Review

Osteoclast migration, differentiation and function: novel therapeutic targets for rheumatic diseases

Junichi Kikuta¹² and Masaru Ishii¹²

Abstract

RA is a chronic autoimmune disease characterized by joint synovial inflammation and progressive cartilage/bone destruction. Although various kinds of RA drug have been developed worldwide, there are currently no established methods for preventing RA-associated bone destruction, the most severe outcome of this disease. One of the major pathogenic factors in arthritic bone destruction is the enhanced activity of osteoclasts at inflammatory sites. Osteoclasts are bone-resorbing giant polykaryons that differentiate from mononuclear macrophage/monocyte-lineage haematopoietic precursors. Upon stimulation by cytokines, such as M-CSF and RANK ligand, osteoclast precursor monocytes migrate and attach onto the bone surface (migration). They then fuse with each other to form giant cells (differentiation) and mediate bone resorption (function). In this review, we summarize the current understanding regarding the mechanisms underlying these three dynamic steps of osteoclastic activity and discuss novel lines of osteoclast-targeted therapies that will impact future treatment of RA.

Key words: osteoclast, bone erosion, rheumatoid arthritis, cellular dynamics, chemokine, RANKL, cathepsin, imaging.

Introduction

RA is a chronic autoimmune disease that is characterized by joint synovial inflammation and progressive cartilage and bone destruction [1]. RA can cause pain, swelling, stiffness and severe impairment of joints, thereby resulting in disability and deformity owing to permanent joint destruction. The onset of RA frequently occurs around the age of 30–50 and the disease can last a lifetime. Over the past decade, the remarkable advances of anti-RA therapies, including biologic agents, have improved the symptoms, long-term outcomes and quality of life of RA patients [2]. Despite enhanced treatment of this intractable disease, there remain a large number of underprivileged patients who cannot utilize these advances. These patients continue to suffer from severe bone destruction and the resultant dysfunction in arthritic joints.

Various cell types, such as macrophages, T/B lymphocytes and synovial fibroblasts, have been reported to be involved in the pathogenesis of chronic inflammation in RA [3, 4]. However, bone destruction is considered to be mainly mediated by enhanced activation of osteoclasts, the only somatic cell type capable of resorbing bone matrices [5]. The mode of action of osteoclasts consists of several processes including migration, differentiation and bone-resorptive function [6–8]. In this review, we summarize the current knowledge regarding these dynamic processes in osteoclast activity and discuss novel therapeutic strategies for the treatment of RA by targeting these steps.

Migration of osteoclast precursors

Osteoclast precursors originate from mononuclear macrophage/monocyte-lineage haematopoietic cells and usually exist in the bone marrow cavity and blood stream [8, 9]. Osteoclasts can differentiate in vitro from bone marrow-resident monocytoid lineage progenitors, such as common monocytoid progenitors as well as circulating monocytes. To date, there have been several reports characterizing the entity of osteoclast precursors, although whether there are specific monocytoid populations committed to differentiating exclusively into osteoclasts remains to be elucidated [10–12]. Monocytes are plastic and various kinds of monocytoid cell, including CD11c⁺ dendritic cells, can differentiate into osteoclast-like cells.

¹Laboratory of Cellular Dynamics, WPI-Immunology Frontier Research Center, Osaka University, 3-1 Yamada-oka, Suita, Osaka and ²CREST, Japan Science and Technology, Chiyoda-ku, Tokyo, Japan.

Correspondence to: Masaru Ishii, Laboratory of Cellular Dynamics, Immunology Frontier Research Center, Osaka University, 3-1 Yamada-oka, Suita, Osaka 565-0871, Japan.

E-mail: mishii@ifrec.osaka-u.ac.jp

Submitted 29 March 2012; revised version accepted 10 August 2012.
in vitro when supplemented with adequate conditioned media [13]. Regardless, monocyteid cells, including osteoclast precursors, seem to circulate systemically and migrate into the bone surfaces targeted for resorption (Fig. 1).

**CXCL12 and CX3CL1**

What regulates the migration and positioning of osteoclast precursors in vivo? The recruitment of osteoclast precursors toward bone-lining osteoblasts possessing RANK ligand (RANKL) on their surface is critical for osteoclast differentiation. Recent studies have revealed the involvement of several chemokines in controlling osteoclast precursor migration from the circulation into bone or within the bone cavity. One of the best characterized chemoattractants for controlling osteoclast precursors is CXCL12/SDF-1, stromal cell-derived factor-1 [14, 15]. CXCL12 is highly expressed by specific stromal cells enriched in perivascular regions in the bone marrow cavity [CXCL12-abundant reticular (CAR) cells] [16]. The chemokine receptor CXCR4, on the other hand, is expressed on a wide variety of haematopoietic cells, including circulating monocytes and osteoclast precursors. CXCL12 has been shown to promote chemotactic recruitment, development and survival of osteoclast precursors, which express large amounts of CXCR4 [17].

Recently, CX3CL1 (fractalkine), a different chemokine, has also been shown to be involved in osteoclast precursor migration [18]. CX3CL1 is expressed by osteoblasts, whereas osteoclast precursors preferentially express its cognate receptor CX3CR1. CX3CL1 was suggested to play an important role in recruitment into the bone marrow cavity as well as firm adhesion onto the endosteum of osteoclast precursors. In summary, both the CXCL12–CXCR4 and CX3CL1–CX3CR1 axes may be novel targets for the treatment of RA.

**Sphingosine-1-phosphate**

Sphingosine-1-phosphate (S1P), a lipid mediator enriched in blood, is known to be a critical regulator of lymphocyte chemotaxis in a similar manner as chemokines [19–21]. In mammals, there are five types of receptors for S1P, S1PR1–S1PR5 [21]. We recently showed that osteoclast precursors express S1PR1 and S1PR2, and that S1P dynamically regulates migration and localization of osteoclast precursors in vivo [22, 23]. S1PR1 and S1PR2 have reciprocal effects on the migration of osteoclast precursors [19, 24]. S1PR1 promotes chemotactic movements into blood circulation (where S1P is high), whereas S1PR2 requires a higher concentration of S1P for activation. S1PR2 also negatively regulates the S1PR1 response or even induces chemotaxis to the opposite direction of the S1P gradient, called chemorepulsion, when activated [25]. In a higher S1P concentration environment, such as in blood vessels, S1PR2 activity is overwhelming and osteoclast precursors can enter into bone spaces by chemorepulsion mediated by S1PR2. After the precursors enter a lower S1P environment, such as in bone marrow, S1PR1 is reactivated and some of the osteoclast precursors may return to blood vessels according to S1P gradients. The number of osteoclast precursors on the bone surface is finely determined by the balance between the trafficking of osteoclast precursors to and from the circulation. This is a novel point of control for osteoclastogenesis in vivo [23].

**Therapeutic target for S1P**

S1P controls the migratory behaviour of osteoclast precursors. This critical control point in osteoclastogenesis is also an attractive target for treating RA and osteoporosis. We previously showed that FTY720, an agonist for four of the five S1P receptors (excluding S1PR2, and preferably acting on S1PR1), relieved ovariectomy-induced osteoporosis in mice by reducing the number of mature osteoclasts attached to bone surfaces [22]. We also revealed that FTY720 suppressed collagen antibody-induced arthritis and recovered the ovariectomy-induced bone density loss simultaneously in ovariectomized mice with arthritis [26]. These results clearly suggest that S1P-targeted therapy, such as with S1P receptor agonists, would be beneficial for treating elderly female RA patients who suffer from post-menopausal bone loss and arthritic inflammation simultaneously. Moreover, the mechanism of action of S1P is completely different from that of conventional treatments such as bisphosphonates, which suppress mature osteoclasts [27]. Therefore, synergistic therapeutic effects would be expected if these drugs were administered simultaneously.
Differentiation of osteoclast precursors into mature osteoclasts

The differentiation of haematopoietic progenitor cells into bone-resorbing mature osteoclasts is regulated by the sequential exposure of specific factors, which activate intracellular signalling cascades. Macrophage-colony stimulating factor (M-CSF) and RANKL are two major factors essential for osteoclast differentiation [28]. M-CSF and RANKL activate multiple intracellular signalling pathways resulting in the transcriptional/expressional regulation of osteoclast-specific genes (Fig. 2).

M-CSF signalling

M-CSF is critical for the proliferation and survival of osteoclast precursors [29, 30]. M-CSF is produced by osteoblasts and stromal cells and binds to its cognate receptor c-Fms/CSF1-R/CD115 expressed on early osteoclast precursors [31]. PU.1, a member of the E-26 (ETS) family of transcription factors, regulates the differentiation and commitment of precursor myeloid cells to monocytic osteoclast lineages by stimulating the expression of c-Fms [32-34]. c-Fms, a member of the receptor tyrosine kinase superfamily, becomes phosphorylated on tyrosine residues upon stimulation by M-CSF [35]. Phosphorylated c-Fms then activates extracellular signal-regulated kinase (ERK) through the growth factor receptor bound protein 2 (Grb-2)-son of sevenless (SOS) complex and Akt through phosphoinositide 3-kinase (PI3K) [36]. M-CSF also acts as a survival factor of osteoclast precursors by activating microphthalmia-associated transcription factor (Miff), which induces anti-apoptotic B-cell leukaemia/lymphoma-associated gene 2 (Bcl-2) [37-39]. Furthermore, M-CSF stimulates the expression of RANK in osteoclast precursors [40].

RANKL–RANK–osteoprotegerin axis

RANKL, a member of the TNF-α superfamily, is induced in osteoblasts and stromal cells by hormones and factors such as 1,25 (OH)2 vitamin D3, PTH, IL-1β and TNF-α [41-43]. RANKL binds to its receptor RANK on osteoclast precursors [44], which leads to the expression of various osteoclast genes including tartrate-resistant acid phosphatase (TRAP), cathepsin K, calcitonin receptor, α/β3-integrin and MMP-9.

Osteoprotegerin (OPG) is a soluble decoy receptor that blocks RANKL binding to RANK, thereby inhibiting osteoclastogenesis induced by RANKL [45, 46]. OPG is produced by osteoblasts and stromal cells in response to anabolic agents such as oestrogens and TGF-β-related BMPs [47, 48]. The ratio of RANKL to OPG is critical for controlling osteoclast differentiation and bone-resorptive function. A relative abundance of OPG blocks osteoclast production and leads to osteopetrosis, whereas a relative abundance of RANKL results in enhanced bone remodelling and osteoporosis [45, 49].

RANKL has been studied as a potential target for the treatment of RA. Denosumab is a fully humanized anti-RANKL mAb. It can reduce bone turnover markers and increase BMD in post-menopausal women with low BMD [50-52]. It can also reduce fracture risk in women with post-menopausal osteoporosis and inhibit structural damage in patients with RA when added to ongoing MTX treatment [53].

RANK signalling

During osteoclastogenesis, binding of RANK to RANK induces the recruitment of adaptor molecules such as the TNF receptor-associated cytoplasmic factors (TRAFs) to sites on the cytoplasmic domain of RANK [54-56]. Among TRAF family proteins, TRAF6 is critical for osteoclast differentiation and activation [57-59]. The downstream signalling pathways from TRAF6 include IκB kinase (IκK), nuclear factor κB (NF-κB), c-Jun N-terminal kinase (JNK), Akt, c-Src, p38, ERK, activator protein 1 (AP-1), and nuclear factor and activator of transcription (NFATc1) [60]. Signalling via RANK requires co-stimulation via one of two immunoreceptor tyrosine-based activation motif (ITAM)-containing receptors, DNAX-activating protein of molecular mass 12 kDa (DAP12) and FcγR7 [61, 62].

NFATc1 is an important target for controlling excessive osteoclastogenesis. LIF inhibits osteoclastogenesis by suppressing the induction of NFATc1 in osteoclast precursors [63, 64]. The calcineurin inhibitors FK506 and CSA, which suppress NFATs, have been approved for the treatment of RA [65, 66]. However, their direct effects on osteoclastogenesis contributing to their clinical efficacy remain to be elucidated. Combinatory use of DMARDs can also reduce inflammation and bone destruction, although the mechanism by which DMARDs exert their bone-protective effects is also poorly understood. It has...
been shown using an in vitro culture system that MTX, buccillamine and SSZ inhibit osteoclastogenesis by interfering with RANKL-mediated induction of NFATc1 in osteoclast precursors [67].

**TGF-β**

TGF-β is a multifunctional cytokine that regulates ubiquitous proliferation and differentiation activity in a variety of cells and is abundantly present in bone [68]. TGF-β binds to two different types of serine/threonine kinase receptors, termed type I and type II receptors. A type I receptor is activated by a type II receptor upon ligand binding and transduces signals basically through Smad family proteins by inducing phosphorylation with their kinase activity [69]. TGF-β acts directly on bone marrow macrophages and promotes osteoclastogenesis, whereas it inhibits osteoclastogenesis indirectly through the effect on stromal cells surrounding bone marrow macrophages [70–72]. Recently, it has been reported that TGF-β regulates RANKL-induced osteoclastogenesis through molecular interaction between Smad3 and TRAF6 [73]. TGF-β type I receptor kinase inhibitors do not only suppress RANKL-induced osteoclastogenesis but prevent anti-collagen type II antibody-induced arthritis in mice [74]. The blockade of TGF-β signalling may become an alternative strategy for the treatment of RA.

**Protein kinase CβIII**

Protein kinase C (PKC) proteins play roles in proliferation, differentiation and survival in various cell types [75]. PKC family enzymes have 10 isotypes. Among them, PKC-β can generate two isoforms (PKC-βI and βII) [76, 77]. Expression of PKC-βI and βII is increased during osteoclast differentiation, and PKC-βI has been shown to have a pivotal role in regulating differentiation, cell fusion and bone-resorptive function of mature osteoclasts by participating in the ERK signalling pathway of M-CSF and RANKL [78].

**Cell–cell fusion**

Osteoclast precursors fuse with one another and become multinucleated during maturation under the influence of RANKL. Dendritic cell-specific transmembrane protein (DC-STAMP), a seven-transmembrane domain protein, has been shown to be an essential regulator of osteoclast precursor cell fusion [79–81]. DC-STAMP-deficient mice exhibited impaired osteoclastic bone resorption despite expressing normal osteoclast markers [79, 82]. Vacular type H⁺-ATPase is a ubiquitous multisubunit complex mediating the adenosine triphosphate (ATP)-dependent transport of protons. The d2 isoform of vacular type H⁺-ATPase (V-ATPase) V0 domain (Atp6v0d2), which secretes H⁺ from osteoclasts, has also been shown to regulate osteoclast fusion and bone formation. Atp6v0d2-deficient mice show defective osteoclasts and enhanced bone formation [83]. Since NFATc1 regulates the expression of DC-STAMP and Atp6v0d2, osteoclast cell–cell fusion is induced along with the differentiation of osteoclasts [82, 84].

**Osteoclast function**

Osteoclasts function through the degradation and removal of both the inorganic mineral and organic matrix. To resorb bone effectively, osteoclasts attach tightly to the bone surface, become polarized and reorganize their cytoskeleton and membrane to form the sealing zone and the ruffled border, between the osteoclast and bone surface, separate from bone marrow cavity. Bone degradation is localized within this isolated area (Fig. 3).

**Cytoskeleton**

Osteoclast attachment to the bone surface occurs through the activity of integrins, particularly αvβ3-integrin, a vitronectin receptor [85, 86]. αvβ3-integrins form a distinct actin complex known as the podosome [87, 88] and recognize proteins in the organic matrix of bone that include Arg-Gly-Asp (RGD) amino acid motifs [89]. Podosomes contain a F-actin core and actin regulatory proteins including cortactin, Wiskott–Aldrich Syndrome Protein (WASP) and Arp2/3. The area surrounding the actin core is also rich in kinases such as c-Src and Pyk2, and the Rho family small GTPases, Rac and Cdc42.

For rearrangement of the cytoskeleton during osteoclast polarization, integrins recruit many kinds of protein such as non-receptor tyrosine kinases c-Src, Pyk2 and Syk, and ubiquitin ligase c-Cbl [90–95]. M-CSF also plays a role in controlling integrin signalling. The key components of the signal cascades from c-Fms to the osteocytoskeleton are c-Src, Syk, PI3K and Cbl. DAP12, a transmembrane adapter molecule, is a shared player of growth factor and integrin signals [96, 97]. The spleen tyrosine kinase (Syk)/DAP12 association represents a point of convergence between M-CSF and αvβ3 signalling to the cytoskeleton.

c-Src phosphorylates Syk, which leads to cytoskeletal re-organization by promoting the activation of Vav3, a guanine nucleotide exchange factor (GEF) for the Rho

![Fig. 3 Bone-resorptive function of mature osteoclasts.](image-url)
family small GTPase [98] and subsequently of Rac and Cdc42. These small GTPases stimulate WASP and cortactin, known activators of the ARP2/3 complex that is required for actin nucleation and polymerization.

Nitrogen-containing bisphosphonates are therapeutically effective inhibitors for bone resorption. These drugs target farnesyl diphosphate (FPP) synthase, an enzyme in the cholesterol biosynthesis pathway required for the post-translational modification of small GTPases (including Rho, Rac and Cdc42). As appropriate localization of these small GTPases at the membrane is critical for regulation of the osteoclast cytoskeleton, bisphosphonates disrupt the osteoclast cytoskeleton, reduce resorptive activity and induce apoptosis.

Acidification

To activate matrix-degrading enzymes and dissolve the mineral component of bone, acidification of the lacunar space below the ruffled border membrane is required [99]. A specialized resorptive organelle, the ruffled membrane, is formed by fusion of secretory vesicles into the plasma membrane within the sealing zone. This directional vesicular trafficking occurs along microtubules, and then microfilaments, mediated by the small GTPases Rab7, Rab3D and Rac1 [100, 101]. The vesicles contain the V-ATPase and the ClC-7 chloride channel. H+ and Cl− ions are expelled though the ruffled border into the sealed zone [102] and the low pH is created within the resorption lacunae by the V-ATPase proton pump [103, 104].

To demonstrate the acidification by bone-resorbing osteoclasts in vivo, we have recently developed a pH-sensing fluorescent chemical probe [105]. By using this probe, we have succeeded in the visualization of bone resorption by osteoclasts, and we have suggested that the pH value in the resorption pit created by osteoclasts should be within the range of 4–6.

Matrix-degrading enzymes

In addition to the low pH of resorption lacunae, efficient degradation of bone is dependent on the production and activation of various matrix-degrading enzymes including cathepsin K [106], several MMPs [107, 108] and TRAP [109]. Cathepsin K is an osteoclast-specific enzyme that degrades type I collagen under acidic conditions. MMP-9 exerts little effect on the bony matrix, rather it initiates bone degradation by removing bone-lining collagen, allowing cathepsin K to begin digestion [110].

Cathepsin K has been studied as a potential target for the treatment of RA. The cathepsin K inhibitor odanacatib has demonstrated a clear and potent specificity for cathepsin K [111]. Administration of odanacatib is associated with a considerable reduction in levels of bone resorption markers and increased bone density at the lumbar spine and hip of post-menopausal women with low BMD [112]. Inhibition of cathepsin K by NC-2300 suppresses not only osteoclastic bone resorption, but also autoimmune joint inflammation in a rat model [113]. Furthermore, cathepsin K regulates Th17 cell differentiation by mediating the activation of dendritic cells by Toll-like receptor 9 (TLR9) and the production of cytokines, such as IL-6 and IL-23 [113]. However, the possibility that NC2300 exerted anti-arthritis effects through other cells cannot be excluded. Cathepsin K is an interesting molecule that was originally found in bone and subsequently shown to regulate the immune system.

Conclusions and future perspectives

There is an urgent need for more effective therapeutics for preventing RA-associated bone destruction. As osteoclasts are key players in joint and bone destruction, it is of critical importance to understand their modes of migration, differentiation and function in vivo. Recent studies have demonstrated that osteoclast-targeted therapies, such as FTY720, denosumab, and cathepsin K inhibitor, could be quite beneficial for the treatment of both RA-associated bone destruction and post-menopausal osteoporosis. Although these novel drugs are in need of further study, many additional advances in our knowledge could bring about drug-free remission or cure in RA patients in the near future.

**Rheumatology key messages**

- Osteoclast is the characteristic cell type destroying bone tissues in arthritic joints.
- Therapeutics targeting migration, differentiation and function of osteoclasts are critically important for treating RA.

**Funding:** This work was supported by Grants-in-Aid for Encouragement of Young Scientists (A) (22689030), for Scientific Research on Innovative Areas (22113007) and by a Funding Program for World-Leading Innovative R&D on Science and Technology (FIRST Program) from the Ministry of Education, Science, Sports and Culture of Japan, by a Grant-in-Aid for Research on Allergic Disease and Immunology (H21-010) from the Ministry of Health, Labor and Welfare of Japan, a Grant from the International Human Frontier Science Program (RGY0077/2011), and by Grants from Takeda Science Foundation, from Mochida Memorial Foundation for Medical and Pharmaceutical Research, from Astellas Foundation for Research on Metabolic Disorders, from Kanai Foundation for the Promotion of Medical Science and from Pfizer Health Research Foundation.

**Disclosure statement:** The authors have declared no conflicts of interest.

**References**

44 Nakagawa N, Kinosaki M, Yamaguchi K et al. RANK is the essential signaling receptor for osteoclast differentiation factor in osteoclastogenesis. Biochem Biophys Res Commun 1998;253:395–400.
Osteoclasts as a target for RA treatment


73 Yasui T, Kadono Y, Nakamura M et al. Regulation of RANKL-induced osteoclastogenesis by TGF-beta through molecular interaction between Smad3 and Traf6. J Bone Miner Res 2011;26:1447-56.


Bone marrow oedema of the radius presenting as tennis elbow

A 39-year-old, right-handed woman was referred to our rheumatology clinic after having 7 years of pain in her right elbow. Her general practitioner had prescribed hydrocortisone injections and an epicondylitis clasp for tennis elbow; however, her pain progressed and affected her activities of daily living. On physical examination there was severe tenderness over the right lateral epicondyle, exacerbated by right arm extension and resisted supination. US examination did not reveal any significant changes of the extensor tendon. An MRI of the elbow was requested to rule out osseous causes for her pain. The MRI showed a small joint effusion and marked increased medullary signal in the proximal radius, extending distally to at least mid-radius. There was no evidence of an enthesopathy or tendinopathy. The conclusion was bone marrow oedema within the radius, of which the cause was uncertain on this scan (Fig. 1).

The treatment of bone marrow oedema consists of treating the underlying condition; however, we were unable to find the underlying cause in our patient. She was treated with i.v. pamidronate 60 mg repeated a week later, with dramatic improvement within a day of the second infusion.

Disclosure statement: The authors have declared no conflicts of interest.

Rebecca Lee¹, Nasra K. Al-Adhoubi¹ and Ali S. M. Jawad¹

¹Department of Rheumatology, Royal London Hospital, London, UK.