of the fate of mesenchymal progenitors, due to its ability to inhibit the expression of other lineage-specific transcription factors. Studies with a clonal cell line (2T3) suggest that BMP-2 induces osteoblast or adipocyte differentiation in mesenchymal precursors, depending on whether the BMP receptor type IA or IB is activated. Therefore, BMP receptors may also play a critical role in both the specification and reciprocal differentiation of osteoblast and adipocyte progenitors (96).

V. Serial and Parallel Models of Osteoblast and Osteoclast Development

Even though millions of small packets of bone are constantly remodeled, bone mass is preserved thanks to a remarkably tight balance between the amount of bone resorbed and formed during each cycle of remodeling. In any established BMU, bone resorption and formation are happening at the same time; new osteoblasts assemble only at sites where osteoclasts have recently completed resorption, a phenomenon referred to as coupling, and formation begins to occur while resorption advances. The end result is a new packet of bone, either a cylindrical osteon or Haversian system, or a plate-like hemiosteon, that has replaced the older bone that was removed (97).

As the BMU advances, cells are successively recruited at each new cross-sectional location. Osteoblasts do not arrive until the osteoclasts have moved on. However, during the longitudinal progression of the BMU as a whole, new osteoclasts and new osteoblasts are needed simultaneously, although not at the same location. Two models of osteoblast recruitment, a serial and a parallel (Fig. 1), can explain the distinction between the cross-sectional and longitudinal events during BMU progression (38). According to the serial model, factors released from resorbed bone or the local increase in mechanical strain resulting from bone resorption, stimulate osteoblast precursor cell proliferation and differentiation (98–100). According to the parallel model, osteoblast and osteoclast precursor proliferation and differentiation occur concurrently in response to whatever signal conveys the need for initiation of new BMUs, and whatever hormone prolongs their progression (10, 38). With either model, new osteoblasts must be directed to the right location.

Concurrent osteoblast and osteoclast production makes teleological sense as at least one of the means of maintaining a balance between bone formation and resorption under normal conditions. In support of the existence of a parallel pathway of osteoblast and osteoclast formation, it is well established that osteoclasts cannot be formed *in vitro* unless appropriate stromal cells, analogous to the bone marrow stromal cells that support hematopoiesis, are present to provide essential support. The precise phenotype of the cells that support osteoclast development remains unknown, but they are clearly related to both the osteoblast and the bone marrow stromal/adipocytic lineages (101–104). Interestingly, bone marrow-derived cells with both osteoblastic and adi-

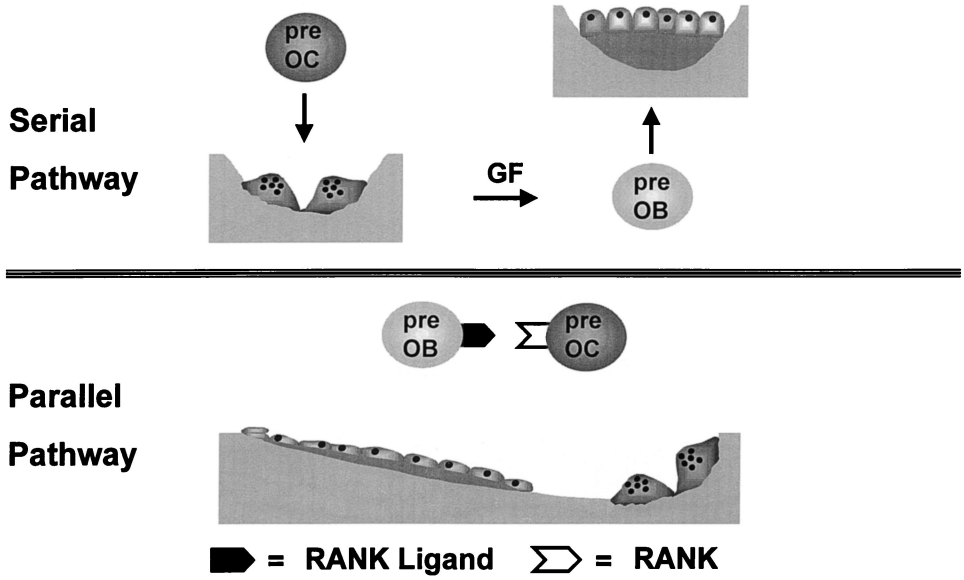

FIG. 1. Serial and parallel models of osteoblast and osteoclast development. For explanation, please see text. PreOC, Preosteoclast; GF, growth factors released from the matrix of resorbed bone; preOB, osteoblast progenitors. The expressions of RANK ligand and RANK on preosteoblasts and preosteoclasts, respectively, are depicted to indicate their critical contribution in osteoclastogenesis and thereby the dependency of osteoclastogenesis on preosteoblastic cells.

pogenic characteristics support the formation of osteoclasts, but marrow-derived cells that exhibit a purely osteoblastic or adipocytic phenotype do not (94). More to the point, noggin, a BMP antagonist, blocks not only osteoblastogenesis but also osteoclastogenesis in murine bone marrow cultures, indicating that commitment of mesenchymal progenitors to the osteoblastic lineage is prerequisite for osteoclastogenesis (23). This evidence suggests that the early less differentiated progeny of common mesenchymal progenitors of the osteoblastic and adipocytic lineage can support osteoclast development, but more differentiated cells that have committed to either the osteoblast or the adipocyte pathway lose this property. It is possible, but as yet untested, that the cells that provide support for osteoclast development are a distinct progeny of mesenchymal progenitors, which displays permanently a phenotype with mixed adipocytic/osteoblastic characteristics, but never progresses to a terminally differentiated osteoblast or adipocyte. For convenience and lack of a better term, the cells that support osteoclast development are frequently referred to as stromal/osteoblastic to indicate their similarities to both bone marrow stromal cells and osteoblasts.

In full agreement with the *in vitro* evidence for the dependency of osteoclast development on support by cells related to osteoblasts, mice lacking osteoblasts due to Cbfa1 deficiency also lack osteoclasts (26). In addition, marrow cells from SAMP6 mice, a strain with defective osteoblastogenesis, exhibit decreased osteoclastogenesis (105) and do not exhibit the expected increase in osteoclastogenesis nor do they lose bone after loss of sex steroids (106).

The molecular mechanism of the dependency of osteoclastogenesis on cells of the mesenchymal lineage has been elucidated during the last 2 yr with the discovery of three proteins involved in the TNF signaling pathway (reviewed in Ref. 107). Two of these proteins are membrane-bound cytokine-like molecules: the receptor activator of nuclear factor-κB (NF-κB) (RANK) and the RANK-ligand. Other names used in the literature for RANK are osteoprotegerin ligand (OPG-L) and TRANCE. RANK is expressed in hematopoietic osteoclast progenitors, while RANK-ligand is expressed in committed preosteoblastic cells and T lymphocytes (108–110). RANK-ligand binds to RANK with high affinity. This interaction is essential and, together with M-CSF, sufficient for osteoclastogenesis. 1,25-$(OH)_2D_3$, PTH, PTHrP, gp130 activating cytokines (*e.g.*, IL-6, IL-11), and IL-1 induce the expression of the RANK-ligand in stromal/osteoblastic cells (50, 107). Osteoprotegerin (OPG), the third of the three proteins, unlike the other two, is a secreted disulfide-linked dimeric glycoprotein. A hydrophobic leader peptide and three and one-half TNF receptor-like cysteine-rich pseudorepeats characterize the amino terminus of this protein. Unlike other members of the TNF receptor family, OPG does not posses a transmembrane domain. OPG has very potent inhibitory effects on osteoclastogenesis and bone resorption *in vitro* and *in vivo* (111). Consistent with an important role of OPG in the regulation of osteoclast formation, OPG transgenic mice develop osteopetrosis, whereas OPG knockout mice exhibit severe osteoporosis (112). The antiosteoclastogenic property of OPG is due to its ability to act as a decoy by binding to RANK-ligand and blocking the RANK-ligand/RANK interaction. In addition to skeletal metabolism, the RANK/RANK-ligand/OPG circuit may regulate several other biological systems. Indeed, OPG is produced by many tissues other than bone, including skin, liver, stomach, intestine, lung, heart, kidney, and placenta as well as hematopoietic and immune organs. Consistent with this, mice deficient in RANK-ligand completely lacked lymph nodes as well as osteoclasts (113). Moreover, OPG is also a receptor for the cytotoxic ligand TRAIL (TNF-related apoptosis-inducing ligand) to which it binds with high affinity and inhibits TRAIL-mediated apoptosis in lymphocytes (114) and also regulates antigen presentation and T cell activation (115).

Osteoblastic cells and T lymphocytes, the two cell types that express high levels of RANK-ligand, are also the two cell types that express high levels of the osteoblast-specific transcription factor Cbfa1 (24). More intriguingly, both the murine and human RANK-ligand genes contain two functional Cbfa1 sites, and mutation of these sites abrogates the tran-

scriptional activity of the RANK-ligand gene promoter (116). Therefore, the cell-specific expression of RANK-ligand in cells of the stromal/osteoblastic lineage and concurrent differentiation of osteoblasts and osteoclasts might be dictated, at least in part, by interaction between an osteoblast-specific transcription factor and RANK-ligand. BMP 2 and -4 stimulate Cbfa1 expression. Based on these lines of evidence, it has been postulated that the molecular underpinnings of the control of the rate of bone regeneration and the concurrent production of osteoclasts and osteoblasts could well be a **BMP→Cbfa1→RANK-ligand** gene expression cascade in cells of the bone marrow stromal/osteoblastic lineage (117, 118). According to this hypothesis, BMPs may provide the tonic baseline control of both processes, and thereby the rate of bone remodeling, upon which other inputs (*e.g.*, biomechanical, hormonal, etc.) operate.

Studies with transgenic and knockout animal models as well as models with spontaneous genetic mutations have identified at least three transcription factors that are required for osteoclast differentiation: PU-1, fos, and NF-κb. A review of the precise role of these factors is beyond the scope of this article, but the reader is referred to a recent excellent review of the topic (119).

VI. Function of the Mature Cells

A. Osteoblasts

The fully differentiated osteoblasts produce and secrete proteins that constitute the bone matrix (120). The matrix is subsequently mineralized under the control of the same cells. A major product of the bone-forming osteoblast is type I collagen. This polymeric protein is initially secreted in the form of a precursor, which contains peptide extensions at both the amino-terminal and carboxyl ends of the molecule. The propeptides are proteolytically removed. Further extracellular processing results in mature three-chained type I collagen molecules, which then assemble themselves into a collagen fibril. Individual collagen molecules become interconnected by the formation of pyridinoline cross-links, which are unique to bone. Bone-forming osteoblasts synthesize a number of other proteins that are incorporated into the bone matrix, including osteocalcin and osteonectin, which constitute 40% to 50% of the noncollagenous proteins of bone. Mice deficient in osteocalcin develop a phenotype marked by higher bone mass and improved bone quality, suggesting that osteocalcin functions normally to limit bone formation without compromising mineralization (121). Conversely, mice deficient in osteonectin exhibit decreased osteoclast and osteoblast numbers and bone remodeling and profound osteopenia, suggesting that, under normal conditions, this protein may play a role in the birth or survival of these cells (122). Other osteoblast-derived proteins include glycosaminoglycans, which are attached to one of two small core proteins: PGI (or biglycan) and decorin; the latter has been implicated in the regulation of collagen fibrillogenesis. A number of other minor proteins such as osteopontin, bone sialoprotein, fibronectin, vitronectin, and thrombospondin serve as attachment factors that interact with integrins.

In addition to being the cells that produce the osteoid matrix, mature osteoblasts are essential for its mineralization, the process of deposition of hydroxyapatite (123, 124). Osteoblasts are thought to regulate the local concentrations of calcium and phosphate in such a way as to promote the formation of hydroxyapatite. In view of the highly ordered, well aligned, collagen fibrils complexed with the noncollagenous proteins formed by the osteoblast in lamellar bone, it is likely that mineralization proceeds in association with, and perhaps governed by, the heteropolymeric matrix fibrils themselves. Osteoblasts express relatively high amounts of alkaline phosphatase, which is anchored to the external surface of the plasma membrane. Alkaline phosphatase has been long thought to play a role in bone mineralization. Consistent with this, deficiency of alkaline phosphatase due to genetic defects leads to hypophosphatasia, a condition characterized by defective bone mineralization (125). However, the precise mechanism of mineralization and the exact role of alkaline phosphatase in this process remain unclear. Bone mineralization lags behind matrix production and, in remodeling sites in the adult bone, occurs at a distance of 8–10 μm from the osteoblast. Matrix synthesis determines the volume of bone but not its density. Mineralization of the matrix increases the density of bone by displacing water, but does not alter its volume.

B. Osteocytes

Some osteoblasts are eventually buried within lacunae of mineralized matrix. These cells are termed osteocytes and are characterized by a striking stellate morphology, reminiscent of the dendritic network of the nervous system (126, 127). Osteocytes are the most abundant cell type in bone: there are 10 times as many osteocytes as osteoblasts. Osteocytes are regularly spaced throughout the mineralized matrix and communicate with each other and with cells on the bone surface via multiple extensions of their plasma membrane that run along the canaliculi; osteoblasts, in turn, communicate with cells of the bone marrow stroma which extend cellular projections onto endothelial cells inside the sinusoids. Thus, a syncytium extends from the entombed osteocytes all the way to the vessel wall (128) (Fig. 2). As a consequence, the strategic location of osteocytes makes them excellent candidates for mechanosensory cells able to detect the need for bone augmentation or reduction during functional adaptation of the skeleton, and the need for repair of microdamage, and in both cases to transmit signals leading to the appropriate response; albeit this remains hypothetical (129). Osteocytes evidently sense changes in interstitial fluid flow through canaliculi (produced by mechanical forces) and detect changes in the levels of hormones, such as estrogen and glucocorticoids, that influence their survival and that circulate in the same fluid (130–132). Therefore, disruption of the osteocyte network is likely to increase bone fragility.

C. Lining cells

The surface of normal quiescent bone (*i.e.*, bone that is not undergoing remodeling) is covered by a 1–2-μm thick

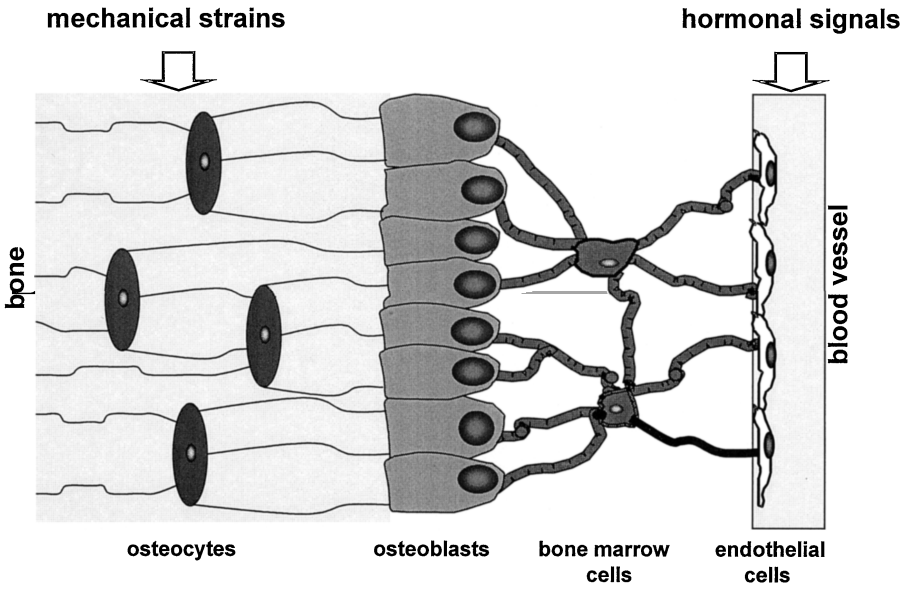

FIG. 2. Functional syncytium comprising osteocytes, osteoblasts, bone marrow stromal cells, and endothelial cells. [Adapted from G. Marotti and reproduced with the permission of the Editor of *Journal of Clinical Investigation* 104: 1363–1374, 1999 (219).

layer of unmineralized collagen matrix on top of which there is a layer of flat and elongated cells. These cells are called lining cells and are descendents of osteoblasts (13). Conversion of osteoblasts to lining cells represents one of the fates of osteoblasts that have completed their bone forming function; another being entombment into the matrix as osteocytes. Osteoclasts cannot attach to the unmineralized collagenous layer that covers the surface of normal bone. Therefore, other cells, perhaps the lining cells, secrete collagenase, which removes this matrix before osteoclasts can attach to bone. It has been proposed that targeting of osteoclast precursors to a specific location on bone depends on a "homing" signal given by lining cells; and that lining cells are instructed to do so by osteocytes, the only bone cells that can sense the need for remodeling at a specific time and place (133).

D. Osteoclasts

Mature osteoclasts are usually large (50 to 100 μm diameter) multinucleated cells with abundant mitochondria, numerous lysosomes, and free ribosomes. Their most remarkable morphological feature is the ruffled border, a complex system of finger-shaped projections of the membrane, the function of which is to mediate the resorption of the calcified bone matrix (17, 123). This structure is completely surrounded by another specialized area, called the clear zone. The cytoplasm in the clear zone area has a uniform appearance and contains bundles of actin-like filaments. The clear zone delineates the area of attachment of the osteoclast to the bone surface and seals off a distinct area of the bone surface that lies immediately underneath the osteoclast and which eventually will be excavated. The ability of the clear zone to seal off this area of bone surface allows the formation of a microenvironment suitable for the operation of the resorptive apparatus.

The mineral component of the matrix is dissolved in the acidic environment of the resorption site, which is created by the action of an ATP-driven proton pump (the so-called vacuolar H+-ATPase) located in the ruffled border membrane. The protein components of the matrix, mainly collagen, are degraded by matrix metalloproteinases, and cathepsins K, B, and L are secreted by the osteoclast into the area of bone resorption (134). The degraded bone matrix components are endocytosed along the ruffled border within the resorption lacunae and then transcytosed to the membrane area opposite the bone, where they are released (135, 136). Another feature of osteoclasts is the presence of high amounts of the phosphohydrolase enzyme, tartrate-resistant acid phosphatase (TRAPase). This feature is commonly used for the detection of osteoclasts in bone specimens (137). Mice deficient in TRAPase exhibit a mild osteopetrotic phenotype (due to an intrinsic defect of osteoclastic resorptive activity) and defective mineralization of the cartilage in developing bones (138).

VII. Death of Bone Cells by Apoptosis

The average lifespan of human osteoclasts is about 2 weeks, while the average lifespan of osteoblasts is 3 months (Table 1). After osteoclasts have eroded to a particular distance, either from the central axis in cortical bone or to a particular depth from the surface in cancellous bone, they die and are quickly removed by phagocytes (139). The majority (65%) of the osteoblasts that originally assembled at the remodeling site also die (140). The remaining are converted to lining cells that cover quiescent bone surfaces or are entombed within the mineralized matrix as osteocytes (Fig. 3A). Both osteoclasts and osteoblasts die by apoptosis, or programmed cell death, a process common to several regenerating tissues (141). As in other tissues, bone cells undergoing apoptosis are recognized by condensation of chromatin, the degradation of DNA into oligonucleosome-sized fragments, and the formation of plasma and nuclear membrane blebs (Fig. 4). Eventually the cell breaks apart to form so-called apoptotic bodies. Osteoblast apoptosis explains the fact that 50–70%

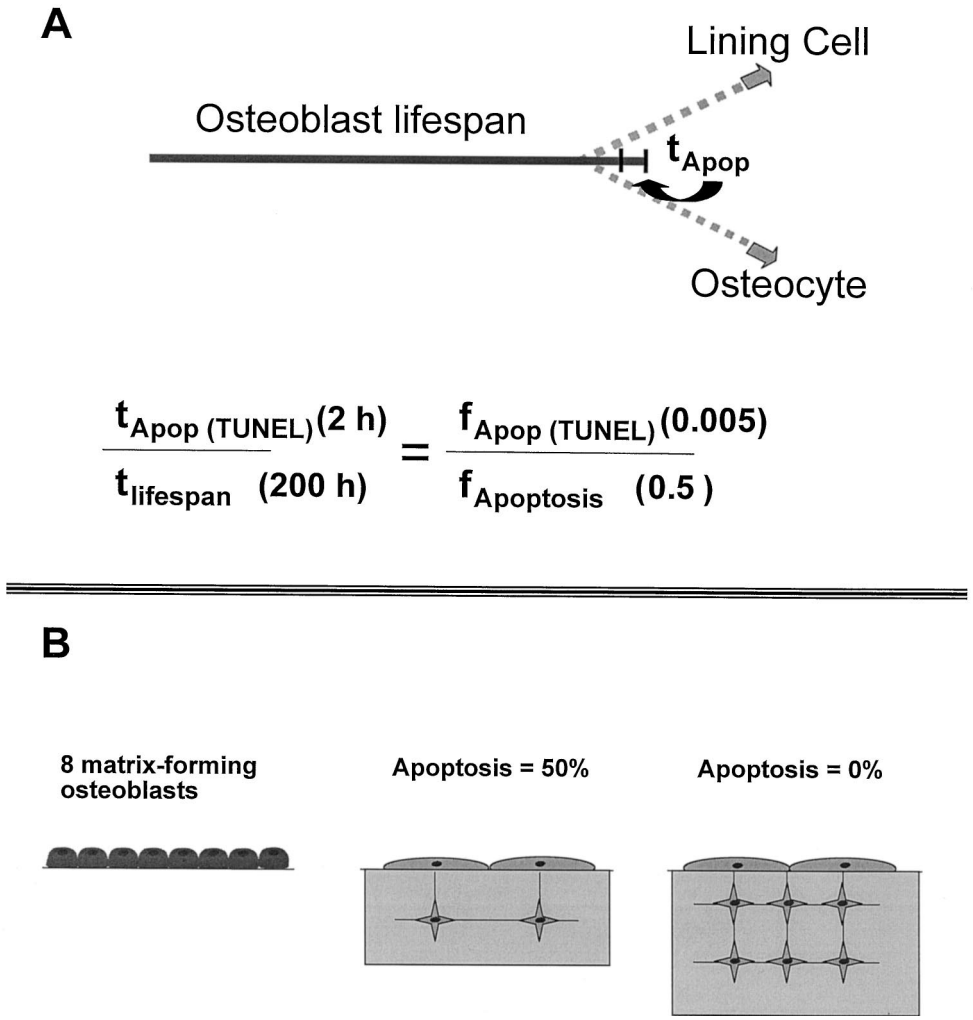

FIG. 3. Osteoblast apoptosis and its implications. *A,* The average life span of a matrix forming osteoblast (~200 h in the mouse) is indicated by the *continuous line*. The process of apoptosis represents only a small fraction of this time period. The alternative two fates of osteoblasts are to become lining cells or osteocytes. The fraction of osteoblasts that undergo apoptosis *in vivo* ($f_{Apoptosis}$) can be estimated from a bone biopsy specimen. The duration of the apoptosis phase that can be observed in the specimen (t_{Apop}) depends on the sensitivity of the detection method. For example, in the case of the TUNEL technique (without $CuSO_4$ enhancement), the TUNEL-labeled phase of apoptosis is estimated to be approximately 2 h. In a steady state, the fraction of cells at a particular stage is the same as the corresponding fraction of time spent in that stage. Assuming an apoptosis detection time of 2 h and a 200-h life span, a prevalence of TUNEL positive osteoblasts in the biopsy of 0.005 indicates that half of the osteoblasts die by apoptosis. B, A change in the timing and extent of osteoblast apoptosis ($f_{Apoptosis}$) from 50% to zero should increase the number of osteoblasts present at the site of bone formation and thereby the work output, *i.e.,* the amount of bone formed by a given team of matrix-forming osteoblasts. It will also lead to an increase in the density of osteocyte apoptosis, as illustrated by the example shown.

of the osteoblasts initially present at the remodeling site of human bone cannot be accounted for after enumeration of lining cells and osteocytes (142). Moreover, the frequency of osteoblast apoptosis *in vivo* is such that changes in its timing and extent could have a significant impact in the number of osteoblasts present at the site of bone formation (130). Osteocytes are long-lived but not immortal cells; some die by apoptosis (132, 143, 144). Osteocyte apoptosis could be of importance to the origination and/or progression of the BMU. Indeed, osteocytes are the only cells in bone that can sense the need for remodeling at a specific time and place. Moreover, osteocytes are in direct physical contact with lining cells on the bone surface, and targeting of osteoclast precursors to a specific location on bone depends on a "homing" signal given by lining cells (133).

The same growth factors and cytokines that stimulate osteoclast and osteoblast development can also influence their apoptosis. For example, TGFβ promotes osteoclast apoptosis while it inhibits osteoblast apoptosis. IL-6 type cytokines have antiapoptotic effects on animal and human osteoblastic cells (and at least *in vitro* they antagonize proapoptotic effects of glucocorticoids) as well as on osteoclasts and their progenitors (54, 140, 145, 146).

VIII. Regulation of Bone Cell Proliferation and Activity

A large body of literature suggests that growth factors, cytokines, hormones, and drugs regulate the proliferation of

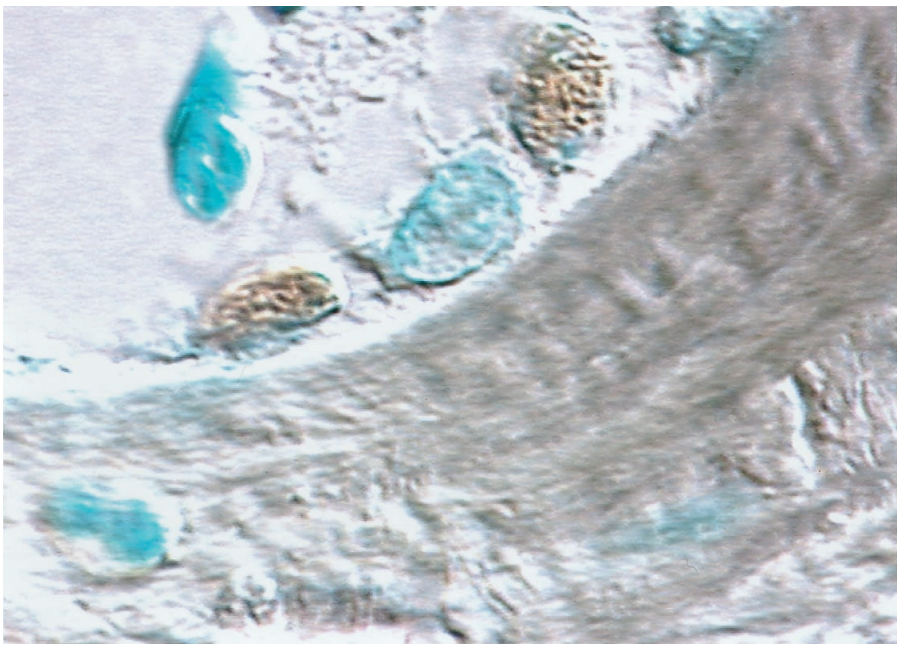

FIG. 4. Two osteoblasts undergoing apoptosis in a section of murine cancellous bone (TUNEL staining with toluidine blue counterstain, ×630). Apoptotic osteoblasts (shown in *brown*) are adjacent to an intact osteoblast (shown in *blue*), on the surface of a trabecula occupying the *right lower portion* of the picture in which two intact (*blue stained*) osteocytes are also seen. Apoptotic osteoblasts display nuclear condensation and fragmentation. [Photomicrograph provided by Robert S. Weinstein, M.D., University of Arkansas for Medical Sciences.]

committed cells or the biosynthetic and functional activity of the differentiated osteoblasts and osteoclasts. Hence, in addition to cell number, alterations in the functional activity of individual cells, *i.e.*, cell vigor, may contribute to changes in the rate of bone resorption and formation. However, because of the inherent difficulty in demonstrating changes in individual cell vigor *in vivo*, the vast majority of such literature and its conclusions rely heavily, if not exclusively, on *in vitro* experimentation. A detailed discussion of this work is beyond the scope of this review, and the reader is referred to other articles (30, 147–151). Nonetheless, some general aspects merit discussion here, as they are important for putting the significance of birth rate and apoptosis into a broader perspective.

In regenerating tissues, the initial commitment of a stem cell progeny is followed by amplification with several or many rounds of cell division. In most tissues, division of stem cells is infrequent, and almost all of the divisions that produce the final population of differentiated cells occur in the so-called transit compartment (152). This notion is obscured by the frequent practice of showing linear diagrams representing the transition from one cell type to another and ignoring completely the amplification during the transition. In general, terminally differentiated cells do not divide, and an osteoblast, defined as a cell making bone matrix, and osteoclast, as a cell resorbing bone, are in this category. Therefore, the *in vivo* relevance of much of the *in vitro* evidence on the regulation of osteoblastic or osteoclastic cell proliferation, using established cell lines or primary cultures of isolated cells, must be largely confined to changes in this transit compartment. Irrespective of whether a given regulatory factor, be that a cytokine or a hormone, influences the initial commitment of a stem cell, or the subsequent amplification of its progeny, or both, the end result is a change in the rate of production and therefore the number of cells available for the execution of the biological task. For the sake of simplicity, the terms birth, rate of birth, osteoblastogenesis, or osteoclastogenesis, as used in this article, implicitly combine commitment and amplification.

The *in vivo* relevance of numerous reports of *in vitro* observations of changes in the biosynthetic activity of osteoblastic cells, *e.g.*, changes in the level of expression of osteocalcin or alkaline phosphatase in response to a given agent, is also a matter of conjecture. Most likely, given the nature of commonly used *in vitro* cell models which, by and large, represent preterminally differentiated cells, such observations might be more relevant to postcommitment differentiation events, than to altered activity of the fully differentiated cell. Moreover, even if some agents can alter the function of terminally differentiated cells in short-term cultures *in vitro*, heretofore there is no evidence that short-term change in the rate of collagen production or bone matrix digestion, for example, are ultimately translated into differences in the amounts of bone matrix formed or resorbed.

In the bone literature there is considerable ambiguity when using the term "activation." It is important that one distinguishes between activation as a switch from an off state to an on state, and activation as modulation of activity of an already active cell. Morphological evidence summarized elsewhere does not support the notion that completely inactive osteoclasts are waiting for a stimulus to make them active (117). However, this evidence does not address the issue of whether there are variations in the rate of bone matrix dissolution by individual osteoclasts after they started work.

Studies with the widely used bone slice pit bioassay have shown that several regulatory factors can cause a decrease or increase in the resorptive ability of individual osteoclasts (153–155). However, it is not clear to what extent these observations reflect a change in cell vigor as opposed to a change in the precariously short lifespan of osteoclasts *in vitro*. In estrogen deficiency, individual osteoclasts are seemingly more "active" as they dig deeper resorption cavities often leading to trabecular perforation, but it has been con-

vincingly argued that this is due to delayed apoptosis (133). In Paget's disease osteoclasts are certainly far more aggressive than normal, perhaps as a result of their uniquely large size and number of nuclei (156). Today, the most compelling evidence in support of the notion that the vigor of individual osteoclasts may not always be maximal comes from *in vitro* as well as *in vivo* experiments with soluble RANK-ligand (157). Specifically, it has been found that RANK-ligand acts on mature rat osteoclasts *in vitro* to stimulate more frequent cycles of resorption and induce rearrangements of the cytoskeleton. Moreover, intravenous administration of RANK-ligand to mice elevates the circulating concentration of ionized calcium within 1 h. RANK-ligand has potent anti-apoptotic effects on cultured osteoclasts (Ref. 158 and William Boyle, personal communication). Therefore, definitive conclusions regarding RANK-ligand's ability to modulate osteoclast vigor will have to await dissection of the contribution of its effects on osteoclast survival *in vivo*. Similar to osteoclasts, the bone-forming ability of osteoblasts may not be always maximal, as suggested by the evidence that PTH can rapidly enhance bone formation when administered by subcutaneous injections to rats (159).

To conclude this section, it is intuitive that the amount of bone resorbed or formed by a team of osteoclasts and osteoblasts should be a function of the total cell number as well as the vigor of individual cells. However, whereas cell number can be quantified on bone sections from animals and humans with conventional histomorphometric techniques, quantification of individual cell vigor cannot. This situation makes it difficult to judge at present whether cell vigor is or is not a critical component in the pathogenesis of abnormal skeletal regeneration in common acquired metabolic bone diseases such as postmenopausal, senile, or steroid-induced osteoporosis. For this reason and space limitations, changes in bone cell vigor or other potential mechanisms of osteoporosis resulting from changes in extraskeletal tissues, for example altered calcium absorption or excretion, will not be discussed in the following section. This also reflects the author's intention to focus on the dynamics of bone cell number, rather than a dismissal of other mechanisms.

IX. Pathogenesis Of Osteoporosis

From the brief discussion of the principles of physiological bone regeneration and the role of osteoblasts and osteoclasts in the process, it is obvious that the rate of supply of new osteoblasts and osteoclasts and the timing of the death of these cells by apoptosis are critical determinants of the initiation of new BMUs and/or extension or shortening of the lifetime of existing ones. Recent advances in our understanding of the pathogenesis of the various forms of osteoporosis have confirmed this truism by revealing that over- or undersupply of these cells relative to the need for remodeling are the fundamental problems in all these conditions (160) (Table 2).

A. Sex steroid deficiency

The mechanism of action of sex steroids on the skeleton is not fully understood. At menopause (or after castration in men), the rate of bone remodeling increases precipitously. This fact may be explained by evidence, derived primarily from studies in mice, that loss of sex steroids up-regulates the formation of osteoclasts and osteoblasts in the marrow by up-regulating the production and action of cytokines that are responsible for osteoclastogenesis and osteoblastogenesis (21, 161, 162). Indeed, both estrogen and androgen suppress the production of IL-6, as well as the expression of the two subunits of the IL-6 receptor, IL-6Rα and gp130, in cells of the bone marrow stromal/osteoblastic lineage (40, 163). Suppression of IL-6 production by estrogen or selective estrogen receptor modulators (SERMs), such as raloxifene, does not require direct binding of the estrogen receptor to DNA. Instead, it is due to protein-protein interaction between the estrogen receptor and transcription factors such as NF-Kβ and C/EBP. This mechanism provides a model that best fits current understanding of the molecular pharmacology of estrogen and SERMs (164). Consistent with the suppressive effect of sex steroids on IL-6 and its receptor, several, albeit not all, studies have shown that the level of expression of IL-6, as well as IL-6Rα and gp130, is elevated in estrogen-

TABLE 2. Cellular changes and their culprits in the three most common causes of bone loss

	Cellular changes	Probable culprits
Sex steroid deficiency	↑ Osteoblastogenesis	Increased IL-6; TNF; IL-1RI/IL-RII
	↑ Osteoclastogenesis[a]	MCSF; decreased TGFβ; OPG
	↑ Lifespan of osteoclasts	Loss of pro- and antiapoptotic effects of sex steroids, respectively
	↓ Lifespan of osteoblasts	
	↓ Lifespan of osteocytes	
Senescence	↓ Osteoblastogenesis[b]	Increased PPARγ2, PGJ2, noggin; Deceased IL-11, IGFs
	↓ Osteoclastogenesis	
	↑ Adipogenesis	
	↓ Lifespan of osteocytes	
Glucocorticoid excess	↓ Osteoblastogenesis	Decreased Cbfal and TGFβ R1; and BMP-2 and IGF1 action
	↓ Osteoclastogenesis	
	↑ Adipogenesis	Increased PPARγ2
	↑ Lifespan of osteoclasts[c]	
	↓ Lifespan of osteoblasts	Decreased Bcl-2/BAX ratio
	↓ Lifespan of osteocytes	

[a] Oversupply of osteoclasts relative to the need for remodeling.
[b] Undersupply of osteoblasts relative to the need for cavity repair.
[c] Osteoclast numbers may transiently increase in the earlier stages of steroid therapy, without an increase in osteoclastogenesis, indicating increased lifespan.

deficient mice and rats as well as in humans, in the bone marrow and in the peripheral blood (165–168). Furthermore, neutralization of IL-6 with antibodies or knockout of the IL-6 gene in mice prevents the expected cellular changes in the marrow and in trabecular bone sections and protects the mice from bone loss after loss of sex steroids (51, 67). Consistent with its pathogenetic role in the bone loss caused by loss of sex steroids, IL-6 seems to play a similar role in several other conditions associated with increased bone resorption as evidenced by increased local or systemic production of IL-6 and the IL-6 receptor in patients with multiple myeloma, Paget's disease, rheumatoid arthritis, Gorham-Stout or disappearing bone disease, hyperthyroidism, primary and secondary hyperparathyroidism, as well as McCune Albright Syndrome (66, 68, 169–174).

In line with the fact that loss of sex steroids increases the rate of bone remodeling, in addition to up-regulating osteoclastogenesis, loss of sex steroids increases the number of osteoblast progenitors in the murine bone marrow. These changes are temporally associated with increased bone formation and parallel the increased osteoclastogenesis and bone resorption (175). As IL-6 type cytokines can stimulate osteoblast development and differentiation (54, 55, 146), increased sensitivity to IL-6 and other members of this cytokine's family may account also for the increased osteoblast formation that follows the loss of gonadal function. In view of the fact that mesenchymal cell differentiation and osteoclastogenesis are tightly linked, stimulation of mesenchymal cell differentiation toward the osteoblastic lineage after sex steroid loss may be the first event that ensues after the hormonal change, and increased osteoclastogenesis and bone loss might be downstream consequences of this change (106).

In addition to IL-6, estrogen also suppress TNF and M-CSF (176, 177), and estrogen loss may increase the sensitivity of osteoclasts to IL-1 by increasing the ratio of the IL-1RI over the IL-1 receptor antagonist (IL-RII) (178). As in the case of IL-6, the effects of estrogen on TNF and M-CSF are mediated via protein-protein interactions between the estrogen receptor and other transcription factors. In agreement with the evidence that IL-1 and TNF play a role in the bone loss caused by loss of estrogen, administration of IL-1RA and/or a TNF soluble receptor ameliorates the bone loss caused by ovariectomy in rats and mice (179–181). Because of the interdependent nature of the production of IL-1, IL-6, and TNF, a significant increase in one of them may amplify, in a cascade fashion, the effect of the others (161). Interestingly, recent *in vitro* studies with human osteoblastic cells indicate that OPG production is stimulated by estrogen, suggesting that this cytokine may also play an important role in the antiosteoclastogenic (antiresorptive) action of estrogen on bone (182).

Increased remodeling, resulting from up-regulation of osteoblastogenesis and osteoclastogenesis, alone can cause a transient acceleration of bone mineral loss because bone resorption is faster than bone formation, and new bone is less dense than older bone. However, in addition to increased bone remodeling, loss of sex steroids leads to a qualitative abnormality: osteoclasts erode deeper than normal cavities (133, 183). In this manner, sex steroid deficiency leads to the removal of some cancellous elements entirely; the remainder are more widely separated and less well connected. An equivalent amount of cancellous bone distributed as widely separated, disconnected, thick trabeculae is biomechanically less competent than when arranged as more numerous, connected, thin trabeculae. Concurrent loss of cortical bone occurs by enlargement and coalescence of subendocortical spaces, a process due to deeper penetration of endocortical osteoclasts.

This deeper erosion can be now explained by evidence that estrogen acts on mature osteoclasts to promote their apoptosis; consequently, loss of estrogen leads to prolongation of the lifespan of osteoclasts (133). Specifically, estrogen promotes osteoclast apoptosis *in vitro* and *in vivo* by 2- to 3-fold, an effect seemingly mediated by TGFβ (139). In direct contrast to their proapoptotic effects on osteoclasts, estrogen (as well as androgen) exerts antiapoptotic effects on osteoblasts and osteocytes; consequently, loss of estrogen or androgen leads to shorter lifespan of osteoblasts and osteocytes (184). Extension of the working life of the bone-resorbing cells and simultaneous shortening of the working life of the bone-forming cells, can explain the imbalance between bone resorption and formation that ensues after loss of sex steroids. Furthermore, the increase in osteocyte apoptosis could further weaken the skeleton by impairment of the osteocyte-canalicular mechanosensory network. The increase in bone remodeling that occurs with estrogen deficiency would partly replace some of the nonviable osteocytes in cancellous bone, but cortical apoptotic osteocytes might accumulate because of their anatomic isolation from scavenger cells and the need for extensive degradation to small molecules to dispose of the osteocytes through the narrow canaliculi. Hence, the accumulation of apoptotic osteocytes caused by loss of estrogen, or glucocorticoid excess (130), could increase bone fragility even before significant loss of bone mass, because of the impaired detection of microdamage and repair of substandard bone.

In conclusion, the increased rate of bone remodeling in estrogen deficiency may be due to increased production of both osteoclasts and osteoblasts, and the imbalance between bone resorption and formation is due to an extension of the working lifespan of the osteoclast and shortening of the working lifespan of the osteoblast. Moreover, a delay of osteoclast apoptosis seems responsible for the deeper resorption cavities and thereby the trabecular perforation associated with estrogen deficiency.

Clinical observations of decreased bone mass in a male with mutant estrogen receptor (185), and increased bone mass after treatment with estrogen in two males with P-450 aromatase deficiency (186, 187), have raised the possibility that estrogen derived by peripheral aromatization of androgens is critical for the maintenance of bone mass in men as well as in women (188). However, in all three cases, the decreased bone mass in young males with estrogen deficiency in the face of androgen sufficiency could be due to failure in achieving peak bone mass from defects occurring during development or growth, not to loss of bone mass, as it is the case with common forms of osteoporosis. In addition, individuals with complete androgen insensitivity, due to mutations in the androgen receptor gene on the X chromosome and increased testosterone and estrogen production,

have decreased bone mass, in spite of the elevated estrogen levels (189). Moreover, androgens, including nonaromatizable ones, have identical effects to those of estrogen on the biosynthetic activity and the birth as well as the death of bone cells *in vitro* and *in vivo*, at least in rodents (67, 106, 190). It is therefore more likely that both estrogen and androgen are important for the maintenance of bone mass in the adult male skeleton.

B. Senescence

The amount of bone formed during each remodeling cycle decreases with age in both sexes. This is indicated by a consistent histological feature of the osteopenia that occurs during aging, namely a decrease in wall thickness, especially in trabecular bone (191–193). Wall thickness is a measure of the amount of bone formed in a remodeling packet of cells and is determined by the number or activity of osteoblasts at the remodeling site.

Studies measuring bone turnover by histomorphometry (194), or indirectly by circulating markers (195–197), have suggested that in aging women, even in extreme old age, bone turnover is most likely increased by secondary hyperparathyroidism or by the continuing effect of estrogen deficiency. Increased turnover and reduced wall thickness are not inconsistent, as the former is the result of increased activation frequency, and the decreased wall thickness—an index of decreased bone formation by osteoblasts—in senescence is local and relative to the demand created by resorption.

Changes in the birth of bone cells in the bone marrow provide a mechanistic explanation for the contribution of senescence to bone loss, independently from sex steroid deficiency. Specifically, using SAMP6 mice, a murine model of age-related osteopenia (but sufficient in sex steroids and with intact reproductive function), a tight association among reduced number of osteoblast progenitors, decreased bone formation, and decreased bone mass has been established (105). Decreased osteoblastogenesis with advancing age has been confirmed in the human bone marrow (198, 199). Importantly, the decrease in osteoblastogenesis is accompanied by increased adipogenesis and myelopoiesis, as well as decreased osteoclastogenesis, the latter most likely caused by a reduction in the stromal/osteoblastic cells that support osteoclast formation (105, 200). This suggests that in aging there must be changes in the expression of genes that favor the differentiation of multipotent mesenchymal stem cells toward adipocytes at the expense of osteoblasts. The evidence that PPARγ2 induces the terminal differentiation of marrow cells with both osteoblastic and adipocytic characteristics to adipocytes, and simultaneously suppresses Cbfa1 expression and terminal differentiation to osteoblasts (94), raises the possibility that increased expression of PPARγ2 or its ligands, *e.g.*, PGJ2, may be some of the culprits responsible for the reciprocal change between adipogenesis and osteoblastogenesis with advancing age (Table 2).

Uptake of oxidized low-density lipoproteins (LDL) play an important role in foam cell formation and the pathogenesis of atherosclerosis. Two of the major components of oxidized LDL, 9-hydroxy-9,11-octadecadienoic acid (HODE) and 13-HODE, are endogenous ligands and activators of PPARγ (201), and PPARγ promotes monocyte/macrophage differentiation and uptake of oxidized LDL (202). Taken together with these findings, the evidence that activated PPARγ2 promotes adipocyte differentiation at the expense of osteoblastogenesis in the bone marrow by suppressing Cbfa1 (94) suggests a mechanistic link among dietary fat/lipoproteins, bone marrow stromal cell differentiation, osteoporosis, and atherogenesis. In support of the existence of such a link, activation of PPARγ by thiazolidinediones or oxidatively modified LDL inhibits osteoblastogenesis of bone marrow-derived stromal cells *in vitro* (94, 203, 204). Moreover, high fatty acid content in rabbit serum or high fat diet of mice for 4 months decreases osteogenic cell differentiation in *ex vivo* bone marrow cell cultures (86, 204). These new advances may explain clinical observations that atherosclerosis and osteoporosis coexist (205, 206).

Quantitative trait loci (QTLs) analysis of osteopenia-associated loci using closely related mouse strains have mapped five loci to regions of chromosomes 2, 7, 11, and 16 (207). Association of these same loci with bone mineral density has been reproduced in crosses of different recombinant-inbred mouse strains (208, 209). Such recurrent appearance of QTL, especially in crosses involving distantly related strains, implies that polymorphism at these loci may be favored by evolution and might underlie variation in peak bone density among humans. Intriguingly, of the more than 12 genes affecting bone homeostasis that were localized near these QTLs, 2 are prostaglandin synthases, a third is the BMP-2/4 antagonist noggin, a fourth is the proapoptotic protein bax, and the fifth is IL-11. Hence, the transcription factor PPARγ2 and its ligand, PGJ2, noggin, and IL-11 are potentially responsible for the decreased osteoblastogenesis with advancing age. This contention is supported by the evidence that the reciprocal relationship between decreased osteoblastogenesis and increased adipogenesis in the SAMP6 mouse may be explained by a change in the expression of PPARγ or its ligands in early mesenchymal progenitors; that BMP-2/4, in balance with noggin, may determine the tonic baseline control of the rate of osteoblastogenesis; and that IL-11 is a potent inhibitor of adipogenesis, which stimulates osteoblast differentiation and the expression of which is reduced in SAMP6 mice. In addition to these factors, growth factors such as IGFs have also been implicated in the bone loss associated with senescence (210, 211). Irrespective of the identity of the precise mediator, the reciprocal change between adipogenesis and osteoblastogenesis can explain the association of decreased relative bone formation and the resulting osteopenia with the increased adiposity of the marrow seen with advancing age in animals and humans (105, 142, 212–215).

C. Glucocorticoid excess

The cardinal histological features of glucocorticoid-induced osteoporosis are decreased bone formation rate, decreased wall thickness of trabeculae (a strong indication of decreased work output by osteoblasts), and *in situ* death of portions of bone. Increased bone resorption, decreased osteoblast proliferation and biosynthetic activity, and sex-steroid deficiency, as well as hyperparathyroidism resulting from decreased intestinal cal-

cium absorption and hypercalciuria due to defective vitamin D metabolism, have all been proposed as mechanisms for the loss of bone that ensues with glucocorticoid excess (216).

The decreased bone formation and osteonecrosis can now be explained by evidence that glucocorticoid excess has a suppressive effect on osteoblastogenesis in the bone marrow and also promotes the apoptosis of osteoblasts and osteocytes (118). Indeed, mice receiving glucocorticoids for 4 weeks, a period equivalent to ~3–4 yr in humans, exhibit decreased bone mineral density associated with a decrease in the number of osteoblast, as well as osteoclast, progenitors in the bone marrow and a dramatic reduction in cancellous bone area and in trabecular width compared with placebo controls. These changes are associated with a significant reduction in osteoid area and a decrease in the rates of mineral apposition and bone formation. More strikingly, glucocorticoid administration to mice causes a 3-fold increase in the prevalence of osteoblast apoptosis in vertebrae and induced apoptosis in 28% of the osteocytes in metaphyseal cortical bone. Nevertheless, even though there is a significant correlation between the severity of the bone loss and the extent of reduction in bone formation, some of the bone loss may be due to an early increase in bone resorption as evidenced by an early increase in osteoclast perimeter of vertebral cancellous bone after 7 days of steroid treatment. *In vivo* studies with mice from this author's group show that at this early time point (7 days after glucocorticoid administration) osteoclastogenesis in *ex vivo* bone marrow cultures is decreased by half, while the number of osteoclasts in bone sections doubles (Robert Weinstein, personal communication), suggesting that an early effect of glucocorticoid excess might be increased osteoclast survival. *In vitro* studies by others, on the other hand, have shown that glucocorticoids inhibit OPG and concurrently stimulate the expression of RANK-ligand in human osteoblastic, primary, and immortalized bone marrow stromal cells (217). Taken together, these lines of evidence suggest that the initial rapid phase of bone loss with glucocorticoid treatment could be due to an extension of the lifespan of preexisting osteoclasts, mediated by RANK-ligand (117).

The same histomorphometric changes that have been found in mice after a 4-week treatment with steroids have been confirmed in biopsies from patients receiving long-term glucocorticoid therapy. Moreover, as in mice, an increase in osteoblast and osteocyte apoptosis is found in human biopsies. Compared with osteoblast apoptosis, apoptotic osteocytes are far more prevalent, at least in metaphyseal cortices, probably because of the anatomical isolation of osteocytes from scavenger cells. Consistent with these findings, glucocorticoids promote osteoblast and osteocyte apoptosis *in vitro* (218, 219). Decreased production of osteoclasts can explain the reduction in bone turnover with chronic glucocorticoid excess, whereas decreased production and apoptosis of osteoblasts can explain the decline in bone formation and trabecular width. Accumulation of apoptotic osteocytes may also explain the so-called "osteonecrosis," also known as aseptic or avascular necrosis, another manifestation of steroid-induced osteoporosis that causes collapse of the femoral head in as many as 25% of patients (220). This contention is supported by evidence that whole femoral heads obtained from patients with glucocorticoid-induced osteoporosis exhibit abundant apoptotic osteocytes adjacent to the subchondral fracture crescent (221). Glucocorticoid-induced osteocyte apoptosis, a cumulative and unrepairable defect, could uniquely disrupt the proposed mechanosensory role of the osteocyte network and thus promote collapse of the femoral head.

At this time, the mediators of the cellular changes caused by glucocorticoid excess are only a matter of conjecture. Nonetheless, glucocorticoids directly suppress BMP-2 and Cbfa1-2—two critical factors for osteoblastogenesis—and may also decrease the production of IGFs while they stimulate the transcriptional activity of PPARγ2 (222–225) (Table 2).

X. Pharmacotherapeutic Implications of Osteoblast and Osteocyte Apoptosis

Estrogen replacement therapy (ERT), various bisphosphonates (*e.g.*, alendronate), the SERM raloxifene, calcitonin, and sodium fluoride, as well as calcium and vitamin D, are approved modalities for the prevention and treatment of bone loss, irrespective of its cause. Decreased osteoclast progenitor development and/or decreased osteoclast recruitment and promotion of apoptosis of mature osteoclasts leading to a slowing of the rate of bone remodeling are thought to be the main mechanisms of the so-called "antiresorptive" agents estrogen, bisphosphonates, SERMs, and calcitonin. Sodium fluoride has anabolic properties, but its therapeutic range is very narrow. Calcium and vitamin D are rarely sufficient on their own, but they are considered a very useful supplementation in any regimen for osteoporosis.

A. Intermittent PTH administration

The ideal therapy for osteoporosis, especially in elderly patients who already have advanced bone loss, would be an anabolic agent that will increase bone mass by rebuilding bone. It is well established that daily injections of low doses of PTH—an agent better known for its role in calcium homeostasis—increases bone mass in animals and humans (226–231) as does the PTH-related protein (PTHrP), the only other known ligand of the PTH receptor (232). Indeed, although constant, high levels of PTH cause increased bone resorption and osteitis fibrosa cystica, low and intermittent doses of PTH, too small to affect serum calcium concentrations, promote bone formation and increase bone mineral density at the lumbar spine and hip. This so-called anabolic effect can be now explained by evidence that PTH increases the life span of mature osteoblasts *in vivo* by reducing the prevalence of their apoptosis from 1.7–2.2% to as little as 0.1–0.4% rather than by affecting the generation of new osteoblasts (218). The antiapoptotic effect of PTH in mice was sufficient to account for the increase in bone mass and was confirmed *in vitro* using rodent and human osteoblasts and osteocytes. Like PTH, PGE inhibits periosteal cell apoptosis via cAMP-dependent stimulation of sphingosine kinase (233). Interestingly, whereas PTH inhibits apoptosis in cells overexpressing Gs, an activator of adenylate cyclase, PTH stimulates apoptosis via G protein-coupled receptors in cells

overexpressing Gq (an activator of JNK and calcium signaling), suggesting that the antiapoptotic effects of PTH are mediated by signals transduced through the Gs pathway (234).

Osteocytes in the newly made lamellar cancellous bone in the mice receiving daily PTH injections were closer together and more numerous than those found in the animals receiving vehicle alone (218). The closely spaced, more numerous osteocytes are the predictable consequence of protecting osteoblasts from apoptosis (Fig. 3B). The antiapoptotic effect of PTH on osteoblasts as well as osteocytes has been confirmed *in vitro* using primary bone cell cultures and established cell lines. Elucidation of this mechanism provides for the first time proof that inhibition of osteoblast apoptosis may represent a novel therapeutic strategy for augmenting bone mass. Be that as it may, several alternative mechanisms, including activation of lining cells, have been proposed, and they may also contribute to the anabolic effect of PTH (235–237). Nonetheless, lining cells cover at least 3 times more surface than osteoblasts. Therefore, conversion of lining cells to bone-forming osteoblasts alone would be insufficient to cover the increased cancellous bone area observed in rats and to account for the expanded bone perimeter and the increased osteocyte number and density observed with PTH treatment in mice (218).

Daily subcutaneous injections of PTH are safe and effective in the treatment of patients with corticosteroid-induced osteoporosis (230). The elucidation of the importance of osteoblast and osteocyte apoptosis in the mechanism of glucocorticoid-induced osteoporosis, and the elucidation of the importance of preventing apoptosis in the anabolic effects of PTH on bone, readily explain how PTH can be such an effective therapy in this condition. Hence, PTH and perhaps future PTH mimetics represent, for the first time, pathophysiology-based, *i.e.*, rational as opposed to empirical, pharmacotherapies for osteopenias, in particular, those in which osteoblast progenitor formation is suppressed. In any case, future studies to assess the antifracture efficacy of these compounds will be needed before their effectiveness for the management of osteoporosis can be established.

B. Bisphosphonates and calcitonin

Bisphosphonates, stable analogs of pyrophosphate, and calcitonin are potent inhibitors of bone resorption and effective therapies for the management of osteoporosis and other diseases characterized by bone loss (238, 239). The main mechanism of the antiresorptive actions of these agents is decreased development of osteoclast progenitors, decreased osteoclast recruitment, and promotion of apoptosis of mature osteoclasts leading to a slowing rate of bone remodeling (133, 240–242). Nonetheless, the antifracture efficacy of these agents is disproportional to their effect on bone mass (243), suggesting an additional effect on bone strength unrelated to effects on bone resorption. Moreover, long-term treatment of human and nonhuman primates with bisphosphonates has been shown to increase wall thickness, an index of increased osteoblast numbers or activity (244–246), raising the possibility that they may not only inhibit bone resorption, but may also have a positive effect on bone formation. An explanation for this evidence is now provided by studies demonstrating that bisphosphonates such as etidronate, alendronate, pamidronate, olpadronate, or amino-olpadronate (IG9402, a bisphosphonate that lacks antiresorptive activity), as well as calcitonin have antiapoptotic effects on osteoblasts and osteocytes (219). These effects are associated with a rapid increase in the phosphorylated fraction of extracellular regulated signal kinases (ERKs) and are blocked by specific inhibitors of ERK activation. In agreement with the *in vitro* results, alendronate abolishes the increase in the prevalence of vertebral, cancellous bone osteocyte and osteoblasts apoptosis induced by administration of prednisolone in mice. These findings raise for the first time the possibility that increased survival of osteoblasts and osteocytes may both contribute to the efficacy of bisphosphonates and calcitonin in the management of disease states due to loss of bone.

If both "antiresorptive" and "anabolic" agents [*e.g.*, intermittent PTH] prevent osteoblast and osteocyte apoptosis, why is increased formation so much more apparent in the case of the "anabolic" agents? The discussion of physiological bone regeneration in the beginning of this review article provides an answer. Bone formation occurs only on sites of previous osteoclastic bone resorption, *i.e.*, on sites undergoing remodeling. Each remodeling cycle is a transaction that, once consummated, is irrevocable. Therefore, agents with antiapoptotic properties that do not have antiresorptive/antiremodeling properties, *i.e.*, they do not decrease the number of remodeling units, are expected to rebuild more bone and therefore increase the overall bone mass, because of the greater number of profitable transactions. Hence, by decreasing the prevalence of osteoblast apoptosis, agents with pure antiapoptotic properties, such as intermittent PTH, can expand the pool of mature osteoblasts at sites of new bone formation and allow these cells more time to make bone, to a much greater degree than the antiresorptive agents that also slow remodeling. However, in the case of either class of agents, upholding the osteocyte-canalicular network by preventing osteocyte apoptosis, should contribute to antifracture efficacy, over and above that resulting from their effects on bone mass (Fig. 5). Therefore, the distinction between antiresorptive and anabolic agents may be more apparent than real when it comes to antifracture efficacy.

C. Novel pharmacotherapeutic strategies

Based on the understanding of the role of growth factors on osteoblast development, proliferation, and differentiation, several of them (*e.g.*, GH, the insulin-like growth factors I and II, TGFβ, BMPs, and FGF) have been advocated as potential future therapeutic agents for the management of bone loss (247, 248). However, with the exception of BMPs, which may be of value in local augmentation of bone mass and acceleration of fracture healing, none of them has been shown to be efficacious (let alone safe or convenient and practical) for the management of common metabolic bone disorders such as osteoporosis. The recent elucidation of the mechanism of the anabolic effects of PTH, and specifically the demonstration of increased work output of a cell pop-

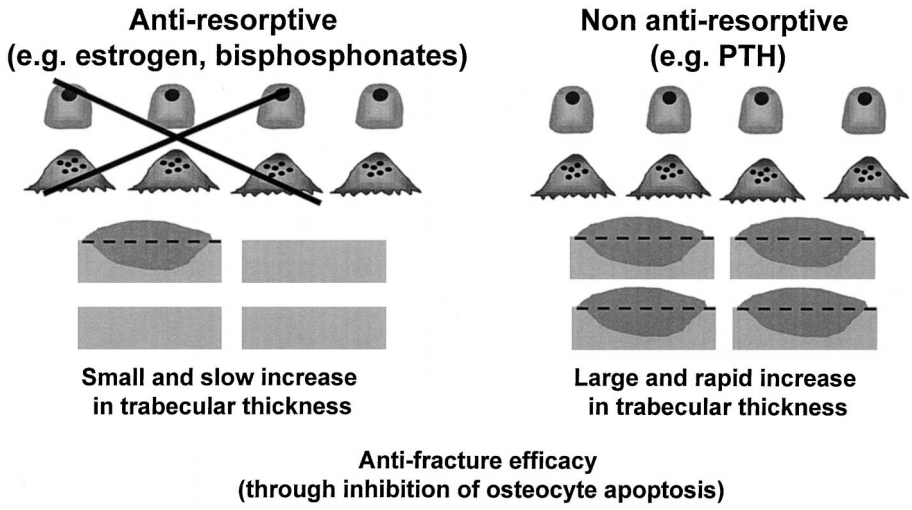

FIG. 5. Hypothetical model of the implications of the effects of antiresorptive (*i.e.*, antiremodeling) *vs.* non-antiresorptive agents on prolonging the life span of mature osteoblasts and osteocytes. For explanation, please see text.

ulation by suppressing apoptosis to augment tissue mass, points to an entirely new avenue for future drug discovery. Indeed, in addition to PTH and PTH mimetics, which by virtue of their peptidic nature carry the inconvenience of daily injections, one can for the first time envisage nonpeptide inhibitors of proapoptotic pathways in osteoblasts and osteocytes as therapeutic agents for osteopenias and especially those in which osteoblast progenitors are low, such as age-related and glucocorticoid-induced osteoporosis.

As discussed in *Section VIII* in this review, estrogen and androgen deficiency increase osteoblast and osteocyte apoptosis in humans, rats, and mice; and these changes have been shown to be reversed by replacement therapy, at least in mice (132, 144, 184, 190). In full agreement with these *in vivo* observations, 17β-estradiol inhibits osteoblast and osteocyte apoptosis *in vitro*. The antiapoptotic effect of 17β-estradiol on osteoblasts and osteocytes require the presence of the estrogen receptor-α or -β (249). Nonetheless, unlike the classical mechanism of estrogen receptor action that involves direct or indirect interaction with the transcriptional apparatus, the estrogen receptor-dependent antiapoptotic effect of 17β-estradiol is due to rapid (within 5 min) phosphorylation of ERKs (250). Moreover, the antiapoptotic effect of 17β-estradiol can be reproduced by 17α-estradiol, a compound thought of as an inactive analog of 17β-estradiol, as well as a membrane-impermeable conjugate of 17β-estradiol with BSA (17βE$_2$-BSA). Numerous effects of estrogen have been observed over the last few years in a variety of cell types, including osteoblasts, the rapidity of which makes a genomic mechanism of action unlikely (251–256). Many of these rapid actions have been attributed to the ability of estrogen to act at the cell membrane on a membrane-associated estrogen receptor (257–260). The antiapoptotic effects of estrogen on osteoblasts and osteocytes fall into this category of "nongenomic" actions. Based on this, the term "activators of non-genomic estrogen-like signaling" (ANGELS), has been coined for compounds that mimic the nongenomic effects of estrogen, but have reduced classical estrogenic actions (261). A paradigm of such agents is the synthetic compound estratriene-3-ol, which has decreased transcriptional activity as compared with 17β-estradiol (262, 263), is a potent neuroprotective compound (264–266), and does exhibit potent antiapoptotic effects on osteoblasts and osteocytes *in vitro*. In support of the hypothesis that ANGELS can be used as a novel, advantageous mode of therapy for the augmentation of bone mass and/or fracture prevention in diseases characterized by low bone mass and increased fragility, preliminary evidence indicates that estratriene-3-ol increases BMD and bone strength in both estrogen-replete and estrogen-deficient mice (261). In view of this preclinical finding and the evidence that androgen (190), as well as estrogen, have antiapoptotic effects on osteoblasts and osteocytes, one is encouraged to think that estrogenic, androgenic, or even nonsteroidal compounds that can activate antiapoptotic, but not antiremodeling, signals on osteoblasts and osteocytes, are candidates for future osteoporosis treatments that, unlike existing ones that prevent or retard bone loss, may augment bone mass.

XI. Summary and Conclusions

In 1995, it was proposed that "changes in the numbers of bone cells, rather than changes in the activity of individual cells, form the pathogenetic basis of osteoporosis"; and that "excessive osteoclastogenesis and inadequate osteoblastogenesis are responsible for the mismatch between the formation and resorption of bone in postmenopausal and age-related osteopenia" (21). Since then, this paradigm shift of thinking has led to important new discoveries that, along with several other independent breakthroughs, refine the concept of "cell number" and broaden its relevance to the physiology and pathophysiology of bone at large. Moreover, these discoveries provide a new landscape for critical reevaluation of our current thinking about therapeutic strategies for bone diseases. Indeed, it is now clear that bone cells must be continually replaced, and the number present depends not only on their birth rate, which reflects the frequency of cell division of the appropriate precursor cell, but also on the life span, which most likely reflects the timing of death by apoptosis. The process of replacement of osteoblasts and osteoclasts is tightly coordinated and orchestrated at the

early progenitor level. Changes in the birth rate and/or apoptosis of bone cells may account for previously unexplained bone diseases, such as the osteoporosis caused by sex steroid deficiency, old age, and glucocorticoid excess. Moreover, attenuation of the rate of apoptosis of osteoblastic cells may be a key mechanism for the effects of anabolic agents, such as PTH. Proof of the principle that the work performed by a cell population can be increased by suppression of apoptosis provides clues for the development of novel pharmacotherapeutic strategies for pathological conditions such as osteoporosis in which tissue mass diminution has compromised functional integrity. Nevertheless, changes in cell birth and death, as well as other mechanisms including changes in bone cell activity, need to be investigated in humans more extensively before definitive conclusions on the pathogenesis of the various causes of bone loss and the development of osteoporosis can be reached.

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