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Cell Biology of Osteoclasts and Osteoblasts and the Hormones and Cytokines That Control Their Development and Activity

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Alterations in the development of osteoclasts and/or osteoblasts underlie the pathophysiology of diseases of bone, including osteoporosis, which can result from the bone loss that occurs after menopause, during aging, or after administration of such immunosuppressive agents as glucocorticoids, and arthritis, which is associated with bone destruction. Estrogen replacement therapy at menopause, or the use of calcitonin or bisphosphonates, to inhibit osteoclastic bone resorption are the main treatments for conditions associated with bone loss. To develop improved therapies, the mechanisms that control the development, activity, and life span of bone cells under normal as well as pathologic conditions must be better understood. During the First Joint Meeting of the International Bone and Mineral Society and the European Calcified Tissue Society held in Madrid, Spain, June 5-10, 2001, several presentations provided new information to advance the state of knowledge in these areas. This article highlights some of them.

Control of Osteoclast Differentiation and Activity

A few years ago, the seminal discovery was made that osteoclast differentiation from hematopoietic progenitors depends on activation of a receptor, called RANK (receptor activator of NF-kappaB signaling), consistent with evidence that transcriptional regulation by NF-kappaB is important for osteoclast development. Stromal/osteoblastic cells express on their surface RANKL, the ligand for RANK.

Bone-resorbing hormones, parathyroid hormone (PTH), for example, and such cytokines as interleukin-1 (IL-1) and tumor necrosis factor (TNF), promote osteoclast differentiation by stimulating the synthesis of RANKL in stromal/osteoblastic cells. RANKL then engages RANK on osteoclast progenitors to stimulate differentiation. This process also requires expression of macrophage colony-stimulating factor (M-CSF) by stromal/osteoblastic cells, which binds to specific receptors on osteoclast progenitors. Stromal/osteoblastic cells also secrete a soluble RANK decoy called osteoprotegerin (OPG), which thus acts as a potent inhibitor of osteoclast formation by binding to RANKL and preventing its interaction with RANK on osteoclast progenitors.

Several presentations demonstrated that other factors can substitute for RANKL and M-CSF in stimulating osteoclast formation, and they may be of importance for the pathophysiology of the bone destruction associated with inflammation. N. Takahashi,[1] Showa University, Tokyo, Japan, reviewed evidence that bacterial lipopolysaccharide (LPS), an intensely inflammatory substance, can stimulate osteoclastogenesis and bone resorption by binding to a protein called Toll-like receptor 4 to activate NF-kappaB in osteoclastic cells. This effect is not mediated indirectly via upregulation of IL-1 or TNF,

which are known to stimulate RANKL; further, OPG was unable to block LPS-induced bone resorption.

In a similar vein, R. L. van Bezooijen and colleagues,[2] Leiden University Medical Center, Leiden, The Netherlands, showed that in combination with TNF, IL-17, a T-cell-derived cytokine found in fluid from osteoarthritic joints, stimulated osteoclast differentiation and bone and cartilage degradation in a manner that could not be blocked by OPG. Moreover, the stimulatory effect of IL-17 did not depend on NF-kappaB activation. T cells from arthritic joints also produce RANKL; thus, cytokines such as IL-17 likely act in combination with RANKL.

However, at least part of the pathophysiology of bone diseases associated with inflammation may be due to the fact that the osteoclastic bone resorption driven by T-cell-derived cytokines or LPS cannot be influenced by factors such as OPG. Additional studies will be needed to unravel the complexities of these new pathways, which are of obvious importance for understanding and managing these diseases. Indeed, some T-cell products inhibit bone resorption, as shown by M. T. Gillespie and coworkers,[3] St. Vincent's Institute of Medical Research, Melbourne, Australia, who demonstrated that IL-12 induces the secretion of an undefined soluble T-cell product that strongly synergizes with IL-18 to inhibit osteoclastogenesis.

T cells may also play a role in the increased osteoclast formation and bone loss caused by estrogen deficiency. Thus, C. Roggia and colleagues,[4] Washington University School of Medicine, St. Louis, Missouri, used a transplantation approach to extend previous studies which indicate that T-cell-derived TNF is required for ovariectomy-induced bone loss in mice. Estrogen does not appear to directly regulate the biosynthesis of TNF but rather the number of T cells that synthesize TNF; however, neither proliferation nor apoptosis of mature T cells was affected by estrogen in ex vivo cultures of bone marrow cells. Thus, the estrogen-regulated events underlying this response remain to be established.

Advances in the understanding of osteoclast function were also presented. H.K. Vaanannen,[5] University of Turku, Turku, Finland, reported studies on the molecular basis of secretory and endocytosis control. The osteoclast is unusual in that the ruffled border portion of the plasma membrane, which is in direct contact with the bone surface being resorbed, is a site of extensive secretion of lysosomal enzymes, as well as a site of extensive endocytosis of the products of bone matrix degradation. The latter are eventually transcytosed to the apical surface membrane for secretion into the extracellular fluid.

It is now evident that these 2 processes are accomplished by specific membrane domains in the ruffled border region of the osteoclast plasma membrane. The exocytotic vesicles are associated with f-actin-containing filaments, whereas endocytotic vesicles are associated with tubulin-containing microtubular filaments. Specific members of the Rab GTPase family of proteins play a pivotal role in organizing and targeting intracellular membrane trafficking along each of these pathways.[6] The clinical significance of these findings is that they point to new osteoclast-specific targets for inhibition of bone resorption. Indeed, F.P. Coxon, University of Aberdeen, Aberdeen, Scotland, and colleagues[7] identified a phosphocarboxylate analogue of risedronate that inhibits bone resorption, not by inhibiting farnesyl phosphate synthase as bisphosphonates do, but by specifically inhibiting geranylgeranyl transferase II, the enzyme involved in prenylation of Rab proteins.

Regulation of Osteoblast Differentiation, Activity, and Life Span

Available therapies for osteoporosis, such as estrogens, selective estrogen receptor modulators (SERMs), bisphosphonates, and calcitonin, are anticatabolic, ie, they prevent bone loss by inhibiting resorption and suppressing remodeling. However, there is a need for an anabolic therapy to treat end-stage osteoporotic individuals with multiple fractures, with the aim of preventing further fractures. Thus, substantial effort has been devoted to investigation of the molecular basis of osteoblast differentiation and the growth factors that promote this process, as well as the mechanisms that underlie the well established, but poorly understood, anabolic effect of intermittent PTH on the skeleton.

Cbfa1 is one of the principal transcription factors responsible for the genetic determination of the osteoblast phenotype during differentiation from primitive mesenchymal progenitors. To probe the range of actions of this transcriptional regulator, it has been overexpressed using cell-specific promoters in chondroblasts and in immature or mature osteoblasts. The results of these studies were presented by P. Ducy,[8] Baylor College of Medicine, Houston, Texas, who showed that, in addition to the role Cbfa1 has in the differentiation process, both osteoblasts and chondroblasts require Cbfa1 activity for proper biosynthetic function.

Besides absence of osteoblasts and a mineralized skeleton, mice lacking Cbfa1 as a result of gene deletion are characterized by a poorly organized growth plate, lack of hypertrophic chondrocytes, and lack of subchondral vascular invasion during development. Overexpression of Cbfa1 in chondroblasts using the type II collagen promoter rescued the growth plate phenotype of the knockout mice but did not rescue the osteoblast deficiency.

Moreover, Cbfa1 overexpression in mature osteoblasts using the OG2 promoter results in the development of mice with significantly increased bone density, suggesting that the transcription factor has the capacity to stimulate osteoblast activity.[8] Nevertheless, bone loss was still evident in these mice after ovariectomy or during aging. On the other hand, expression of a dominant negative form of Cbfa1 in osteoblasts using the OG2 promoter resulted in an osteoporotic phenotype.

In contrast to the anabolic effect of Cbfa1 overexpression in mature osteoblasts using the OG2 promoter, M. Kneissel, Novartis Pharma, AG, Basel, Switzerland, and colleagues[9] reported that overexpression in earlier osteoblast progenitors using a type I collagen promoter resulted in severe osteopenia. Hindlimb fractures were commonly observed, apparently attributable to thin cortical bone resulting from increased endosteal bone resorption. Of interest, cancellous bone was unaffected. Increased osteocyte apoptosis was also observed, perhaps contributing to the bone fragility syndrome in these mice.

The contrasting phenotypes of the OG2-Cbfa1 and Col1A1-Cbfa1 transgenic mice may well be due to increased stromal/osteoblastic cell support of osteoclast formation. Indeed, it is commonly believed that RANKL synthesis is restricted to cells in an early phase of osteoblast differentiation -- a time at which collagen synthesis has begun, but before the onset of osteocalcin synthesis. The importance of these findings is that strategies to increase bone formation by modulating the synthesis of lineage-specifying transcription factors will have to be carefully orchestrated. Future studies are needed to identify the molecular basis of the restriction of RANKL expression to stromal/osteoblastic cells.

It has long been known that bone morphogenetic proteins (BMPs), especially BMP-2 and BMP-4, exert potent prodifferentiation effects on early osteoblast progenitors. Thus, there has been considerable effort to develop strategies for inducing its synthesis at, or for delivering it to, skeletal sites where bone formation is needed. D. Chen, University of Texas Health Science Center at San Antonio, and colleagues[10] reported that increased BMP-2 secretion underlies the ability of proteosome inhibitors to stimulate osteoblast differentiation. They seem to do so by preventing the degradation of the transcription factor Gli. In the undegraded form, Gli stimulates the activity of a BMP-2 promoter, whereas in the degraded low-molecular-weight form, Gli acts to suppress the activity of this promoter. V. Wright and colleagues,[11] University of Pittsburgh, Pittsburgh, Pennsylvania, also reported successful transduction of mesenchymal stem cell progenitors isolated from adult murine muscle with a retrovirus expressing BMP-4. Transplantation into syngeneic mice resulted in bone formation by osteoblasts derived from the transplanted progenitors.

The possibility of other means of stimulating bone formation was revealed by genetic studies demonstrating that loss-of-function mutations of the *SOST* gene[12] in humans were associated with sclerosteosis, which is characterized by generalized osteosIcerosis and hyperostosis of the skeleton. This finding suggests that the protein, secreted by osteoblasts, normally restrains bone formation. Thus, an opportunity to stimulate bone formation is provided by blocking the production and/or activity of this negative regulator.

The most common forms of osteoporosis are diseases of bone remodeling, in which the production and life span of osteoclasts and osteoblasts are altered in such a way as to result in excessive bone resorption and/or inadequate bone formation.[13] It was recently shown that the majority of osteoblasts die by apoptosis[14] and that part of the pathophysiology of osteoporosis resulting from sex-steroid deficiency or glucocorticoid excess involves increased osteoblast apoptosis.[13] Thus, prolongation of osteoblast life span by inhibiting apoptosis represents a potentially effective means of increasing bone formation. R.H. Jilka,[15] University of Arkansas for Medical Sciences, Little Rock, Arkansas, summarized evidence that sex steroids, bisphosphonates, and PTH activate intracellular signaling pathways that prevent apoptosis in osteoblasts.

Both androgen and estrogen rapidly induce Src activation via either the androgen or estrogen receptor (ER); the Src activation in turn culminates in the generation of ERK-dependent antiapoptosis signaling.[16] This nongenotropic sex nonspecific signaling by the ER is completely independent of its transcription-modulating activity, as it can be seen with a fragment lacking the DNA-binding domain and when the receptor is targeted to membranes via prenylation; but it is lost when targeted to the nucleus. What's more interesting, some ER ligands fail to influence the transcriptional activity of the receptor, but they still inhibit osteoblast apoptosis. This opens the possibility of mechanism-specific, as opposed to tissue-specific (ie, SERM), activation of ER action, as a means of preventing postmenopausal bone loss.

The bisphosphonate alendronate also stimulates Src/ERK-dependent antiapoptotic pathways in osteoblasts, and this effect appears to be exerted via connexin 43 hemichannels.[17] These channels consist of transmembrane connexin 43 hexamers that are normally responsible for intercellular communication as gap junctions. In this case,

however, the connexin 43 protein may be acting as a signal transducer -- a function that was previously not suspected.

In contrast to sex steroids and alendronate, PTH inhibits osteoblast apoptosis via cAMP-dependent protein kinase A (PKA) activation. PKA in turn phosphorylates the proapoptotic protein Bad to inactivate its death function and also phosphorylates a transcription factor called CREB to activate its activity. Indeed, new protein synthesis, along with Bad phosphorylation, is required for the apoptosis-inhibiting action of PTH. However, PTH-induced suppression of osteoblast apoptosis is a transient phenomenon. This finding suggests that the regimen of intermittent administration of PTH may be required for its anabolic effect because it generates repeated bursts of transient antiapoptosis signaling in osteoblasts. The transient nature of these signals is likely caused by negative feedback inhibition. Consistent with this, it was also reported by R.M Locklin and colleagues,[18] Mayo Clinic and Mayo Foundation, Rochester, Minnesota, that short-term, but not long-term, exposure of marrow-derived osteoblast progenitors to PTH caused an increase in IGF and Cbfa1.

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