Osteoclasts: Crucial in Rheumatoid Arthritis

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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...
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 secretes RANKL and stimulate osteoclastogenesis [27]. However, it is also reported that mice lacking B cells have osteoporosis [28]. B cells suppressing osteoclastogenesis, because B cells produce osteoprotegerin, 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 Tcirg1 loss of function mutation, have impaired osteoclasts number.
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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 osteopetrosis [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, FcγRII, and FcγRIII) 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
I on osteoclast induces CD8 

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 from 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+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+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 α/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].
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CONFLICT OF INTEREST

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

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