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The Osteoclast: A Multinucleated, Hematopoietic-Origin, Bone-Resorbing Osteoimmune Cell

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Abstract Osteoclasts are multinucleated cells that derive from hematopoietic progenitors in the bone marrow which also give rise to monocytes in peripheral blood, and to the various types of tissue macrophages. Osteoclasts are formed by the fusion of precursor cells. They function in bone resorption and are therefore critical for normal skeletal development (growth and modeling), for the maintenance of its integrity throughout life, and for calcium metabolism (remodeling). To resorb bone, the osteoclasts attach to the bone matrix, their cytoskeleton reorganizes, and they assume polarized morphology and form ruffled borders to secrete acid and collagenolytic enzymes and a sealing zone to isolate the resorption site. Identification of the osteoclastogenesis inducer, the receptor activator of nuclear factor-κB ligand (RANKL), its cognate receptor RANK, and its decoy receptor osteoprotegerin (OPG), has contributed enormously to the dramatic advance in our understanding of the molecular mechanisms involved in osteoclast differentiation and activity. This explosion in osteoclast biology is reflected by the large number of reviews which appeared during the last decade. Here I will summarize the ''classical'' issues (origin, differentiation, and activity) in a general manner, and will discuss an untouched issue (multinucleation) and a relatively novel aspect of osteoclast biology (osteoimmunology). J. Cell. Biochem. 102: 1130–1139, 2007.

Key words: osteoclast; bone-resorption; osteoimmunology; cell differentiation; cell fusion

"It may at once be stated that an osteoclast is a composite mass consisting of the fused bodies of two, three, or more cartilage cells containing any number of osteoblasts-in short, it is a meso-ectodermal syncytium" [Geddes, 1913]. "In so far as no particulate matter resembling bone matrix was seen within the cells, nor free within the medium, the present experiments support the views. . .that osteoclasts have no osteolytic properties. . ." [Hancox, 1946].

Obviously, these two statements regarding the origin and the function of the osteoclast are considered wrong today and demonstrate the advances in our understanding of osteoclast biology and activity since first described [Kolliker, 1873]. We have known for a long time that the osteoclast is an osteolytic cell, and in fact is the major, if not the exclusive, bone-resorbing cell, and that the multinucleated osteoclast is of hematopoietic origin. Several factors critical for osteoclast differentiation and/or function have been discovered using genetically manipulated mice. However, a specific osteoclast differentiation factor, now called receptor activator of NF-κB ligand (RANKL), was identified only less than a decade ago [Fuller et al., 1998; Lacey et al., 1998; Yasuda et al., 1998]. This discovery enabled us to study in vitro the differentiation of osteoclast from its mononuclear precursor without the need for other supporting cells, and therefore to study in details cellular signaling and other molecular mechanisms participating in osteoclast differentiation and function.

A large number of extensive reviews on osteoclasts in health and disease, and in particular focusing on osteoclast origin, differentiation and function, have been published in recent years [Boyle et al., 2003; Teitelbaum and Ross, 2003; Greenfield and Rubin, 2005; Asagiri and Takayanagi, 2007]. In this review it is intended that some of the "traditional" issues (origin, differentiation, and resorption), as well

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as relatively less discussed aspects of osteoclast biology (multiple nuclei, osteoimmunology), will be summarized.

OSTEOCLAST ORIGIN AND DIFFERENTIATION

The restoration of bone resorption in osteopetrotic mice (mice with impaired resorptive activity) by normal bone marrow and spleen cells transplantation [Walker, 1975], and the analyses of osteoclast and osteoblast lineages using a chimera of chick and quail embryonic tissue [Kahn and Simmons, 1975] provided the first convincing evidence regarding the hematopoietic origin of the osteoclast. Many laboratories contributed to the current hypothesis regarding the steps from the first identifiable osteoclast precursor to the mature active resorbing cells [reviewed in: Boyle et al., 2003; Teitelbaum and Ross, 2003; Greenfield and Rubin, 2005] as illustrated in Figure 1A. A hematopoietic stem cell (HSC) that is common to lymphocytes, red blood cells, platelets, granulocytes and mononuclear phagocytes, progresses through the colony-forming unit for granulocytes and macrophages (CFU-GM) and the CFU for macrophages (CFU-M), to the pre-osteoclast and multinucleated cell. Finally, the mature active resorbing osteoclast is formed. The major breakthrough in the last decade was the discovery that RANKL is critical for osteoclastogenesis, leading to an understanding of the steps from CFU-M to osteoclast. CFU-M has been known for a long time as the immediate precursor of macrophages. However, RANKL activation of its receptor, RANK, "instructs" the CFU-M to undergo osteoclastic differentiation (in the presence of M-CSF and absence of GM-CSF) or to become a dendritic cell (in the presence of GM-CSF) [Miyamoto et al., 2001]. Until 1998, in vitro osteoclastogenesis was thought possible only in the presence of accessory cells in addition to the precursors. The realization that RANKL is mostly a membrane protein of the osteoblast provided an explanation for the requirement of accessory cells for in vitro osteoclastogenesis. To date, the combination of the soluble recombinant extracellular portion of RANKL, together with M-CSF, is a powerful mean of inducing in vitro osteoclastogenesis without a need for other cells. The divergence from the macrophage/dendritic cell towards the osteoclast ("zooming" on the differentiation from CFU-M to the osteoclast lineage) is shown in Figure 1B. The figure illustrates the binding of two osteoblast-derived factors, the released M-CSF (1) and the membrane RANKL (2) to their cognate receptors in the CFU-M. Osteoprotegerin (OPG), also released by osteoblasts (but not exclusively), binds RANKL (3), competes with RANK on the osteoclast differentiation inducer, and thus inhibits osteoclastogenesis [Simonet et al., 1997; Lacey et al., 1998]. GM-CSF binding to its receptor in the CFU-M (4) also inhibits osteoclastogenesis and induces differentiation into dendritic cells. Additional cytokines and growth factors affect osteoclastogenesis, reviewed in Greenfield and Rubin [2005] and Takayanagi [2007].

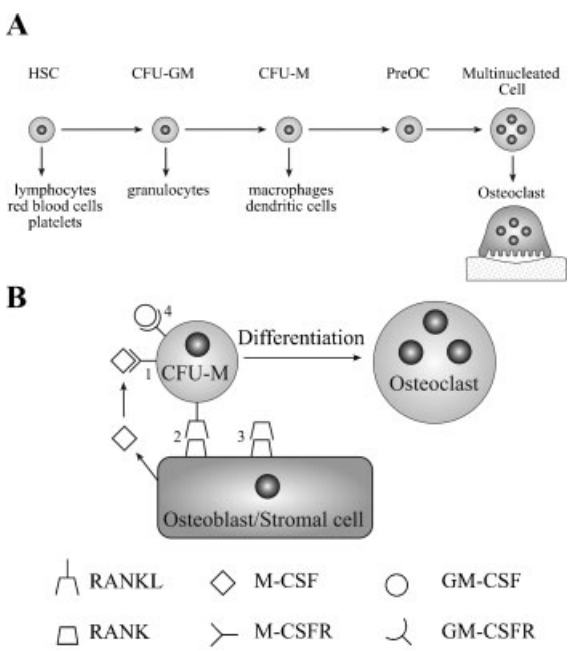

Fig. 1. Osteoclast differentiation. **A**: A schematic showing the main stages during differentiation from the hematopoietic stem cell to the osteoclast. **B**: A schematic showing the osteoblast/stromal cell-dependent osteoclastic differentiation.

Many of the molecules involved in regulation of osteoclastogenesis were identified as such in either naturally occurring or genetically-modified osteopetrotic mice. These molecules include growth/differentiation factors, receptors and transcription factors operating prior to CFU-M (e.g., PU.1, M-CSF and its receptor), as well as genes operating from CFU-M and beyond (e.g., M-CSF and its receptor, RANKL, RANK, TRAF6, NF-κB [p50/p52], c-Fos) [reviewed in Greenfield and Rubin, 2005]. Although a deficiency in molecules belonging to these two groups impairs resorption, the effects of the

molecules belonging to the latter group are more osteoclast-specific. This is exemplified by the development of both osteoclasts and macrophages which is arrested in PU.1-deficient mice [Tondravi et al., 1997], while the severe osteopetrosis in mice lacking c-Fos that develops due to a complete block of osteoclast differentiation is, in fact, associated with an increase in macrophage number [Grigoriadis et al., 1994].

THE OSTEOCLAST: A BONE RESORBING CELL

Bone resorption is the process by which the two phases of the bone matrix, the mineral and the organic, are dissolved and degraded, respectively. Resorption functions in bone modeling during growth, and is necessary for tooth eruption. Throughout life, resorption is critical to the bone remodeling, together with the bone formation, executed by the osteoblasts. This process is the preventive maintenance of mechanical strength by continuously replacing fatigued bone by new "fresh" bone. The bone is the main reservoir of calcium ions, and the remodeling is critical for Ca^{2+} fluxes into and from the extracellular fluid to maintain an appropriate level of blood calcium.

The osteoclast is the exclusive bone resorptive cell, and its morphological features are adopted accordingly. Moreover, the osteoclast expresses genes whose activities are critical for resorption. The osteoclast is a polykaryon and is unusually big. The mammalian osteoclast normally contains up to eight nuclei, but around 100 nuclei are found in osteoclasts of Paget's disease patients [Roodman and Windle, 2005]. Its size (in vitro diameter can reach ~300 μm as compared to 10–20 μm for a macrophage) enables the osteoclast to cover a relatively large matrix area and thereby operate efficiently. In order to resorb bone, it is essential for the osteoclast to attach to the bone surface and to assume a polarized morphology. In the membrane domain facing the matrix, the sealing zone that encircles a highly convoluted membrane domain, the ruffled border that serves as the actual resorbing membrane creates a tight sealing with matrix. The opposite membrane domain can be divided into basolateral domain (BLD) and functional secretory domain (FSD) [Mulari et al., 2003; Vaananen, 2005]. A schematic of a resorbing osteoclast is shown in Figure 2. The process of resorption can be divided into the following steps: (1) Fusion of the mononuclear precursors to form the polykaryon and targeting to the site of resorption. (2) Attachment to the mineralized bone matrix, reorganization of actin and formation of the "actin ring" and the sealing zone. (3) Formation of the ruffled border, encircled by the sealing zone. (4) Release of acid and acidic collagenolytic enzymes into the space enclosed by the matrix, sealing zone and ruffled border resulting in mineral dissolution and organic matrix degradation. (5) Removal of the resorption products from the resorption lacuna to the functional secretary domain by transcytosis and their secretion into the circulation. Then, the osteoclast is detached from the matrix, loses its polarized structure and either relocates to a new resorption site or undergoes apoptosis.

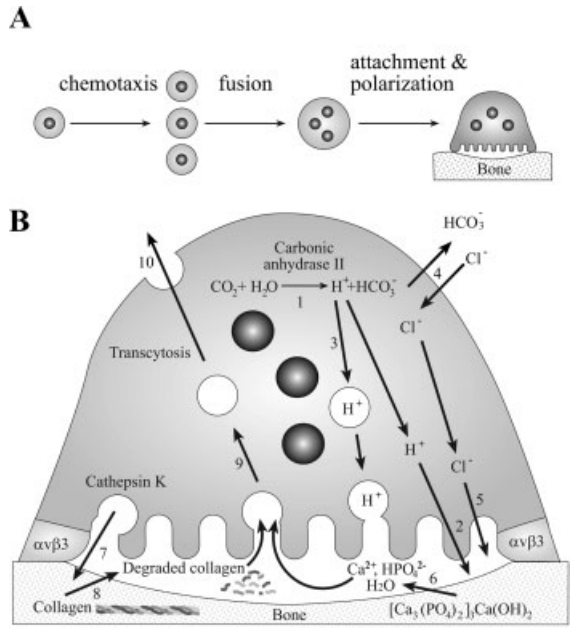

Fig. 2. Bone resorption. **A**: A schematic showing the attraction, attachment and polarization of the resorbing cell. **B**: A schematic showing the events within the osteoclast participating in the resorption. Carbonic anhydrase II catalyzes the hydration of CO_2 (1), resulting in supply of protons that accumulate in the resorption area by the proton pump (2) and through vesicular transport (3). The HCO_3^- produced together with the proton is exchanged for chloride ion (4) that is transferred through a chloride channel (5) to the resorption area. The HCl dissolves the hydroxyapatite (6), and cathepsin K exocytosed from the cell (7) degrades the collagen (8). The ions and collagen degradation products are endocytosed by the ruffled membrane (9), the vesicles are fused to the membrane opposite to the ruffled membranes (10) and the resorption products are disposed.

Migration and Targeting

It is not entirely clear what attracts the resorptive cell to the remodeling site. Based on in vitro chemotaxis assays using modified Boyden chambers, it was shown that type I collagen peptides, α2HS glycoprotein and osteocalcin evoke a dose-dependent chemotactic response in human monocytes. It was therefore suggested that osteoclast precursors (monocytes) are mobilized by chemotaxis, and chemoattractants responsible for this activity derive from the bone matrix or, in the case of collagen and osteocalcin, directly from the osteoblasts that produce them [Malone et al., 1982]. Another potential chemoattractant for recruiting osteoclast precursor is stromal cell-derived factor-1 (SDF-1), which is produced also by immature osteoblasts within bone [Yu et al., 2003]. The spatial connection between micro cracks to ongoing remodeling raised the suggestion that dying osteocytes may signal to the attraction of osteoclast precursors [Noble et al., 2003]. An additional hypothesis is that healthy osteocytes inhibit resorption, and when these signals are stopped the osteoclast precursors migrate toward the site [Heino et al., 2002]. Matrix metalloproetinases (MMPs) produced by osteoclast lineage cells were found to be critical for the migration of the precursor cells. MMP14 is required for the intrinsic ability of the osteoclast to move through collagen/osteoid (non-mineralized bone matrix) and MMP9 probably releases chemoattractants like vascular endothelial growth factor (VEGF) [Delaisse et al., 2003]. (The fusion will be discussed later).

Attachment and Polarization

The formation of a microenvironment isolated from the extracellular space between the osteoclast and the underlying bone matrix is essential for resorption. Based on in vitro and in vivo approaches it was proposed that $\alpha_v\beta_3$ integrin mediates the attachment of the osteoclast to the bone matrix through the recognition of an RGD sequence presents in bone proteins such as osteopontin and bone sialoprotein [Ross and Teitelbaum, 2005]. The contact of the osteoclast with the bone matrix is associated with the formation of the ruffled border, the highly convoluted membrane facing the matrix, and polarization of F-actin to a circular structure ("actin ring"). The plasma membrane beneath the actin ring forms a tight attachment to the matrix—the sealing zone [Vaananen, 2005]. Unlike most other cells attaching to matrices and forming focal adhesions at the attachment sites, the primary adhesion mediating structures of osteoclasts are dot-like, actin-rich structures known as podosomes [Jurdic et al., 2006]. Podosomes are highly dynamic structures formed not only in osteoclasts, but also in other monocyte-derived cells, such as macrophages and dendritic cells, as well as in smooth muscle cells, endothelial cells, transformed fibroblasts and certain epithelial cells [Linder and Aepfelbacher, 2003]. The non-receptor tyrosine kinase (NRTK) c-Src is critical for the formation of the ruffled border [Miyazaki et al., 2004]. The most striking phenotype of $Src^{-/-}$ mouse is an osteopetrotic phenotype that is characterized by inactive osteoclasts rather than reduced osteoclast number [Soriano et al., 1991]. In fact, osteoclasts express especially high levels of c-Src, consistent with the important role of the kinase in these cells. The lack of ruffled border in Src-deficient osteoclasts suggests impaired vesicle trafficking that is required for the acidification and the secretion of collagenolytic enzymes into the resorption lacuna. An important resorption-related function of c-Src is regulation of the dynamics of the podosomes.

Mineral Dissolution and Organic Phase Degradation

The unique mode of osteoclast attachment to bone, forming an isolated space demarcated by the bone matrix, the sealing zone and the ruffled border, creates a compartment isolated from the general extracellular space. This compartment, the site of resorption, is acidified to a pH of ~4.5 by fusion of acidic vesicles with the ruffled border and by an electrogenic proton pump (H^+-ATPase) coupled to a Cl^- channel. The functional separation of the ruffled border from the rest of the cell membrane by the sealing zone enables concentration of the proton pump and the chloride channel in the ruffled border, and thus the directional release of the proton and the chloride ion to the isolated compartment. The HCl dissolves the solid hydroxyapatite, $[Ca_3(PO_4)_2]_3Ca(OH)_2$, to Ca^{2+}, HPO_4^{2-}, and H_2O. To enable continuous release of HCl into the resorption area, protons are continuously produced by the activity of carbonic anhydrase II, an enzyme that is highly expressed in osteoclasts and facilitates the hydration

of CO_2, resulting in production of protons and HCO_3^-. The latter ion is exchanged to chloride by the chloride-bicarbonate exchanger located in the basolateral membrane. The osteoclast is characterized also by a high number of mitochondria required to produce the energy for the resorption process. The organic matrix is degraded probably by more than one enzyme. However, it seems, at least from the available information, that cathepsin K is the main bone matrix-degrading enzyme [Gowen et al., 1999]. This enzyme is the predominant proteinase in human osteoclasts, but other proteinases (such as cathepsin D, B, and L) are also present in these cells [Drake et al., 1996].

Disposal of Resorption Products

Efficient resorption requires the simultaneous removal of the ions and the collagen fragments produced by the resorption. A specific plasma membrane domain located in the upper part of the cell (opposite to the ruffled border), termed the functional secretory domain (FSD) is the area where degradation products are targeted [Vaananen, 2005]. The degradation products are endocytosed by the osteoclast, the endocytic vesicles (derived from the ruffled border) are targeted to and fused with the FSD, and the degradation products are released into the extracellular fluid. This process, transcytosis, can take place because of the functional separation between the ruffled border and the rest of the cell membrane.

THE OSTEOCLAST: A MULTINUCLEATED CELL

It is reasonable to hypothesize that multinucleation increases resorption efficiency. Otherwise, it would be difficult to understand the reason for the energy investment required for the fusion of the mononuclear precursors to form the large osteoclast. For example, according to this hypothesis the resorption of one osteoclast with five nuclei is more efficient than resorption of five mononuclear osteoclasts.

The multinucleation characterizing the osteoclast is the most striking morphological feature distinguishing the osteoclast from its precursor. The membrane protein, dendritic cell-specific transmembrane protein (DC-STAMP), was found to be critical for fusion of the mononuclear precursors to form the multinucleated osteoclast. Interestingly, DC-STAMP-deficient cells other than failing to fuse, exhibit normal or near normal characteristics of osteoclasts, including formation of actin-ring and ruffled border. However, the resorption efficiency in vitro (normalized per nucleus) was reduced in DC-STAMP-deficient mononuclear osteoclasts and DC-STAMP-deficient mice exhibited increased bone mass [Yagi et al., 2005]. If the absence of DC-STAMP indeed affects only fusion, these data are direct evidence that multinucleation results in a more efficient resorption per nucleus.

Additional support for the connection between the size of the osteoclast and the efficiency of resorption is obtained by the comparing avian and mammalian osteoclasts. Avian osteoclasts contain many more nuclei than mammalian osteoclasts, and have a higher capacity for resorbing bone [Vaananen, 2005]. The remarkable resorption capacity of the bird cells probably developed to accommodate the need for rapid release of calcium to calcify eggshells in laying hens. However, there are no available data comparing the activity of avian and mammalian osteoclasts per size or per nucleus.

A small number of studies directly addressed the connection between the size of the osteoclast and its resorptive activity per nucleus. In one study [Piper et al., 1992] a positive correlation between the number of nuclei per osteoclast and the volume of the pit made was demonstrated, but there was a trend for the volume resorbed per nucleus to decrease with an increase in the number of nuclei per osteoclast. On the other hand, Lees et al. [2001] found no difference in resorption per nucleus when comparing large (containing >10 nuclei) to small (containing 2–5 nuclei) osteoclasts. These investigators instead observed a striking difference between the proportions of actively resorbing osteoclasts of the two populations. While approximately 40% of the large osteoclasts were actively resorbing, only 6% of the small osteoclasts exerted this activity.

Paget's disease of bone provides an example of osteoclasts containing substantially more nuclei than do normal osteoclasts, up to 100 nuclei per cell. This disease is a localized disorder of bone remodeling. The process is initiated by increases in osteoclast-mediated bone resorption, with subsequent compensatory increases in new bone formation, resulting in a disorganized mosaic of woven and lamellar bone at affected skeletal sites. The initiating lesion in

Paget's disease is an increase in bone resorption [Roodman and Windle, 2005]. Quantitative analyses [Weinstein, 1995] revealed two- to ninefold increases in the resorption rate by Pagetic versus normal osteoclasts. Obviously, normalizing to resorptive activity per nucleus, the Pagetic osteoclasts are less active.

The limited literature addressing the connection between the size of osteoclast and the resorptive activity per nucleus does not allow us to reach a solid conclusion. At this point there is no support for the hypothesis that there is a direct correlation between number of nuclei/osteoclast to the resorptive activity/nucleus ("specific resorption"). The only solid conclusion might be that the resorption/nucleus is greater in multinucleated than in mononuclear osteoclasts, based on experiments with DC-STAMP-deficient cells. The significance of the higher proportion of actively resorbing osteoclasts in the larger osteoclasts requires more study. If proven true also in vivo, it will point to an advantage of the larger cells in "specific resorption" efficiency of a whole population that includes resorbing and not resorbing osteoclasts. It is clear, however, that in the case of pathologically huge osteoclasts, such as in Paget's disease of bone, the resorptive activity per nucleus is reduced, and it would be a disaster if it were not the case. It is possible that the size of the cell is more critical than the number of nuclei to the resorptive activity of the osteoclast. It was recently proposed [Aharon and Bar-Shavit, 2006] that aquaporin 9 (AQP9), the only water channel found in the osteoclast-lineage, controls the size of the osteoclast by allowing water entry during osteoclastogenesis. This was demonstrated by smaller osteoclasts formed in the presence of AQP9 inhibitor, but the effect on resorption is yet to be determined. Trebec et al. [2007] showed increased relative expression in large compared to small osteoclasts of integrins α_v and β_3, proMMP9, procathepsin K, RANK, IL-receptor1, and TNF receptor 1.

There is minimal understanding of functional and/or structural differences among nuclei within the same osteoclast. While intuitively there is an expectation for gene expression in the nuclei that is commensurate with the size and specialized regions of the osteoclast, Boissy et al. [2002] showed that all the nuclei within an osteoclast are transcriptionally active, as measured in situ by 5-bromouridine triphosphate (BrUTP) incorporation. Saltman et al. [2005] showed that each nucleus within the osteoclast contains punctately organized microenvironments where regulatory complexes that support transcriptional and post-transcriptional control reside. Functional equivalency of osteoclast nuclei is reflected by similar representation of regulatory proteins that support ribosomal RNA synthesis (nucleolin), mRNA transcription (RNA polymerase II, bromouridine triphosphate), processing of gene transcripts (SC35), signal transduction (NF-κB), and phenotypic gene expression (Runx1). However, the extent to which gene expression in osteoclast nuclei is linked to specialized regions of the cell is yet to be determined.

THE OSTEOCLAST: A MEMBER OF THE "OSTEOIMMUNE SYSTEM"

The interplay between the immune system and bone metabolism has been recognized as important for both of these systems. Various factors produced and released during immune responses markedly affect bone cells and bone metabolism. The finding that cultured human peripheral blood leukocytes release "bone resorbing activity" in culture [Horton et al., 1972] is the first solid evidence pointing to a possible relationship between the immune and the bone systems. This activity was later identified as that of interleukin 1β (IL-1β) [Dewhirst et al., 1985]. In the decades since this discovery it became clear that large numbers of cytokines, receptors, signaling pathways and transcription factors play pivotal roles in both the immune and skeletal systems. More recently, niches for lymphocytes in bone have been shown to play an important role in the biology of these cells. The osteoclast seems to be the most obvious link between the immune and bone systems. This cell derives from the HSC, as do the classical members of the immune system, B and T lymphocytes, and the more closely related monocytes-macrophages and dendritic cells.

Communication between the immune and the skeletal systems is observed in normal physiological processes, but more obviously in autoimmune and other inflammatory diseases. This interrelationship prompted Arron and Choi [2000] to propose the term "osteoimmunology" to describe the interface between immunology and bone biology. The importance of this term is

in the realization that to understand bone homeostasis and remodeling, as well as inflammatory and metabolic bone diseases, one should understand how the immune system is regulated.

T Cells Modulation of Osteoclasts

Although the immune system communicates also with the bone forming cells, the osteoblasts [Walsh et al., 2006], the discussion in this perspective is limited to the osteoclasts. In addition to IL-1, the first immune cell product recognized for its activation of osteoclastic resorption [Dewhirst et al., 1985], many additional cytokines, such as TNF-α, IL-6, IL-11, IL-15, have also been shown to stimulate resorption. Others that exerted inhibitory effects on resorption include IL-4, IL-10, IL-12, IL-13, IL-18, GM-CSF, and IFN-γ [Walsh et al., 2006].

The most important physiological osteoclast differentiation factor, RANKL, which is mainly expressed in osteoblast-lineage cells, is also expressed in activated T cells [Anderson et al., 1997]. Indeed, it was shown that activated T cells can directly trigger osteoclastogenesis via RANKL in vitro, and that systemic activation of T cells in vivo leads to a RANKL-dependent increase in osteoclastogenesis, followed by bone loss. Convincing evidence for the role of T cells as crucial mediators of bone loss in vivo was provided by a study showing that the blocked osteoclastogenesis in RANKL-deficient mice is restored by transgenic overexpression of RANKL in T cells, and a partial restoration of normal bone marrow cavities is also observed [Kim et al., 2000]. It is important to note, however, that mice which lack T cells still have normal bone cavities and tooth eruption, and these cells are thus probably not required for normal bone homeostasis, since for this basal activity RANKL presented to the osteoclast lineage cells by osteoblasts or stromal cells is sufficient. In contrast, chronic systemic activation of T cells results in increased osteoclastogenesis/resorption via production of RANKL in autoimmune diseases, viral infections, or local inflammation within the bone due to metastasis, infections, and fractures, or joint inflammation in arthritis. In the above-mentioned pathological states activated T cells produce in addition to RANKL, proinflammatory cytokines which can induce RANKL expression in osteoblasts and bone marrow stromal cells [Hofbauer et al., 1999], and T cells therefore promote bone resorption also indirectly via expression of proinflammatory cytokines that mediate RANKL expression in osteoblasts.

Osteoclasts and Innate Immunity

The innate branch of the immune system is a phylogenetically ancient defense mechanism that senses invading pathogens via germline-encoded pattern recognition receptors, the Toll-like receptors (TLRs) [Lemaitre et al., 1996]. To date, eleven human TLRs and thirteen mouse TLRs have been identified [West et al., 2006]. These TLRs are activated by specific pathogen-associated molecular patterns (PAMPs). TLRs are expressed in various immune cells, including macrophages, dendritic cells, B cells and specific types of T cells. Different microbial components are sensed by different TLRs. Detection of the pathogen is only the first step in the host's anti-pathogen reaction. The binding of PAMPs to their corresponding TLRs activates signaling pathways leading to the transcription of distinct target genes required for effective immune responses, such as proinflammatory cytokines. The molecular pathways by which TLRs initiate specific gene programs have been extensively studied and reviewed in recent years [Wu and Arron, 2003; West et al., 2006].

Bone cells (and therefore bone remodeling) are regulated by cytokines [Weitzmann and Pacifici, 2005]. The activation of TLRs in innate immune cells induces the synthesis and release of proinflammatory cytokines [Wu and Arron, 2003; West et al., 2006]. Therefore, when the innate immune system is operating, bone metabolism is affected. In this way, TLRs indirectly modulate bone cell activity. TLRs expressed by innate immune cells are thus important links between the immune and bone systems. In addition, it is established that bone cells, osteoblasts and osteoclasts express TLRs, and therefore PAMPs may interact with bone cells to modulate bone cell activity directly, without the need for mediating innate immune cells. PAMPs probably modulate bone cells through both an indirect route, mediated by cells of the innate immune system, and the direct activation of TLRs in osteoclasts and osteoblasts.

While the activation of TLRs in committed osteoclast precursors, mature osteoclasts and osteoblasts results in increased osteoclastogen-

esis and is probably the mechanism by which pathogen-induced bone loss occurs, activation of TLRs in early osteoclast precursors exerts an anti-osteoclastogenic effect [Zou and Bar-Shavit, 2002; Zou et al., 2002; Amcheslavsky et al., 2005]. The anti-osteoclastogenic effect could serve as a mechanism for down-regulating excessive resorption, and as a switch for promoting the differentiation of common precursor cells into inflammatory cells.

Probably the most novel aspect of osteoclast interactions with the immune system was discovered by Kollet et al. [2006], which demonstrated a role for the osteoclast in the mobilization of progenitor cells from the bone marrow to the circulation. These investigators showed that stress, such as mild bleeding and lipopolysaccharide (LPS) administration, resulted in the appearance of many active osteoclasts along the endosteum, and with the mobilization of hematopoietic progenitors from the bone marrow to the circulation.

The osteoimmune axis, showing the interrelationship between the two systems, is illustrated in Figure 3.

CONCLUDING REMARKS

The realization that RANKL, together with M-CSF, induces osteoclast differentiation without the need for a supportive cell is responsible for the breakthroughs we have witnessed in this field over the last decade. Cellular, biochemical and molecular analyses, together with lessons from genetically modified and naturally occurring mutant mice, have contributed to the identification of osteoclastogenesis inducers and inhibitors, and to signaling pathways and transcription factors regulating osteoclast differentiation and activities. Not much is known, however, about the functional variability among the nuclei within an osteoclast, and if such variability exists.

The rapidly growing field of osteoimmunology is expected to provide in the near future a better understanding of the pathogenesis of accelerated bone resorption characterizing a number of bone diseases. This should lead to the development of novel strategies to treat these diseases.

The more we understand mechanisms involved in osteoclast differentiation, attraction, migration and attachment to bone, degradation of bone, viability and apoptosis, more potential drug targets are generated to inhibit excessive pathological resorption (an issue not discussed here, but the subject of numerous studies and reviews).

The current major research directions in osteoclast biology, cell signaling and osteoimmune interface are far from being exhausted, and are therefore expected to supply major advances in our understanding of osteoclast biology. It will be of interest to decipher some basic mechanisms operating in the dynamics of the podosomes which play a crucial role in sealing zone formation and maintenance, in precursor fusion resulting in the multinucleated osteoclast, and in possible spatial specialization of the nuclei within the osteoclast.

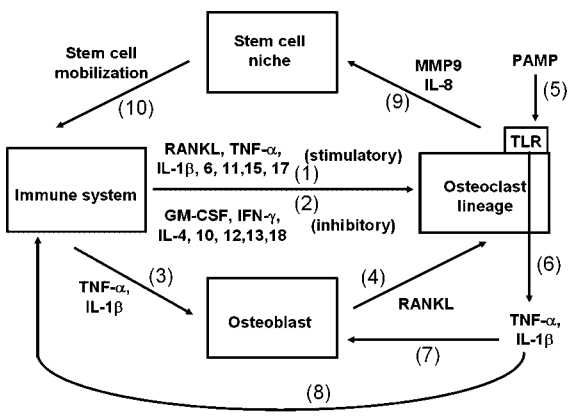

Fig. 3. Crosstalk between the osteoclast and the immune system. Cells of the immune system signal to osteoclasts by releasing stimulatory (1) and inhibitory (2) cytokines that directly interact with osteoclast-lineage cells, as well as cytokines inducing osteoblasts (3), to synthesize RANKL, thereby indirectly affecting osteoclasts (4). Activation of TLRs in osteoclasts (5) induces synthesis and release (6) of cytokines that can modulate osteoblasts (7) as well as immune system cells (8). Upon activation, osteoclasts also release stem cells from their niche (9), modulating the immune system (10).

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