JRD
Journal of Rheumatic Diseases Vol. 23, No. 3, June, 2016
http://dx.doi.org/10.4078/jrd.2016.23.3.141

Review Article

Osteoclasts: Crucial in Rheumatoid Arthritis

Won-Ju Jeong[1], Ha-Jeong Kim[2]
Departments of [1]Orthopedic Surgery and [2]Physiology, Kyungpook National University School of Medicine, Daegu, Korea

Osteoclasts are a major component of bone metabolism in physiologic condition and in rheumatoid arthritis (RA). RA is a chronic, autoimmune, inflammatory disease primarily affecting the joints. Joint inflammation leads to cartilage and bone destruction by osteoclast activation. This osteoclast activation leads to typical RA symptoms and is the therapeutic target. Several kinds of drugs are used for preventing bone loss by osteoclasts in RA patients. However, the bone destructive action of osteoclasts is not the only mechanism in RA pathogenesis. Recent research suggests that the osteoclasts regulate hematopoietic stem cell niches and invoke immune responses in bone. Osteoclasts are derived from bone marrow hematopoietic stem cells, and maintain the hematopoietic stem cell niches contract with osteoblasts. Osteoclasts secret several cytokines to regulate inflammation and T cell differentiation, and present antigen to T cells via major histocompatibility complex class I and class II molecules. Osteoclast concepts in both origins and functions are under major reconsideration and research. In this review, we will discuss these new insights. **(J Rheum Dis 2016;23:141-147)**

Key Words. Osteoclasts, Osteoclastogenesis, Rheumatoid arthritis, RANK ligand, Immunity

INTRODUCTION

Bone is dynamic organ, and it is continuously broken down (resorption) and built-up (synthesis) in life. Osteoblasts and osteoclasts are key players of this bone remodeling and delicate balance between them is important in physiologic condition. A loss balance between osteoblasts and osteoclasts cause most adult bone diseases. For examples, the excessive activity of osteoclasts leads osteoporosis and rheumatoid arthritis (RA), the other hands osteoclasts dysfunction leads osteopetrosis [1].

RA is a chronic inflammatory disease in joint synovium. Even though the exact etiology is not clear, it is widely accepted that RA is caused by breakdown of immune tolerance. The excessive autoreactive $CD4^+$ T cells and impaired regulatory T cells and other inflammatory cells including macrophages, plasma cells, and B cells infiltrate in synovium of RA patients. Infiltrated T cells and other immune cells in synovial tissues secret pro-inflammatory cytokines and induce osteoclastogenesis [2,3]. Increased osteoclasts in inflamed synovium are more activated by several immune regulators such as cytokines, and induce bone loss. It causes joint swelling, stiffness, pain and impairment, thereby resulting disability and deformity [1]. Traditionally it is believed that osteoclasts have a role in bone erosion in RA synovium. Despite a lot of anti-resorptive agent development, RA symptom is not completely improved with them [4].

Osteoclasts are highly specialized cells, and have the unique function for bone resorption. For decades, a lot of research about the cellular and molecular mechanisms for osteoclasts differentiation and bone resolving mechanisms has established [1]. It is also reported that osteoclasts involve in hematopoietic niche maintenance [5-7]. These bone turnover and hematopoietic niche maintenance by osteoclasts occur via continuous crosstalk with osteoblasts and osteocytes. The role of osteoclasts is not only in bone resorption but also in inflammation. Considering osteoclasts are tissue specific macrophages

Received : May 9, 2016, **Revised :** June 14, 2016, **Accepted :** June 14, 2016

Corresponding to : Ha-Jeong Kim, Department of Physiology, Kyungpook National University School of Medicine, 680 Gukchaebosang-ro, Jung-gu, Daegu 41944, Korea. E-mail : kimhajeong@knu.ac.kr

pISSN: 2093-940X, eISSN: 2233-4718

and their origin is monocyte precursors, their inflammatory role is not surprising. Osteoclasts secrete several cytokines and present antigen to T cell [8,9]. This review outlines about osteoclast biology in RA. Recent studies about osteoclasts differentiation, activation and inflammatory role will be highlighted.

MAIN SUBJECTS

Osteoclasts differentiation and activation in rheumatoid arthritis

The osteoclast is a multinucleated cell near the bone surface. Peripheral blood monocyte precursor cells differentiate and fuse to become osteoclasts in bone tissue. Tartrate-resistant acid phosphatase (TRAP) and calcitonin receptor are used as osteoclast markers [3]. The crosstalk between the osteoclasts and neighboring cells (osteoblasts, osteocytes, and hematopoietic cells) is also essential for osteoclastogenesis. In normal bone tissue, various factors regulate osteoclast differentiation and activity [10]. Macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor-kappa B ligand (RANKL), those are mainly secreted from osteoblasts, are essential factor for osteoclast differentiation.

RANKL binds to the RANK receptor on osteoclast precursor cells. RANKL deficient mice lack mature osteoclasts and are protected from bone erosion in the K/BxN serum transfer arthritis model [11]. In RA, RANKL is expressed from osteoblasts and synovial fibroblasts, and its expression is upregulated by pro-inflammatory cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6 and IL-17 [12]. RANKL is also produced certain activated B and T cells [13-17]. This increased RANKL in inflamed synovium leads to osteoclast overfunction, and bone destruction in RA [9].

TNF-α, IL-6, and IL-1 are key pro inflammatory cytokines in RA synovium and induce the expression of RANKL and other pro inflammatory cytokines. Increased TNF-α in RA synovium is major factor in osteoclast over function. TNF-α stimulates the migration of osteoclast precursor cells and the expression of osteoclast-associated immunoglobulin-like receptor on osteoclast precursor cells. Increased osteoclast-associated immunoglobulin-like receptor facilitates osteoclast differentiation [18,19]. IL-6 is produced by activated macrophages and fibroblast-like synoviocytes in synovium [20]. IL-1 is produced by immune cells like monocytes and macrophages [21]. The role of TNF-α, IL-6, and IL-1 in RA pathogenesis has been investigated intensively and a lot of agent is developed based on them. Anti-inflammatory agent used in RA will be mentioned in the last part of this review.

The regulation of osteoclast formation and activity by the immune system is well defined. In RA inflamed synovium, activated T cells, B cells, plasma cells, macrophages and mast cells are infiltrated. The balance of T helper cells type (Th)1/Th2/Th17/regulatory T cell (Treg) lymphocyte subsets is important in immune process. Th1 cells are characterized by secretion of interferon (IFN)-γ and TNF-α, and the other hand Th2 cells produce IL-4, IL-10 and IL-13 [12]. Some of them, especially IFN-γ, IL-4, IL-10 and IL-2, inhibit osteoclastogenesis by suppressing RANKL signaling [10,22]. IL-2 is major factor for T cell proliferation and T cell produces RANKL [13]. It is reported that IL-2 stimulates osteoclastic activity [23]. However, in IL-2 lacking mice develop pronounced osteopenia [24]. Taken these reports, overall effect of IL-2 on osteoclastogenesis is suppressive. RA is caused by Th1 shift, however still Th1 and Th2 cells cannot explain the molecular mechanism of bone damage in RA [25]. IL-17, is secreted from Th17, is increased in RA joints [26]. IL-17 induces RANKL expression and promotes osteoclastogenesis (Figure 1).

The other component of the adaptive immune system, B cell also secrets RANKL and stimulate osteoclastogenesis [27]. However, it is also reported that mice lacking B cells have osteoporosis [28]. B cells suppress osteoclastogenesis, because B cells produce osteoprotegrin, which inhibits RANKL [29]. It is not clear what microenvironment makes this difference.

Osteoclasts are known as tissue-specific macrophages. Like macrophage heterogeneity in inflammatory site, osteoclasts population also has heterogeneity in their origin and phenotype. Osteoclasts are derived from peripheral blood monocyte progenitors in physiologic condition. It is reported that the number of $CD14^+$ monocyte progenitors is increased in peripheral blood in RA patients than healthy controls. Osteoclast differentiation and resorption activity is increased following the increase of monocyte progenitors in RA patients. Osteoclast apoptosis in vitro is also decreased [30,31]. It is also reported that synovial membrane infiltrated macrophages express TRAP and calcitonin receptor. It means macrophages from RA synovium differentiate to osteoclasts [32,33]. Dendritic cells are also transdifferentiated to osteoclasts [34]. *oc/oc* mice, which have a homozygous *Tcirg*1 loss of function mutation, have impaired osteoclasts number

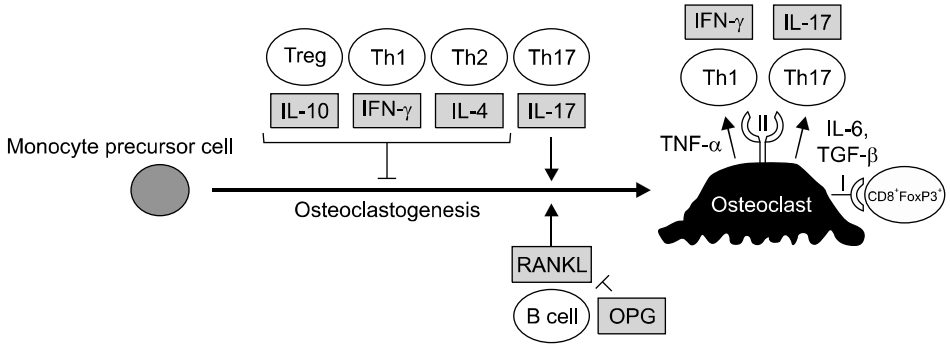

Figure 1. Cross talk between the immune system and osteoclasts in osteoclastogenesis. Osteoclasts are derived from monocyte precursor cells. RANKL from B cell and IL-17 from Th17 cell induces osteoclastogenesis. IL-10 from Treg, IFN-γ from Th1, and IL-4 from Th2 inhibits osteoclastogenesis. I: major histocompatibility complex (MHC) class I, II: MHC class II, IFN: interferon, IL: interleukin, OPG: osteoprotegerin, RANKL: receptor activator of nuclear factor-kappa B ligand, Th1/2/17: T helper cells type 1/2/17, TGF: transforming growth factor, TNF: tumor necrosis factor, Treg: regulatory T cell.

and have osteopeterosis [6,35]. Bone abnormality in *oc/oc* mice is partially corrected by dendritic cell injection [36]. These data suggest osteoclasts are derived from divergent population. Other origin of osteoclasts are synovial mononuclear cells, and subchondral bone cells [37]. Phenotypic differences have been reported between osteoclasts differentiated from dendritic cells or mononuclear cells. The osteoclasts from dendritic cells produce a predominance of pro-inflammatory cytokines, and the osteoclasts from mononuclear cells produce a predominance of anti-inflammatory cytokines [38]. The heterogeneity of the osteoclast population is not solely related with cell origins. Sometimes, osteoclast phenotype differences are dependent on its location in the skeleton or on the type of the local ossification process they participate in [37,39]. Osteoclasts secret the metalloproteinase for intramembranous ossification to break down extracellular matrix, whereas osteoclasts secret cathepsin K for endochondral ossification [40]. It is also reported that the responsiveness to bisphosphonate is different between osteoclasts on cortical cone and them on cancellous bone [41]. Some osteoclasts, found in subchondral bone of RA patients, have similar phenotype with follicular dendritic cells [42]. These data suggest that there are origin and phenotype diversity of osteoclasts. The relation between origin and phenotype is not fully understood, further investigation is needed.

Osteoclasts as an immune cell

It is reported that several skeletal phenotypes are reported in immune-deficient mice, and chronic inflammation itself is independent risk factor of bone loss [43]. Even though number is few, immune deficient phenotype is also established in osteo-compromised gene deficient mice [13-17]. It suggests the bone cells themselves directly regulate the immune cell development.

Osteoclasts are innate immune cells in bone. Osteoclasts directly regulate the immune response via the release of cytokines and perhaps also via antigen presentation to lymphocyte [8]. They express innate immune receptors like macrophages and dendritic cells, which derive from the same lineage as osteoclasts. Toll-like receptors (TLRs) recognize the conserved pathogen-associated molecular patterns (PAMPs) on several pathogens. Murine osteoclast progenitors express TLR 1 through 9, and its expression level is decreased during osteoclasts differentiation. On osteoclast, only TLR2 and TLR4 are expressed. Lipopolysaccharides and peptidoglycans, the ligands of TLR 4 and TLR 2, increase osteoclasts survival [44]. However, it is also reported that the ligand binding against TLR 2, 3, 4, and 9 inhibits osteoclastogenesis [45]. These evidences suggest TLR has biphasic effect. To understand its role in RA, more studies are needed.

Osteoclasts express various Fc gamma receptors (FcγR) which recognize immune complex. The expression level of immune activating receptors (FcγRI, FcγRIII, and FcγRIV) is lower on osteoclasts than other innate immune cells, however the expression lever of immune inhibitory receptors (FcγRII) is similar with other innate immune cells [46,47]. Most immune complex, except anti-citrullinated peptide antibodies (ACPA), inhibits osteoclast differentiation [48]. ACPA rich immune complexes induce TNF-α secretion from macrophages in RA

patient, and it stimulates osteoclastogenesis directly and indirectly [49]. It is reported that ACPA directly enhance osteoclast differentiation through IL-8 autocrine secretion [50,51]. These data suggest the final effect in RA may be dependent on the balance between them. Taken those reports, osteoclasts may play a role as inflammatory regulators like other innate immune cells. However the direct evidence is not, more studies are needed to clarify.

Human osteoclasts express major histocompatibility complex (MHC) class I and class II molecules, and also express co-stimulatory molecules to induce both $CD4^+$ and $CD8^+$ T cell responses [52,53]. However, murine osteoclasts express only MHC class I molecules. MHC class I on osteoclast induces $CD8^+FoxP3^+$ T cell differentiation, even though there is co-stimulatory receptor [54]. RANKL stimulate MHC class I expression during osteoclastogenesis [54,55]. Like macrophages and dendritic cells, MHC class II molecules on osteoclasts are increased by IFN-γ and LPS, but not TNF-α or IL-1. A recent study suggests that antigen presentation by osteoclasts derived by dendritic cell transdifferentiation may promote the activation of naïve $CD4^+$ T cells toward the Th1 phenotype [38]. It is also reported that osteoclast can do phagocytosis and apoptotic cell clearance [56]. It is not reported that osteoclasts induce Th17 differentiation. Considering that osteoclasts secrete TGF-β, IL-6 and IL-23, which are Th17 differentiated factors [52,57], osteoclasts may have ability to induce Th17 in some condition. Some cytokines and growth factors released from osteoclasts promote B cell maturation (Figure 1) [27].

Osteoclast precursor cells are identified in murine as $CD11b^{low/-}CD115^+$ by Jacquin et al. [58]. Recently, an osteoclast precursor cells are characterized by high expression of Ly6C, it is similar phenotype of myeloid derived suppressor cells (MDSCs). In RA mice model, the adaptive transfer of $CD11b^{low/-}Ly6C^+$ cells attenuates joint inflammation [59]. MDSCs is induced certain microenvironment, however which remains to be identified. These finding may give us new therapeutic implications.

Bone marrow (BM) functions as primary and secondary lymphoid organ, and regulate systemic immune responses [60]. It is well known that osteoclasts and osteoblasts play a role in forming the BM cavity. Thus they may regulate the hematopoietic stem cell niche and immune responses. Even though the function of osteoblasts in hematopoietic stem cell (HSC) development and maintenance is more studied [61], the function of osteoclasts in that is also reported recently [6,7]. $Lin^-Sca1^+cKit^+$ HSCs number is reduced in *oc/oc* mice, and in *Ctsk*$^{-/-}$ mice also have impaired osteoclasts number [6,35]. Those studies addressed that the role of osteoclasts in HSC niche formation, the precise mechanism is not clear. The authors concluded that osteoclasts maintain HSC niche by regulating osteoblasts. To resolve these mechanisms, more studies are needed.

Targeting of cytokines as potential rheumatoid arthritis therapy

Osteoclast differentiation and activation is induced in chronic inflammatory condition. Because the etiology of RA is not clear, the purpose of RA treatment is minimized bone loss by inhibiting osteoclast activity. Currently available anti-cytokine drugs are listed.

1) Anti-TNF-α agent; Adalimumab, Certolizumab pegol, Etanercept, Golimumab, Infliximab

In RA patient, bone mineral density is inversely correlated with TNF-α serum level. TNF-α blockade inhibits activation and cytokine production of osteoclasts, chondrocytes, and endothelial cells. Neutralization of TNF-α decreases proinflammatory cytokine production and induces Treg function. It also improves joint pain and fever by PGE2 synthesis [4]. In osteoclastogenesis, TNF the expression of RANKL and M-CSF in stromal cells and also directly induces osteoclast differentiation. TRAP positive cell number is decreased in TNFR1- or TNFR2-deficient mice, which are TNF-α receptors [62,63]. These evidences suggest that TNF-α blockade may be good therapeutic target against osteoclastogenesis. Currently, five different agents are used in RA therapy. Adalimumab, Infliximab, and Golimumab is anti-TNF-α monoclonal antibody to neutralizing TNF-α. Certolizumab pegol, and Etanercept is modified anti-TNF-α neutralizing antibody [64].

2) Anti-IL-6 agent; Tocilizumab

In vitro, IL-6 blockade reduces osteoclastic differentiation and bone resorption in monocytes cultures stimulated by RANKL or RANKL plus TNF-α. In transgenic mice, formation of osteoclasts is also strongly inhibited by the anti-inflammatory effects of IL-6 blockade [65]. IL-6 deficient mice are resistant to antigen-induced experimental arthritis [66]. Tocilizumab is a monoclonal antibody against IL-6 receptor [67]. The other anti-IL-6 receptor monoclonal antibody is developed and currently clinically tested [4].

3) Anti-IL-1 agent; Anakinra, Canakinumab

IL-1 is also proinflammatory cytokine like TNF-α, and induces several proinflammatory cytokines, except TNF-α. IL-1 directly induces osteoclast differentiation [68] and cytokine secretion for differentiation, multinucleation, and survival of osteoclasts [69,70]. IL-1α-deficient, IL-1β-deficienty, and IL-1α/β double deficient mice have increased cortical thickness and decreased osteoclast number [71]. IL-1 receptor antagonist-deficient mice spontaneously develop autoimmune arthritis {Horai, 2004 #133}. These evidences suggest IL-1 may also be a therapeutic target of RA. Anakinra is the IL-1 type-I receptor antagonist, and is approved, however its effect is less than anti-TNF-α drugs [72]. Canakinumab is an anti-IL-1β monoclonal antibody [73].

4) Anti-RNAKL agent; Denosumab

Denosumab is an anti-RANKL monoclonal antibody for neutralizing RANKL. It is expected that it successfully inhibits osteoclasts activation and protects joint destruction, even though basal pathology is whatever. It is already used in the treatment of osteoporosis and RA. However, Current clinical trials are not fully satisfactory [4]. Denosumab retards bone erosion but not inflammation [74].

CONCLUSION

In this review, the factors, induce osteoclasts differentiation and activation, are listed and the function of osteoclasts as immune regulators is also reported. Considering the origin of osteoclasts is same with immune cells, immune regulatory function of osteoclasts is easily expected. Osteoclasts secrete cytokines and present antigen to induce T and B cell differentiation and activation, however more investigations are needed. An understanding of the role of osteoclast in RA pathogenesis will help to develop novel therapeutic agents.

To improve RA symptom, the therapeutic target of most developed drugs is the osteoclast. Because over function of osteoclasts results in bone destruction in RA. Currently available anti-cytokine drugs are described here. Major concern is they do not cure RA, and their long term effects remain unknown. Therefore, the developing new therapeutic target is needed.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

REFERENCES

1. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. Nature 2003;423:337-42.
2. Firestein GS. Evolving concepts of rheumatoid arthritis. Nature 2003;423:356-61.
3. Shaw AT, Gravallese EM. Mediators of inflammation and bone remodeling in rheumatic disease. Semin Cell Dev Biol 2016;49:2-10.
4. Brzustewicz E, Bryl E. The role of cytokines in the pathogenesis of rheumatoid arthritis—Practical and potential application of cytokines as biomarkers and targets of personalized therapy. Cytokine 2015;76:527-36.
5. Karsdal MA, Martin TJ, Bollerslev J, Christiansen C, Henriksen K. Are nonresorbing osteoclasts sources of bone anabolic activity? J Bone Miner Res 2007;22:487-94.
6. Mansour A, Abou-Ezzi G, Sitnicka E, Jacobsen SE, Wakkach A, Blin-Wakkach C. Osteoclasts promote the formation of hematopoietic stem cell niches in the bone marrow. J Exp Med 2012;209:537-49.
7. Mansour A, Wakkach A, Blin-Wakkach C. Role of osteoclasts in the hematopoietic stem cell niche formation. Cell Cycle 2012;11:2045-6.
8. Yao Z, Xing L, Qin C, Schwarz EM, Boyce BF. Osteoclast precursor interaction with bone matrix induces osteoclast formation directly by an interleukin-1-mediated autocrine mechanism. J Biol Chem 2008;283:9917-24.
9. Charles JF, Aliprantis AO. Osteoclasts: more than 'bone eaters'. Trends Mol Med 2014;20:449-59.
10. Takahashi N, Yamana H, Yoshiki S, Roodman GD, Mundy GR, Jones SJ, et al. Osteoclast-like cell formation and its regulation by osteotropic hormones in mouse bone marrow cultures. Endocrinology 1988;122:1373-82.
11. Pettit AR, Ji H, von Stechow D, Müller R, Goldring SR, Choi Y, et al. TRANCE/RANKL knockout mice are protected from bone erosion in a serum transfer model of arthritis. Am J Pathol 2001;159:1689-99.
12. Takayanagi H. Osteoimmunology and the effects of the immune system on bone. Nat Rev Rheumatol 2009;5:667-76.
13. Kong YY, Feige U, Sarosi I, Bolon B, Tafuri A, Morony S, et al. Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. Nature 1999;402:304-9.
14. Horwood NJ, Kartsogiannis V, Quinn JM, Romas E, Martin TJ, Gillespie MT. Activated T lymphocytes support osteoclast formation in vitro. Biochem Biophys Res Commun 1999;265:144-50.
15. Kotake S, Udagawa N, Hakoda M, Mogi M, Yano K, Tsuda E, et al. Activated human T cells directly induce osteoclastogenesis from human monocytes: possible role of T cells in bone destruction in rheumatoid arthritis patients. Arthritis Rheum 2001;44:1003-12.
16. Weitzmann MN, Cenci S, Rifas L, Haug J, Dipersio J, Pacifici R. T cell activation induces human osteoclast formation via

receptor activator of nuclear factor kappaB ligand-dependent and -independent mechanisms. J Bone Miner Res 2001;16:328-37.
17. Wang R, Zhang L, Zhang X, Moreno J, Celluzzi C, Tondravi M, et al. Regulation of activation-induced receptor activator of NF-kappaB ligand (RANKL) expression in T cells. Eur J Immunol 2002;32:1090-8.
18. Li P, Schwarz EM, O'Keefe RJ, Ma L, Boyce BF, Xing L. RANK signaling is not required for TNFalpha-mediated increase in CD11(hi) osteoclast precursors but is essential for mature osteoclast formation in TNFalpha-mediated inflammatory arthritis. J Bone Miner Res 2004;19:207-13.
19. Herman S, Müller RB, Krönke G, Zwerina J, Redlich K, Hueber AJ, et al. Induction of osteoclast-associated receptor, a key osteoclast costimulation molecule, in rheumatoid arthritis. Arthritis Rheum 2008;58:3041-50.
20. Vervoordeldonk MJ, Tak PP. Cytokines in rheumatoid arthritis. Curr Rheumatol Rep 2002;4:208-17.
21. Chizzolini C, Dayer JM, Miossec P. Cytokines in chronic rheumatic diseases: is everything lack of homeostatic balance? Arthritis Res Ther 2009;11:246.
22. Takayanagi H, Ogasawara K, Hida S, Chiba T, Murata S, Sato K, et al. T-cell-mediated regulation of osteoclastogenesis by signalling cross-talk between RANKL and IFN-gamma. Nature 2000;408:600-5.
23. Ries WL, Seeds MC, Key LL. Interleukin-2 stimulates osteoclastic activity: increased acid production and radioactive calcium release. J Periodontal Res 1989;24:242-6.
24. Ashcroft AJ, Cruickshank SM, Croucher PI, Perry MJ, Rollinson S, Lippitt JM, et al. Colonic dendritic cells, intestinal inflammation, and T cell-mediated bone destruction are modulated by recombinant osteoprotegerin. Immunity 2003;19:849-61.
25. Sato K, Suematsu A, Okamoto K, Yamaguchi A, Morishita Y, Kadono Y, et al. Th17 functions as an osteoclastogenic helper T cell subset that links T cell activation and bone destruction. J Exp Med 2006;203:2673-82.
26. Kotake S, Udagawa N, Takahashi N, Matsuzaki K, Itoh K, Ishiyama S, et al. IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. J Clin Invest 1999;103:1345-52.
27. Sezer O. Myeloma bone disease. Hematology 2005;10 Suppl 1:19-24.
28. Raggatt LJ, Partridge NC. Cellular and molecular mechanisms of bone remodeling. J Biol Chem 2010;285:25103-8.
29. Li Y, Toraldo G, Li A, Yang X, Zhang H, Qian WP, et al. B cells and T cells are critical for the preservation of bone homeostasis and attainment of peak bone mass in vivo. Blood 2007;109:3839-48.
30. Durand M, Boire G, Komarova SV, Dixon SJ, Sims SM, Harrison RE, et al. The increased in vitro osteoclastogenesis in patients with rheumatoid arthritis is due to increased percentage of precursors and decreased apoptosis - the In Vitro Osteoclast Differentiation in Arthritis (IODA) study. Bone 2011;48:588-96.
31. Hirayama T, Danks L, Sabokbar A, Athanasou NA. Osteoclast formation and activity in the pathogenesis of osteoporosis in rheumatoid arthritis. Rheumatology (Oxford) 2002;41:1232-9.
32. Gravallese EM, Harada Y, Wang JT, Gorn AH, Thornhill TS, Goldring SR. Identification of cell types responsible for bone resorption in rheumatoid arthritis and juvenile rheumatoid arthritis. Am J Pathol 1998;152:943-51.
33. Fujikawa Y, Sabokbar A, Neale S, Athanasou NA. Human osteoclast formation and bone resorption by monocytes and synovial macrophages in rheumatoid arthritis. Ann Rheum Dis 1996;55:816-22.
34. Rivollier A, Mazzorana M, Tebib J, Piperno M, Aitsiselmi T, Rabourdin-Combe C, et al. Immature dendritic cell transdifferentiation into osteoclasts: a novel pathway sustained by the rheumatoid arthritis microenvironment. Blood 2004;104:4029-37.
35. Jacome-Galarza C, Soung do Y, Adapala NS, Pickarski M, Sanjay A, Duong LT, et al. Altered hematopoietic stem cell and osteoclast precursor frequency in cathepsin K null mice. J Cell Biochem 2014;115:1449-57.
36. Wakkach A, Mansour A, Dacquin R, Coste E, Jurdic P, Carle GF, et al. Bone marrow microenvironment controls the in vivo differentiation of murine dendritic cells into osteoclasts. Blood 2008;112:5074-83.
37. Le Goff B, Berthelot JM, Maugars Y, Heymann D. Osteoclasts in RA: diverse origins and functions. Joint Bone Spine 2013;80:586-91.
38. Abou-Ezzi G, Ciucci T, Amiot V, Wakkach A, Blin-Wakkach C. Osteoclasts induce a vicious circle between inflammation and bone destruction. Bone 2011;48(Suppl 2):S128.
39. Perez-Amodio S, Jansen DC, Schoenmaker T, Vogels IM, Reinheckel T, Hayman AR, et al. Calvarial osteoclasts express a higher level of tartrate-resistant acid phosphatase than long bone osteoclasts and activation does not depend on cathepsin K or L activity. Calcif Tissue Int 2006;79:245-54.
40. Shorey S, Heersche JN, Manolson MF. The relative contribution of cysteine proteinases and matrix metalloproteinases to the resorption process in osteoclasts derived from long bone and scapula. Bone 2004;35:909-17.
41. Chappard D, Petitjean M, Alexandre C, Vico L, Minaire P, Riffat G. Cortical osteoclasts are less sensitive to etidronate than trabecular osteoclasts. J Bone Miner Res 1991;6:673-80.
42. Bugatti S, Caporali R, Manzo A, Vitolo B, Pitzalis C, Montecucco C. Involvement of subchondral bone marrow in rheumatoid arthritis: lymphoid neogenesis and in situ relationship to subchondral bone marrow osteoclast recruitment. Arthritis Rheum 2005;52:3448-59.
43. Hardy R, Cooper MS. Bone loss in inflammatory disorders. J Endocrinol 2009;201:309-20.
44. Bar-Shavit Z. Taking a toll on the bones: regulation of bone metabolism by innate immune regulators. Autoimmunity 2008;41:195-203.
45. Ji JD, Park-Min KH, Shen Z, Fajardo RJ, Goldring SR, McHugh KP, et al. Inhibition of RANK expression and osteoclastogenesis by TLRs and IFN-gamma in human osteoclast precursors. J Immunol 2009;183:7223-33.
46. van Lent PL, Grevers L, Lubberts E, de Vries TJ, Nabbe KC, Verbeek S, et al. Fcgamma receptors directly mediate cartilage, but not bone, destruction in murine antigen-induced arthritis: uncoupling of cartilage damage from bone erosion and joint inflammation. Arthritis Rheum 2006;54:3868-77.
47. Grevers LC, de Vries TJ, Everts V, Verbeek JS, van den Berg WB, van Lent PL. Immune complex-induced inhibition of osteoclastogenesis is mediated via activating but not in-

hibitory Fcγ receptors on myeloid precursor cells. Ann Rheum Dis 2013;72:278-85.
48. MacLellan LM, Montgomery J, Sugiyama F, Kitson SM, Thümmler K, Silverman GJ, et al. Co-opting endogenous immunoglobulin for the regulation of inflammation and osteoclastogenesis in humans and mice. Arthritis Rheum 2011;63:3897-907.
49. Laurent L, Clavel C, Lemaire O, Anquetil F, Cornillet M, Zabraniecki L, et al. Fcγ receptor profile of monocytes and macrophages from rheumatoid arthritis patients and their response to immune complexes formed with autoantibodies to citrullinated proteins. Ann Rheum Dis 2011;70: 1052-9.
50. Krishnamurthy A, Joshua V, Haj Hensvold A, Jin T, Sun M, Vivar N, et al. Identification of a novel chemokine-dependent molecular mechanism underlying rheumatoid arthritis-associated autoantibody-mediated bone loss. Ann Rheum Dis 2016;75:721-9.
51. Wigerblad G, Bas DB, Fernades-Cerqueira C, Krishnamurthy A, Nandakumar KS, Rogoz K, et al. Autoantibodies to citrullinated proteins induce joint pain independent of inflammation via a chemokine-dependent mechanism. Ann Rheum Dis 2016;75:730-8.
52. Li H, Hong S, Qian J, Zheng Y, Yang J, Yi Q. Cross talk between the bone and immune systems: osteoclasts function as antigen-presenting cells and activate CD4+ and CD8+ T cells. Blood 2010;116:210-7.
53. Grassi F, Manferdini C, Cattini L, Piacentini A, Gabusi E, Facchini A, et al. T cell suppression by osteoclasts in vitro. J Cell Physiol 2011;226:982-90.
54. Kiesel JR, Buchwald ZS, Aurora R. Cross-presentation by osteoclasts induces FoxP3 in CD8+ T cells. J Immunol 2009;182:5477-87.
55. Kiesel J, Miller C, Abu-Amer Y, Aurora R. Systems level analysis of osteoclastogenesis reveals intrinsic and extrinsic regulatory interactions. Dev Dyn 2007;236:2181-97.
56. Harre U, Keppeler H, Ipseiz N, Derer A, Poller K, Aigner M, et al. Moonlighting osteoclasts as undertakers of apoptotic cells. Autoimmunity 2012;45:612-9.
57. Pöllinger B, Junt T, Metzler B, Walker UA, Tyndall A, Allard C, et al. Th17 cells, not IL-17+ $\gamma\ \delta$ T cells, drive arthritic bone destruction in mice and humans. J Immunol 2011;186:2602-12.
58. Jacquin C, Gran DE, Lee SK, Lorenzo JA, Aguila HL. Identification of multiple osteoclast precursor populations in murine bone marrow. J Bone Miner Res 2006;21:67-77.
59. Charles JF, Hsu LY, Niemi EC, Weiss A, Aliprantis AO, Nakamura MC. Inflammatory arthritis increases mouse osteoclast precursors with myeloid suppressor function. J Clin Invest 2012;122:4592-605.
60. Riether C, Schürch CM, Ochsenbein AF. Regulation of hematopoietic and leukemic stem cells by the immune system. Cell Death Differ 2015;22:187-98.
61. Morrison SJ, Scadden DT. The bone marrow niche for haematopoietic stem cells. Nature 2014;505:327-34.
62. Kobayashi K, Takahashi N, Jimi E, Udagawa N, Takami M, Kotake S, et al. Tumor necrosis factor alpha stimulates osteoclast differentiation by a mechanism independent of the ODF/RANKL-RANK interaction. J Exp Med 2000;191: 275-86.
63. Kitaura H, Kimura K, Ishida M, Kohara H, Yoshimatsu M, Takano-Yamamoto T. Immunological reaction in TNF-α-mediated osteoclast formation and bone resorption in vitro and in vivo. Clin Dev Immunol 2013;2013:181849.
64. Monaco C, Nanchahal J, Taylor P, Feldmann M. Anti-TNF therapy: past, present and future. Int Immunol 2015;27: 55-62.
65. Axmann R, Böhm C, Krönke G, Zwerina J, Smolen J, Schett G. Inhibition of interleukin-6 receptor directly blocks osteoclast formation in vitro and in vivo. Arthritis Rheum 2009;60:2747-56.
66. Boe A, Baiocchi M, Carbonatto M, Papoian R, Serlupi-Crescenzi O. Interleukin 6 knock-out mice are resistant to antigen-induced experimental arthritis. Cytokine 1999;11: 1057-64.
67. Vivar N, Van Vollenhoven RF. Advances in the treatment of rheumatoid arthritis. F1000Prime Rep 2014;6:31.
68. Kim JH, Jin HM, Kim K, Song I, Youn BU, Matsuo K, et al. The mechanism of osteoclast differentiation induced by IL-1. J Immunol 2009;183:1862-70.
69. Nakamura I, Jimi E. Regulation of osteoclast differentiation and function by interleukin-1. Vitam Horm 2006;74: 357-70.
70. Jimi E, Nakamura I, Duong LT, Ikebe T, Takahashi N, Rodan GA, et al. Interleukin 1 induces multinucleation and bone-resorbing activity of osteoclasts in the absence of osteoblasts/stromal cells. Exp Cell Res 1999;247:84-93.
71. Lee YM, Fujikado N, Manaka H, Yasuda H, Iwakura Y. IL-1 plays an important role in the bone metabolism under physiological conditions. Int Immunol 2010;22:805-16.
72. Singh JA, Christensen R, Wells GA, Suarez-Almazor ME, Buchbinder R, Lopez-Olivo MA, et al. A network meta-analysis of randomized controlled trials of biologics for rheumatoid arthritis: a cochrane overview. CMAJ 2009;181:787-96.
73. Venkatesha SH, Dudics S, Acharya B, Moudgil KD. Cytokine-modulating strategies and newer cytokine targets for arthritis therapy. Int J Mol Sci 2014;16:887-906.
74. Cohen SB, Dore RK, Lane NE, Ory PA, Peterfy CG, Sharp JT, et al. Denosumab treatment effects on structural damage, bone mineral density, and bone turnover in rheumatoid arthritis: a twelve-month, multicenter, randomized, double-blind, placebo-controlled, phase II clinical trial. Arthritis Rheum 2008;58:1299-309.