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Regulation of Osteoclast Differentiation and Function by Interleukin-1

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Interleukin-1 (IL-1) is a multifunctional cytokine that regulates various cellular and tissue functions. Among tissues, bone is the most sensitive to IL-1. IL-1 is a potent cytokine for bone resorption and participates in the multiple steps of osteoclast recruitment, such as differentiation,

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multinucleation, activation, and survival. On the other hand, considerable evidence has been accumulated over the past 10 years to indicate that this cytokine plays key roles in pathological bone destruction in a variety of human diseases, including rheumatoid arthritis, osteoporosis, and periodontal disease. In this chapter, we review the history of "IL-1 in bone" and the locus of this cytokine "from laboratory bench to bedside." A better understanding of the role of IL-1 in osteoclastic bone resorption would provide opportunities for developing new therapeutics to treat diseases of the bone.

I. INTRODUCTION

Osteoclasts are terminally differentiated multinucleated cells that are responsible for bone resorption (Nakamura *et al.*, 2003; Suda *et al.*, 1999; Teitelbaum, 2000). They are the principal, if not exclusive, resorptive cell of bone, playing a central role in the formation of the skeleton and regulation of its mass. Osteoclastic bone resorption consists of multiple steps: the proliferation of osteoclast progenitors, differentiation of progenitors into mononuclear prefusion osteoclasts (pOCs), fusion of pOCs into multinucleated osteoclasts, clear zone (actin ring) and ruffled border formation (activation), and apoptosis. Findings indicate that several cytokines and hormones including macrophage colony-stimulating factor (M-CSF, also called CSF-1) (Fuller *et al.*, 1993; Yoshida *et al.*, 1990), interleukin-1 (IL-1) (Jimi *et al.*, 1995, 1999a), receptor activator of NF-κB ligand (RANKL) (Jimi *et al.*, 1999b; Lacey *et al.*, 1998; Yasuda *et al.*, 1998), and tumor necrosis factor-α (TNF-α) (Kim *et al.*, 2005; Kobayashi *et al.*, 2000) regulate differentiation, activation, and survival of osteoclasts.

IL-1 is a multifunctional cytokine that regulates various cellular and tissue functions (Dinarello, 1994). This cytokine refers to two polypeptides, IL-1α and IL-1β. Although IL-1α and IL-1β are independent gene products, they recognize the same cell-surface receptors (type I and type II) and elicit similar biological responses through the type I receptor (Dinarello, 1994). A natural inhibitor to IL-1 has been identified (Arend *et al.*, 1990; Dinarello, 1994). This peptide, IL-1 receptor antagonist (IL-1Ra) is a naturally occurring structure variant of IL-1 that binds to, but does not activate, IL-1 receptors. Among the tissues, bone is most sensitive to IL-1. Historically, activated monocytes/macrophages were shown to produce a potent bone-resorbing factor initially termed "osteoclast-activating factor (OAF)" (Gowen *et al.*, 1983), and this factor was later identified as IL-1β (Dewhirst *et al.*, 1985). In the 1980s, several lines of evidence demonstrated that IL-1 exhibits potent bone-resorbing activity in *in vitro* organ cultures (Gowen *et al.*, 1984;

Lorenzo *et al.*, 1987). In the opposite way, IL-1Ra blocks the ability of IL-1 to stimulate bone resorption in organ cultures (Seckinger *et al.*, 1990). IL-1 also stimulates bone resorption when infused *in vivo* and causes a substantial increase in plasma calcium levels (Boyce *et al.*, 1989; Sabatini *et al.*, 1988). At the cellular level, IL-1 participates in the multiple steps of osteoclastic bone resorption, such as differentiation, multinucleation, activation, and survival. IL-1 also plays critical roles in the pathological bone destruction associated with multiple myeloma, rheumatoid arthritis, and osteoporosis (Dinarello, 1994). Here, we discuss the involvement of IL-1 in osteoclast differentiation and function, mainly from the viewpoint of osteoclast cell biology.

II. INTERLEUKIN-1 AND OSTEOCLAST DIFFERENTIATION

The molecular events involved in the differentiation and activation of osteoclasts had not been clarified until recently due to the lack of suitable *in vitro* models to investigate osteoclast biology. However, the development of reliable methods to isolate and culture large number of primary osteoclasts or to generate osteoclasts in *in vitro* culture systems allowed remarkable progress in the field of osteoclast research over the course of the last decade. Takahashi *et al.* (1988) developed an excellent *in vitro* culture system of osteoclast differentiation, so-called "Takahashi's coculture system," in which mouse osteoblastic cells and spleen cells were cocultured in presence of osteotropic factors, such as 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$] and prostaglandin E_2 (PGE_2). This murine coculture system has made it possible to investigate the molecular mechanism of osteoclast differentiation, finally leading to the historic discovery of RANKL, the master molecule for osteoclast differentiation (Lacey *et al.*, 1998; Yasuda *et al.*, 1998). It is now a well-established concept that various osteotropic factors, such as 1α,25$(OH)_2D_3$, PGE_2, parathyroid hormone (PTH), and IL-11 induce RANKL expression on the surface of osteoblasts/stromal cells, leading to the differentiation of hematopoietic cells into osteoclasts (Suda *et al.*, 1999).

The involvement of IL-1 in osteoclast differentiation was analyzed using this murine coculture system. Akatsu *et al.* (1991) clearly demonstrated that IL-1 stimulated osteoclast formation not directly but indirectly via PGE_2 synthesis in cocultures of murine osteoblastic cells and spleen cells. However, in terms of the multinucleation of osteoclasts, IL-1 plays a direct role. Using the culture system of purified pOCs established by Wesolowski *et al.* (1995), we reported that IL-1 induces the fusion of mononuclear prefusion osteoclasts (Jimi *et al.*, 1999a). In this sense, IL-1 is indirectly involved in osteoclast differentiation and directly involved in osteoclast multinucleation (Fig. 1). On the other hand, M-CSF, RANKL, and TNF-α among

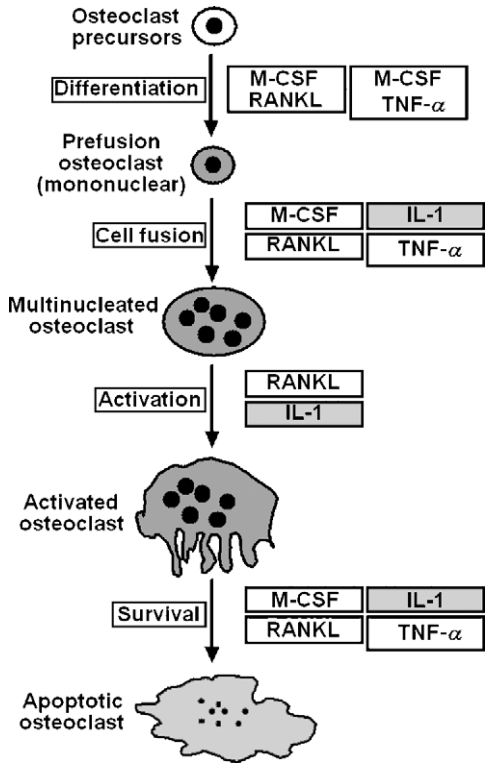

FIGURE 1. The differentiation and activation pathway of osteoclast progenitors into functionally active osteoclasts and the cytokines required for each step.

other related cytokines, have been demonstrated to play essential roles in osteoclast differentiation (Kobayashi *et al.*, 2000; Kong *et al.*, 1999; Yoshida *et al.*, 1990) and induce cell fusion of osteoclasts (Amano *et al.*, 1998; Jimi *et al.*, 1999a,b) (Fig. 1).

III. INTERLEUKIN-1 AND OSTEOCLAST ACTIVATION

A. MECHANISM OF ACTION OF IL-1 IN OSTEOCLAST ACTIVATION AT THE CELLULAR LEVEL

The most important involvement of IL-1 in osteoclasts is the activation of the bone-resorbing activity of these cells. Thomson *et al.* (1986) first reported that IL-1 stimulated the pit-forming activity of isolated rat osteoclasts. They showed that this effect of IL-1 was not due to a direct action of the cytokine,

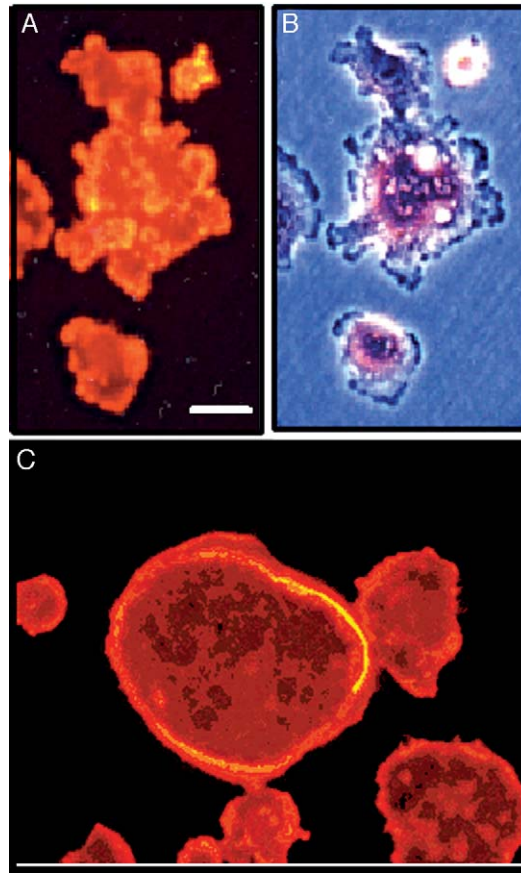

FIGURE 2. Effects of IL-1 on cytoskeleton and cell fusion of prefusion osteoclasts. Prefusion osteoclasts were plated on vitronectin (20 μg/ml)-coated dishes in the absence of serum. After culture for 60 min, cells were treated with IL-1 (10 ng/ml) for 0 (A, B) and 30 (C) min. Cells were fixed and stained for tartrate-resistant acid phosphatase (B) or F-actin (A, C). Note that IL-1 induces actin ring formation and the multinucleation of osteoclasts. Bar = 10 μm

but was mediated by soluble factor(s) secreted by osteoblasts. Nowadays, however, several lines of evidence have clearly demonstrated the expression of IL-1 receptors in mature osteoclasts *in vivo* and *in vitro*, suggesting the direct action of IL-1 on osteoclasts (Jimi *et al.*, 1998; Xu *et al.*, 1996). Activated osteoclasts resorbing bone have a specific ringed organization of F-actin, known as the actin ring, which is now recognized as a marker for osteoclast activation (Lakkakorpi and Väänänen, 1991; Nakamura *et al.*, 1996; Turksen *et al.*, 1988). Using the purified pOC culture system, we have demonstrated that IL-1 directly induces actin ring formation (Fig. 2) and the

activation of osteoclasts at the cellular level (Jimi *et al.*, 1999a; Kobayashi *et al.*, 2000, Nakamura *et al.*, 2002).

B. MECHANISM OF ACTION OF IL-1 IN OSTEOCLAST ACTIVATION AT THE MOLECULAR LEVEL

Then, how is IL-1-initiated signaling transduced in osteoclasts, resulting in actin ring formation and bone resorption? The first molecule to be discussed is TRAF6 (Fig. 3). TRAF, TNF receptor-associated factor family proteins, are adaptor molecules that mediate the intracellular signaling of various cytokine receptors, including the TNF receptor superfamily and the Toll/IL-1 receptor family (Inoue *et al.*, 2000). To date, six members of the TRAF family have been identified. TRAF6 is the only TRAF that is involved in the signal from the Toll/IL-1 receptor family by interacting with the IL-1 receptor-associated kinase (IRAK). The essential role of TRAF6 in physiological bone development has now been clarified by the results from TRAF6-deficient mice, in which the osteopetrotic phenotype was observed. According to the report by Lomaga *et al.* (1999), in these mice, abundant dysfunctional osteoclasts were found in their skeletal tissues, which is compatible with the direct involvement of IL-1 in osteoclast activation, but not in osteoclast differentiation. Armstrong *et al.* (2002) confirmed the involvement of TRAF6 in osteoclast activation in *in vitro* cultures. Kim *et al.* (2005) also showed that TRAF6 is essential for osteoclast function, but not for osteoclast differentiation, using cell cultures from TRAF6 null mice.

Another question to be answered is: What is the next molecule for osteoclast activation? Generally, IL-1 enhances various intracellular signal transduction pathways, including NF-κB activation, ERK activation, and so forth. The interesting aspect was the similar phenotype of TRAF6-deficient

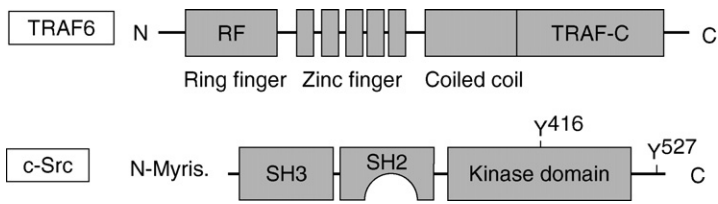

FIGURE 3. Structural features of c-Src and TRAF6. Src kinase contains a unique N-terminal region, an SH3 (Src-homology-3) domain that binds to proline-rich motifs, an SH2 domain that binds to phosphotyrosine-containing motifs, a kinase domain, and a C-regulatory sequence that is phosphorylated by the Csk protein-tyrosine kinase. Domains within TRAF6 include ring finger (RF), zinc finger, coiled coil, and TRAF-C domains. The direct interaction of TRAF6 and c-Src is mediated by the RPTIPRNPK motif (aa 469–477) in TRAF6 and the SH3 domain in c-Src.

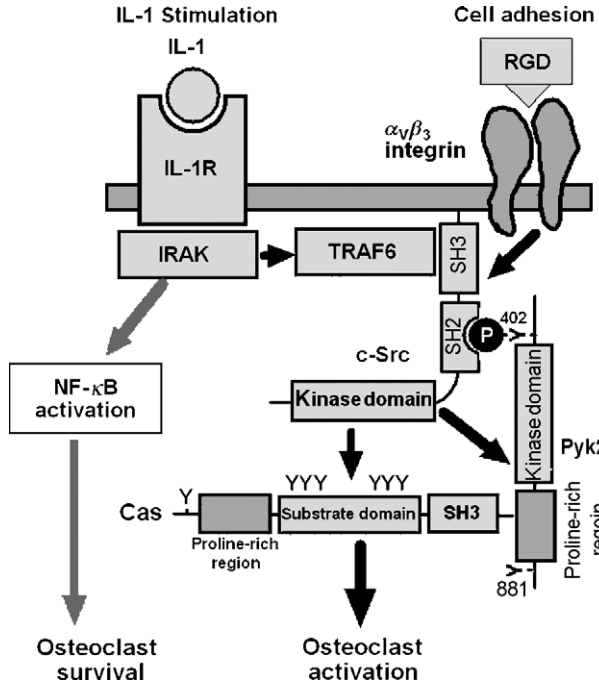

FIGURE 4. Our proposed model of IL-1-induced signaling pathways in osteoclasts. Osteoclast adhesion via $\alpha_V\beta_3$ integrin induces c-Src-dependent tyrosine phosphorylation of Pyk2 and $p130^{Cas}$, leading to the cytoskeletal organization of osteoclasts. IL-1 signaling cross talks with this tyrosine phosphorylation pathway via the association of TRAF6 with c-Src, leading to the formation of a huge molecular complex including TRAF6, c-Src, Pyk2, and $p130^{Cas}$, and results in osteoclast activation. On the other hand, IL-1-induced NF-κB activation plays an important role in osteoclast survival.

mice to that of c-Src-deficient mice. c-*src* was first identified as the normal cellular counterpart of the oncogene encoded by Rous sarcoma virus, v-*src* (Thomas and Brugge, 1997). The protooncogene product c-Src is a 60-kDa protein and belongs to the nonreceptor-type tyrosine kinase family, the structural feature of which is shown in Fig. 3. Soriano *et al.* (1991) reported that the targeted disruption of c-*src* in mice induced osteopetrosis. The absence of c-Src is sufficient to abolish bone resorption *in vivo,* without reducing the osteoclast number (Boyce *et al.*, 1992), suggesting that c-Src plays an essential role in osteoclast activation. The similarity of these two knockout mice suggests that both c-Src and TRAF6 have some mutual relationship in osteoclast function. In fact, we found that IL-1 stimulation induced the activation of c-Src kinase, tyrosine phosphorylation of Pyk2 and $p130^{Cas}$, the substrates of c-Src (Duong *et al.*, 1998; Nakamura *et al.*, 1998), and the formation of a huge molecular complex, including TRAF6,

c-Src, Pyk2, and $p130^{Cas}$, resulting in the actin ring formation and osteoclast activation (Nakamura *et al.*, 2002). In $Src^{-/-}$ osteoclasts, IL-1 did not induce the tyrosine phosphorylation of Pyk2 and $p130^{Cas}$ and did not rescue the bone-resorbing activity of $Src^{-/-}$ osteoclasts, suggesting the functional interaction of TRAF6 and c-Src (Nakamura *et al.*, 2002). Wong *et al.* (1999) also reported the association of TRAF6 and c-Src in the signal transduction pathway of RANKL, which led to the activation of Akt/PKB and osteoclast survival. According to their report, the direct interaction of TRAF6 and c-Src is mediated by the RPTIPRNPK motif (aa 469–477) in TRAF6 and the SH3 domain in c-Src. The molecular mechanism of action of IL-1 in osteoclast activation is proposed in Fig. 4.

Several findings retorting against the concept mentioned earlier were also reported. Naito *et al.* (1999) reported that TRAF6 was an essential transducer for osteoclast differentiation, since their $TRAF6^{-/-}$ mice were defective in osteoclast formation and exhibited severe osteopetrosis. Miyazaki *et al.* (2000) reported that NF-κB activation plays a key role in IL-1-induced osteoclast function, using adenovirus vectors carrying dominant negative IκB kinase 2 (IKK2) and constitutively active IKK2. Further studies will be required to explain this discrepancy. On the other hand, among the other related cytokines, RANKL is also involved in osteoclast function (Burgess *et al.*, 1999; Jimi *et al.*, 1999b), although neither M-CSF nor TNF-α can induce actin ring formation and osteoclast activation (Jimi *et al.*, 1999a; Kim *et al.*, 2005) (Fig. 1).

IV. INTERLEUKIN-1 AND OSTEOCLAST SURVIVAL

Another important involvement of IL-1 in bone resorption is the survival of osteoclasts. When osteoclasts were purified by removing osteoblasts from cocultures, osteoclasts rapidly died within 48 h due to spontaneously occurring apoptosis. The addition of IL-1 to the purified osteoclasts prolonged the survival of these cells (Jimi *et al.*, 1995). Thus, IL-1 is involved in prolonging the lifespan of osteoclasts as well as in the activation of osteoclasts. Besides IL-1, M-CSF (Fuller *et al.*, 1993), RANKL (Jimi *et al.*, 1999b), and TNF-α (unpublished observation) also induce osteoclast survival (Fig. 1).

Although the involvement of IL-1 in osteoclast survival at the cellular level is now well accepted, its molecular mechanism still remains to be discussed. We have shown that IL-1 activated NF-κB in osteoclasts and that the activation of NF-κB is involved in the survival of osteoclasts, using antisense oligodeoxynucleotides to NF-κB (Rel A/p65 and p50) and proteasome inhibitors (Jimi *et al.*, 1996, 1998). On the other hand, M-CSF, another survival factor of osteoclasts, does not activate NF-κB in osteoclasts (Jimi *et al.*, 1996), indicating that some signals other than NF-κB should be

involved in the survival of osteoclasts due to M-CSF. As pointed out by Miyazaki *et al.* (2000), ERK is a candidate molecule for M-CSF-induced osteoclast survival. Akiyama *et al.* (2003) also reported that the proapoptotic BH3-only Bcl-2 family member Bim is critical for controlling osteoclast survival and apoptosis. In spite of distinct pathways for IL-1- and M-CSF-induced cell survival, there must be a common downstream pathway in these signals. In this sense, Okahashi *et al.* (1998) reported that both IL-1 and M-CSF reduce caspase activity, which is known to induce apoptosis in osteoclasts.

There are several reports retorting against our findings. Lee *et al.* (2002) showed the IL-1α-stimulated osteoclast survival through the PI 3-kinase/Akt and ERK pathways. Miyazaki *et al.* (2000) also demonstrated that ERK activation plays a key role in IL-1-induced osteoclast survival, using adenovirus vectors carrying dominant negative Ras and constitutively active MEK1. Further studies are necessary to elucidate the precise role of NF-κB-mediated signals in osteoclast activation and survival.

V. INTERLEUKIN-1 AND RHEUMATOID ARTHRITIS

Considerable evidence has accumulated over the past 10 years to indicate that IL-1 mediates inflammation and pathological bone destruction in a variety of human diseases associated with multiple myeloma, rheumatoid arthritis (RA), osteoporosis, and periodontal disease. Pacifici's group reported that IL-1 is implicated as a potential mediator of bone resorption and increased bone turnover in postmenopausal osteoporosis (Pacifici *et al.*, 1989) and that the *in vivo* administration of IL-1Ra inhibited the bone loss that occurs in ovariectomized rats (Kimble *et al.*, 1994).

On the other hand, one of the most-studied diseases is RA. Eastgate *et al.* (1988) first demonstrated the correlation of plasma IL-1 levels with disease activity in RA. The important involvement of IL-1 in RA has been further confirmed by the results of studies on mice that are genetically engineered to overproduce IL-1Ra (transgenic mice) or those that lack the capacity to make any isoforms of IL-1Ra (knockout mice). Collagen-induced arthritis in mice was reduced in incidence and severity in IL-1Ra transgenic mice, whereas the opposite pattern was observed in IL-1Ra-deficient mice (Ma *et al.*, 1998). Another report showed that IL-1Ra-deficietnt mice, when crossed into the BALB/cA background, spontaneously developed an inflammatory arthritis that exhibited many features of RA (Horai *et al.*, 2000). Moreover, there is the increasing evidence that the ratio of IL-1Ra and IL-1 may be under genetic control and may influence the development or severity of certain disease. Actually, a possible association between polymorphisms in the IL-1Ra gene and various rheumatic diseases has been studied

(Barrera *et al.*, 2001; Cantagrel *et al.*, 1999; Cvetkovic *et al.*, 2002; Genevay *et al.*, 2002; Perrier *et al.*, 1998).

These numerous studies at the genetic, molecular, cellular, and *in vivo* (animal model) levels have indicated that the exogenous administration of IL-1Ra might be therapeutically beneficial for RA. Clinical trials in RA examining the administration of recombinant human IL-1Ra by subcutaneous injection have yielded positive results. A total of 472 patients with active and severe RA were studied in a 24-week, double-blind, randomized, placebo-controlled, multicentered clinical trial (Bresnihan *et al.*, 1998). In patients treated with the highest dose of IL-1Ra (a single daily subcutaneous injection of IL-1Ra at 150 mg), 43% achieved an American College of Rheumatology (ACR) 20 response, compared with 27% in the placebo group. In addition, the IL-1Ra-treated group overall demonstrated a lower rate of radiological progression over 48 weeks (Jiang *et al.*, 2000). Histological studies on serial synovial biopsies revealed a reduction in mononuclear cell infiltration in four patients with a favorable clinical response (Cunnane *et al.*, 2001). This therapy with IL-1Ra has now been approved by the regulatory agencies in the United States and Europe.

VI. PROSPECTS

In the near future, studies of "IL-1 in bone" should be focused on the precise molecular mechanism of action of this cytokine in osteoclast activation. Especially, in terms of the role of NF-κB in osteoclast function, molecular biology studies and the analysis of other knockout mice would allow us to achieve a more precise understanding of the intracellular function of this molecule. In the long term, these continuous trials for the better understanding of osteoclast biology would lead to the availability of new therapeutic agents to treat diseases of bone.

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