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PERSPECTIVES

New Concepts in Osteoclast Pathophysiology

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It has been many years since the "secrets" of osteoclast physiology began to be revealed, largely owing to the contribution of *in vitro* technologies. This fascinating cell has central roles in bone remodeling, and its deregulation severely affects skeletal homeostasis. It is therefore timely to review recent advances into its biology and to set out some perspectives for the treatment of osteoclast diseases.

Osteoclast Diseases

Dysfunctional osteoclasts are responsible for altered bone remodeling, with severe consequences for the quality of life. A lesson was learned from osteoclast failure in osteopetrosis, and this disease has been fundamental for the understanding of the role of specific molecular mechanisms involved in bone resorption and regulation of osteoclastogenesis (1). Geneticists have taken advantage of the knowledge accumulated in animal models and identified subsets of genes involved in human osteopetrosis (2). Genes encoding for molecules implicated in the acidification of resorbing lacuna account for the largest portion of patients affected by osteopetrosis (3,4). The impact of animal studies has been even more important for the understanding of the osteoclast origin of hematopoietic tissue, which opened up an avenue for the cure of osteopetrosis through bone marrow transplantation (5,6). The extreme rarity of patients who lack osteoclasts suggests that defective osteoclastogenesis is generally incompatible with life, at least in humans, and the fact that osteoclasts are not rescued by bone marrow transplantation in these forms (our unpublished observations) indicates an environmental defect that is yet to be identified.

Gradually, since the first pioneering work that defined a root for osteoclast differentiation from the monocyte/macrophage lineage (7), we have learned that osteoclasts not only originate from hematopoietic bone marrow and are regulated by members of the immune system, but also are likely to belong to the immune cell family (8). Significant evidence supports this hypothesis, including (i) negative autoregulatory loops -- for instance, those through the autocrine receptor activator of NF-κB (RANK) signal-dependent interferon β system (9) and the recently discovered endogenous negative regulator interleukin 1 receptor-associated kinase M (IRAK-M), which represents a natural competitor of activating IRAK molecules in the interleukin 1 (IL-1)/Toll-like receptor signal transduction pathway (10); (ii) the need for typical immune cell coreceptors containing the immunoreceptor tyrosine-based activation motif (ITAM) for activating the osteoclast-associated receptor (OSCAR) and triggering receptor expressed on myeloid cells 2 TREM-2) (9); and (iii) the response to numerous inflammatory cytokines (11). It is therefore likely that in the future, osteoclasts will be regarded as peculiar and well-controlled immune cells that react against bone matrices of self. Like other immune cells, osteoclasts would therefore need an efficient suppression mechanism that could control the size and activity of the population, avoiding uncontrolled and unjustified bone resorption.

Several questions arise from these considerations, and it will be challenging to understand what makes a specific bone site suitable for aggression by osteoclasts, how osteoclasts recognize an endogenous matrix for resorption, and what changes a matrix

must undergo to be recognized by osteoclasts. In this context, the inflammatory response that is believed to play a pivotal role in osteoclast recruitment and the induction of bone resorption (11) is likely to be the central event, and the effort of osteoclast biologists should probably converge on this point in the future to address many important questions.

Coupling and Uncoupling

Most of our understanding of osteoclast physiology is based on the notion that osteoclast activity is tightly associated with that of osteoblasts (12). Balanced bone resorption/bone formation cycles are believed to be essential for bone homeostasis and explain well the continuous renovation of skeletal tissues without detrimental effects on bone mass (12). Unbalanced activities of the two cell types are claimed to induce severe consequences, and when resorption exceeds formation, bone becomes porous and prone to fracture. Brittle bones are therefore seen in postmenopausal women and patients with senile osteoporosis, renal failure, and systemic and local inflammatory diseases. Consequences of increased bone resorption, reduced bone formation, or both are similar, resulting in increased risk of fracture (13).

The concept of osteoclast-osteoblast coupling is considered a milestone in bone physiology, and nowadays, several molecular mechanisms are claimed to underlie the concerted activities of the two cell types (14). Currently, not only are factors released from bone matrix believed to activate osteoblast bone formation, but osteoclast cellular products are also hypothesized to stimulate osteoblasts, independent of bone resorption (15). Again, a lesson arises from osteopetrosis in which increased osteoblast numbers and activities can be observed in the forms characterized by increased osteoclasts (16; our unpublished results). In contrast, osteoblast numbers are generally unchanged or even lower than average in the rare forms lacking osteoclasts (our unpublished observations). This finding suggests that osteoclasts could release osteoblast-activating cellular mediators independently from their resorbing activity. It is tempting to envision these apparently new mediators as if they had an anabolic effect on osteoblasts, especially given that osteoclast inhibitors are available and largely used in the therapy of osteoporosis, but osteoblast anabolic agents are not yet accessible, with the sole exception of parathyroid hormone given intermittently (17). Therefore, there is a requirement for the identification of new molecular mediators that could favor bone formation, and in this context, osteoclasts could be considered an important source of osteoblast anabolic factors. The recognition of osteoclast-derived anabolic factors could therefore open up new possibilities for the cure of bone diseases caused by osteoblast failure.

Does the coupling of osteoblasts and osteoclasts explain all activities in bone? Most regulatory molecules are known to affect osteoclasts indirectly through the osteoblast lineage (14), and in the adult life of a healthy individual, this coupling activity ensures preservation of both bone architecture and mass during remodeling. It also explains well the detrimental sequel that occurs in aging, when a number of proosteoclast cytokines produced by immune cells are increased because of removal of the physiologic block by gonadal steroid hormones, causing unbalanced bone remodeling.

A detailed review of the literature, however, shows quite confusing results. Let us consider for a minute the response of the interleukin superfamily to inflammatory cytokines. The same cytokine, for instance IL-6, is found to directly affect osteoclasts, which express IL-6 receptors, or to influence osteoclast activity solely indirectly through osteoblasts. IL-6 is shown to increase osteoclastogenesis *in vitro* and contribute to osteoclast activation in inflammatory diseases and postmenopausal osteoporosis (7;18;19), but is also found to reduce osteoclast formation and activity in animal models (20;21). In a recent study in our laboratory, we clearly observed that osteoclastogenesis and bone resorption are significantly increased in young mice overexpressing hIL-6, whereas in adult

mice, they are considerably reduced (our unpublished observations; 20;21). What makes the response to IL-6 different in the two groups of animals? Our currently still speculative answer takes into account the fact that osteoclast and osteoblast activities in young animals are largely uncoupled for at least three reasons: to favor bone formation, to determine bone modeling, and to form adequate cavities for hematopoietic and nervous tissue development.

Our understanding of the complexity and frequent inconsistencies observed in the literature for IL-6 and other cytokines is that in growing subjects, osteoclasts are able to directly respond to these cytokines, their activity being uncoupled from that of osteoblasts, and pathological overexpression of IL-6 at this stage further dissociates the activities of the two cell types. This point of view is supported by the observation that in all transgenic IL-6 animal models investigated, osteoblast matrix-forming activity is inhibited. It should also be noted that osteoblasts overexposed to IL-6 differentiate normally and are induced to express IL-1, another important osteoclast stimulator. In contrast, RANK ligand, its decoy receptor osteoprotegerin, and tumor necrosis factor α, typically involved in osteoclast coupling to osteoblasts, remain unchanged (our unpublished observation). It is possible, however, that the cellular features and molecular settling of osteoclasts change with age, determining uncoupled activity during growth, when sites of bone formation are frequently different from those of bone resorption and formation must also overwhelm resorption. In adults, to preserve bone mass and architecture, osteoclast and osteoblast activity is required to be quantitatively equilibrated and associated with the same sites, so that each "quantum" of damaged bone resorbed is replaced by an equal "quantum" of good quality, newly formed matrix. The removal of the physiologic block by sex hormones, which allows the release of inflammatory cytokines at menopause or in aging people, causes a "divorce" between osteoclasts and osteoblasts, with unbalanced bone resorption that overrides bone formation. Therefore, we believe that although osteoblast regulators of osteoclast activity, which are typically membrane-bound molecules, could be regarded as coupling factors, osteoclastogenic interleukin superfamily members and other inflammatory cytokines could be considered uncoupling factors and have a physiologic role during growth. However, uncoupling becomes pathologic in growing subjects, when they are overexposed to these cytokines, as in the case of chronic inflammatory diseases, and in aging, when cytokines apparently uncouple a process that should be tightly associated. In support of this point of view, children with chronic inflammation, as well as growing mice overexpressing IL-6, have a bone phenotype in which osteoclasts are activated and osteoblasts are inhibited.

New Osteoclast Pathways

The cure of bone diseases is generally a hard task. All therapies against osteoporosis have pitfalls, and thus far, we cannot efficiently prevent the risk of fracture with any of the currently available treatments (22). Inflammatory diseases are detrimental for the skeleton, and antiinflammatory drugs, such as the glucocorticoids, typically negatively affect bone tissue, worsening the primary effect induced by inflammation. Although antiresorptive drugs are available and largely used in therapy, there is a requirement for the identification of more specific osteoclast targets that could also be stimulated to improve osteoclast performance in osteopetrosis. In fact, although infantile malignant osteopetrosis is cured by bone marrow transplantation, albeit with a high chance of failure and consistent progression of the neurological deterioration, the so-called benign forms, which however frequently present with severe phenotypes, have no cure at the present time.

One of the most specific mechanisms for osteoclasts is the integrin $\alpha V\beta 3$. Its targeted disruption causes osteopetrosis in mice (23), and its decreased expression in *in vitro* osteoclasts or the blockage of its activity by specific antibodies or antagonists are known to reduce bone resorption (24;25). Unfortunately, however, a therapy based on disruption of integrins is not available, and *in vivo* treatments have not yet provided

substantial evidence that they could affect osteoclast activity.

Integrin αVβ3 elicits a yet unknown intracellular signaling pathway. Of interest, in a recent study performed in our laboratory (26), selective activation of integrin by the specific monoclonal antibody LM609 isolated its signal from those of other integrins and made it apparent that a peculiar pathway is generated upon □V□3 ligation. The pathway leads to selective extracellular signal-regulated kinase (ERK) (but not p38 and c-Jun N-terminal kinase) activation in a Ca^{2+}-, PKCα-, c-Src-dependent manner. Most importantly, it is totally independent of the classical Shc-Grb2-Ras-Raf-1 axis, and MEK, the only recognized ERK activator thus far, is not involved. This finding is at variance with what has been recognized for the largest subsets of integrins (27), further increasing interest in the use of αVβ3 signaling as a pharmacological target against osteoclast diseases. Should this new pathway be recognized and verified to be targetable, one could predict the development of a novel therapeutic approach either to block or stimulate bone resorption. Therefore, we believe that there is still room for new avenues in therapy targeted at osteoclasts, and expect in the near future a new generation of drugs to prevent or cure osteoclast diseases.

Conclusions

Many years of investigation in the field of osteoclast biology have been quite fruitful, and nowadays, we can proudly state that our pioneering work aimed at efficiently isolating or generating osteoclasts *in vitro* has been well exploited. It is legitimate to expect further developments and the full elucidation of the molecular mechanisms underlying osteoclast function, which may then be successfully targeted for therapy.

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