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Review Article

The Role of Toll-Like Receptors in Osteoclastogenesis

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Bone homeostasis is maintained by a balance in the levels of osteoclast and osteoblast activity. Osteoclasts are bone-resorbing cells and have been shown to act as key players in various osteolytic diseases. Osteoclasts differentiate from monocyte/macrophage lineage cells in the presence of receptor activator of nuclear factor-κB ligand and macrophage colony-stimulating factor. Osteoblasts support osteoclastogenesis by producing several osteoclast differentiation factors. Toll-like receptors (TLRs) are members of the pattern recognition receptor family that are involved in recognizing pathogen-associated molecular patterns and damage-associated molecular patterns in response to pathogen infection. TLRs regulate osteoclastogenesis and bone resorption through either the myeloid differentiation primary response 88 or the Toll/interleukin-1 receptor domain-containing adapter-inducing interferon-β signaling pathways. Since osteoclasts play a central role in the progression of osteolytic diseases, extensive research focusing on TLR downstream signaling in these cells should be conducted to advance the development of effective TLR modulators. In this review, we summarize the currently available information on the role of TLRs in osteoclast differentiation and osteolytic diseases.

Key Words: Bone diseases · Osteoclasts · Osteolysis

INTRODUCTION

Bone is continuously remodeled throughout life—old bone is resorbed by osteoclasts, while new bone is formed by osteoblasts.[1,2] Osteoclasts are the key players involved in the maintenance of bone homeostasis; however, an excess of osteoclast activity leads to pathological bone loss as seen in diseases, such as osteoporosis, arthritis, periodontitis, and several metastatic cancers.[2] Therefore, understanding osteoclast biology is important from a clinical perspective.

Osteoclasts are derived from monocyte/macrophage lineage cells. Two indispensable factors are required for osteoclast differentiation: receptor activator of nuclear factor (NF)-κB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF).[3,4] M-CSF is essential for the proliferation and survival of osteoclast precursors and guides the early step of osteoclastogenesis.[5] On the other hand, RANKL directly differentiates osteoclast precursors into mature osteoclasts by binding to its receptor RANK.[6] RANKL/RANK binding activates the NF of activated T cells (NFATc1), the master transcription factor for osteoclast differentiation, which induces the expression of various osteoclast-specific genes, such as tartrate-resis-

tant acid phosphatase, cathepsin K, and dendritic cell-specific transmembrane protein.[7-10] Additionally, the synergistic activation of RANKL/RANK signaling by immunoreceptor tyrosine-based activation motif-containing proteins, DNAX-activating protein of 12 kDa, and Fcγ receptor has been reported.[11,12]

Although RANKL is the primary factor responsible for osteoclast differentiation, many studies have validated the involvement of other pathways in osteoclastogenesis. Among them, the presence of Toll-like receptors (TLRs) on osteoclasts and osteoclast precursors has attracted attention due to their regulatory roles in osteolytic diseases. In this review, we will briefly summarize the functions of TLRs, and their roles in osteoclast differentiation.

TLRS AND THEIR LIGANDS

TLRs are members of the pattern recognition receptor family. All TLRs have common structures consisting of an amino (N)-terminal extracellular domain with leucine-rich repeats, a single transmembrane spanning region, and a carboxyl (C)-terminal cytoplasmic Toll/interleukin (IL)-1 receptor (TIR) signaling domain.[13,14] To date, 10 human TLRs (TLR1-10) and 12 mouse TLRs (TLR1-9 and 11-13) have been identified.[15] TLRs are generally divided into 2 subgroups, depending on their cellular locations: TLR1, TLR2, TLR4, TLR5, and TLR6 are mainly present on the surface of plasma membranes, while TLR3, TLR7, TLR8, and TLR9 reside on the intracellular membranes of endosomes, lysosomes, and endolysosomes.[16]

The ligands recognized by TLRs are known as pathogen-associated molecular patterns, and include different types of molecules. For example, the ligands for TLR1, TLR2, TLR6, and TLR10 are bacterial lipoproteins and peptidoglycans. The ligands for TLR4 or TLR5 are bacterial lipopolysaccharides or flagellin. TLR3 is a sensor for viral double-stranded RNA , while TLR7 and TLR8 bind viral single-stranded RNA, TLR9 binds to CpG DNA, and TLR11 and TLR12 recognize profilin from *Toxoplasma gondii*.[17]

Danger-associated molecular patterns are endogenous nucleic acids and proteins released by stressed cells, and they can act as ligands for TLRs—TLR3 detects cellular RNA; TLR7, TLR8, and TLR9 recognize cellular RNA or DNA; while TLR4 senses secreted cellular proteins, such as high-mobility group box1, heat-shock proteins, and S100 family of proteins.[18-20]

TLR SIGNALING

The first step in TLR activation is the formation of hetero- or homo-dimers. TLR2 heterodimerizes with TLR1 and TLR6, [21] and possibly TLR10.[22] In contrast, TLR4 typically forms homodimers.[23,24] After these conformational changes, TLRs recruit cytoplasmic TIR-domain containing adaptor proteins, such as myeloid differentiation factor 88 (MyD88), TIR domain-containing adaptor protein (TIRAP or MyD88 like [Mal]), TIR-domain-containing adapter-inducing interferon (IFN)-β (TRIF), IFN-β-related adaptor molecule (TRAM), and sterile-α and armadillo motif-containing protein.[25] Among them, TLRs mainly use the MyD88 and TRIF pathways. All TLRs, except TLR3, use the MyD88-dependent pathway, whereas TLR3 and TLR4 use the TRIF-dependent pathway.[26] The recruitment of these adaptor proteins leads to the activation of downstream transcription factors via serial association with various ubiquitin ligases and kinases (Fig. 1).

1. The MyD88-dependent pathway

All TLRs, except TLR3, use the MyD88-dependent pathway to activate downstream signaling. TLR2 and TLR4 require an additional adaptor protein, TIRAP (or Mal) for this pathway.[27] MyD88 has 3 structural domains: a death domain, an intermediate domain, and a C-terminal TIR domain.[28] MyD88 associates with TLRs through its TIR domain.[29]

Upon association with TLRs, MyD88 recruits IL-1 receptor-associated kinase (IRAK) 4 and, subsequently, IRAK1 and IRAK2. Activated IRAK1 binds to tumor necrosis factor (TNF) receptor-associated factor (TRAF) 6, an E3 ubiquitin ligase. This IRAK1/TRAF6 complex recruits the protein kinase transforming growth factor-*β*-activated kinase 1 (TAK1), which phosphorylates IκB kinase β, which, in turn, phosphorylates IκBα. The phosphorylation of IκBα releases NF-κB (p65/p50), allowing its nuclear translocation, binding to response elements, and transcription of target genes.[30,31] On the other hand, TAK1 also activates the mitogen-activated protein kinase (MAPK) signaling pathway.[32] The MyD88-dependent activation of NF-κB and MAPKs induces the expression of various inflammatory cytokines such as IL-1β and TNF-α.[30-32]

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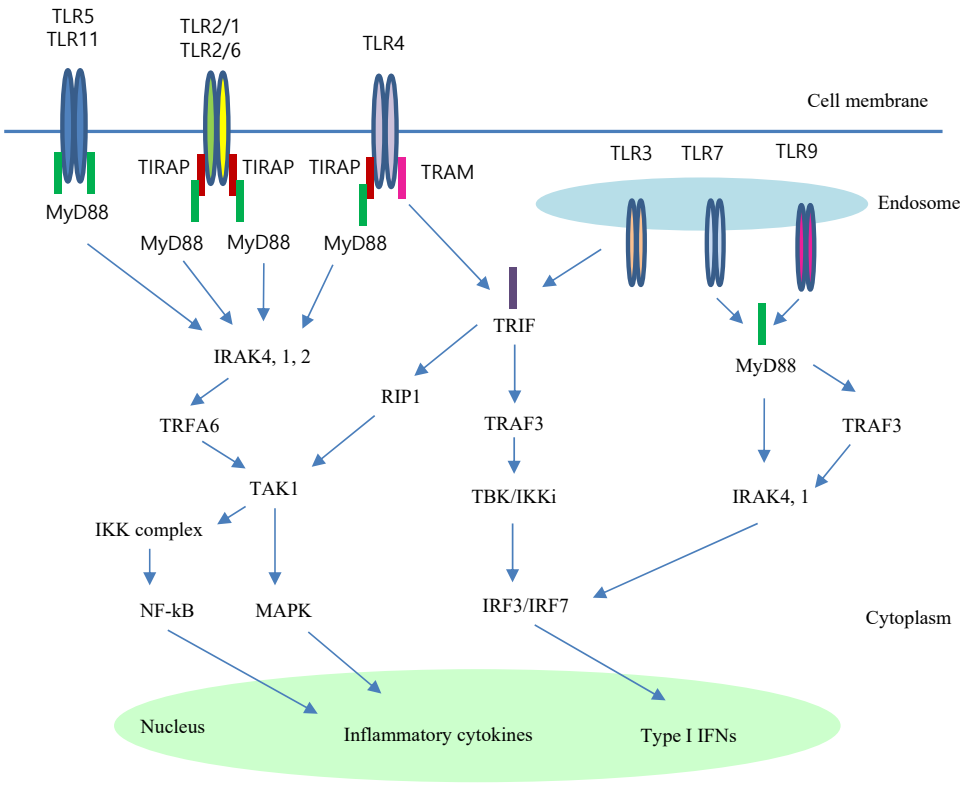

Fig. 1. Toll-like receptor (TLR) signaling pathways. TLRs are located on the surface of cell membranes or endosomal compartments. The activation of different TLRs recruit specific sets of adaptors. All TLRs, except TLR3, use the myeloid differentiation factor 88 (MyD88)-dependent pathway, whereas TLR3 and TLR4 use the TLR domain-containing adapter-inducing interferon-β (TRIF)-dependent pathway. Recruitment of the various signaling adaptors activate downstream signaling pathways to provoke the induction of pro-inflammatory cytokines or the production of type I interferons (IFNs). TIRAP, toll-like receptor domain-containing adaptor protein; IRAK, interleukin-1 receptor-associated kinase; TRF, T-cell replacing factor; TAK, transforming growth factor-β-activated kinase; IKK, IκB kinase; NF-κB, nuclear factor-κB; MAPK, mitogen-activated protein kinase; RIP, receptor-interacting protein; TRAF, tumor necrosis factor receptor-associated factor; IRF, interferon regulatory factor.

2. The TRIF-dependent pathway

The endosomal TLRs (TLR3 and TLR4) mediate the TRIF-dependent pathway to activate NF-κB (TLR4) or to produce IFN-β (TLR3 and TLR4).[33] TLR3 directly, and TLR4 indirectly, recruit TRIF via the association of a TRAM, an adaptor protein. TRIF, in turn, recruits receptor-interacting protein (RIP) 1, TRAF3, and TRAF6, and the polyubiquitination of these proteins activates TAK1.[34] TAK1 induces delayed activation of NF-κB in a manner similar to the MyD88-dependent pathway, leading to the activation of IFN regulatory factor 3 (IRF3).[25,35] Activated IRF3s dimerize, translocate to the nucleus, and bind to IFN-sensitive responsive element to induce transcription of the *IFN-β* gene and the IFN-stimulated genes.[36,37] In addition, TRIF appears to regulate TLR-induced cell death via RIP3 and caspase-8.[38] This mechanism explains how immune cells cause cell death in response to intracellular bacterial infection.[39,40]

THE ROLE OF TLRS IN OSTEOCLAST DIFFERENTIATION

1. Direct effect of TLRs on osteoclastogenesis

Osteoclasts express TLRs, and the expression pattern of TLRs varies depending on the stage of osteoclastogenesis. Osteoclast precursors express TLR1-TLR9; however, during their differentiation into mature osteoclasts, only TLR2 and TLR4 are predominantly expressed,[41] suggesting that these 2 TLRs play a major role during osteoclastogenesis.

The role of TLRs in osteoclast differentiation has been investigated using both *in vitro* and *in vivo* model systems.[42-47] Both positive and negative effects of TLRs on osteoclast differentiation have been reported *in vitro*. These

contradictory effects of TLRs appear to be dependent on the stage of cell differentiation

In mouse bone marrow-derived macrophage and human peripheral blood monocyte cultures, the simultaneous addition of TLR ligands with RANKL inhibits osteoclast formation.[41,48] Therefore, it is possible that TLR signaling sequesters the downstream molecules from the RANKL signaling pathway, resulting in inhibition of osteoclastogenesis.

Despite their negative effects during the early stages of osteoclastogenesis, TLRs play a positive role at the later stages of osteoclast differentiation, once osteoclast precursors are committed to the osteoclast lineage.[49,50] For example, if osteoclast precursors are pre-treated with RANKL for at least 24 hr, the activation of TLRs promoted osteoclastogenesis in the presence of RANKL. Furthermore, once osteoclast precursors are primed with RANKL, simulation of TLRs is sufficient to induce osteoclast formation even in the absence of RANKL. Since RANKL priming alone does not induce osteoclast formation, it is thought to permit differentiation toward osteoclasts, and this commitment in osteoclast lineage is required for the positive role of TLRs in osteoclastogenesis.

NFATc1 is a master transcription factor of osteoclastogenesis, and appears to serve as a gatekeeper for the dual role of TLRs.[9,10] The activation of TLRs in uncommitted osteoclast precursors abolished RANKL-induced NFATc1 expression; however, it enhanced NFATc1 expression in RANKL-primed cells.[41,50,51]

The inhibitory function of TLRs in uncommitted cells is partly mediated by IFN-β production.[52] TLR3 and TLR4 induce IFN-β through a TRIF-dependent signaling mechanism.[26,53] The TRIF-dependent signaling pathway promotes the formation of a homodimer of IRF3,[54] resulting in activation of the IFN-β promoter. Binding of IFN-β to its receptor (IFNAR) triggers the activation of Janus kinase and signal transducer and activator of transcription signaling, resulting in the inhibition of c-Fos expression, further abolishing NFATc1 activation.[55] The IFN-β/c-Fos axis is an important negative feedback mechanism in osteoclast differentiation. Moreover, the deletion of IFN-β or IFNAR increases the number of osteoclasts *in vivo*, further demonstrating the importance of this pathway.[55-57]

On the other hand, the activation of TLRs in committed cells triggers a number of downstream signaling molecules. [50,51,58,59] These pathways allow NF-κB to translocate into the nucleus, stimulate the transcription of NFATc1, and release several inflammatory cytokines, such as TNF-α, IL-1, and IL-6. This pro-osteoclastogenic effect of TLRs appears to be mediated via the MyD88-dependent pathway,[51] since the deletion of MyD88 reduced the number of osteoclasts *in vivo*. In addition, TLRs are believed to have pro-survival effects on mature osteoclasts via the Akt, NF-κB, and extracellular signal-regulated kinase pathways.[50]

Although TLRs have biphasic effects on osteoclast differentiation *in vitro*, they demonstrate a positive effect on osteoclastogenesis and bone destruction *in vivo*. Systemic injection of TLR4 ligand significantly increases the number of osteoclasts, resulting in femoral bone loss *in vivo*.[60,61] In periodontitis models, the injection of TLR2 or TLR4 ligand into the gingiva of the lower mandible also causes alveolar bone loss.[62,63] In rheumatoid arthritis (RA) preclinical models, the positive effects of TLR2, 4, 5, and 7 have been demonstrated. Intraarticular injection of TLR2 or TLR4 ligand induces joint inflammation and chronic destructive arthritis.[64,65] Local injection of TLR5 ligand potentiates joint inflammation and arthritic bone destruction in collagen-induced arthritis (CIA) models.[66] Furthermore, the deletion of TLR7 decreases joint inflammation and the arthritis-induced bone loss in CIA and K/BxN serum transfer arthritis models.[67,68]

In contrast, the functions of TLR3 and TLR9 vary *in vivo*, depending on the experimental conditions. It has been reported that TLR3 is involved in pristane-induced arthritis.[69] Furthermore, activation of TLR3 promotes joint inflammation, while deletion of TLR3 reduces K/BxN serum transfer arthritis.[68,70] However, systemic injection of TLR3 ligand attenuates collagen antibody-induced arthritis via anti-inflammatory type I IFNs.[71] The dual function of TLR9 has also been demonstrated *in vivo*. The activation of TLR9 induces arthritis via the upregulation of pro-inflammatory cytokines [72]; however, it reduces disease manifestation in K/BxN serum transfer arthritic models.[73]

2. Indirect effect of TLRs on osteoclastogenesis

Osteoblasts are derived from mesenchymal stem cells and can modulate osteoclast formation via direct contact with osteoclast precursors.[74,75] RANKL is essential for osteoclast differentiation, and its effect is abrogated by osteoprotegerin (OPG), a soluble decoy receptor for RANK.

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Among many factors expressed by osteoblasts, the ratio of RANKL to OPG is thought to be critical for the regulation of osteoclast formation.[75]

TLRs are expressed on the surface of osteoblasts and indirectly modulate osteoclast differentiation.[76] The activation of TLR2, 4, 5, and 9 in osteoblasts enhances osteoclast formation and bone loss by increasing the RANKL/OPG ratio.[77-81] Myd88-dependent activation of MAPK and NF-κB is generally considered to be responsible for the stimulatory effect of TLRs on RANKL expression.[78] TLRs in osteoblasts also stimulate the production of inflammatory cytokines IL-1β, TNF-α, and IL-6, as well as prostaglandins, which further contribute to osteoclast differentiation by osteoblasts.[49,82] Recently, it has been reported that bacterial infection induces periodontal bone loss via increased RANKL production by the osteocytes.[83] Therefore, the TLR regulation of osteoclastogenesis via osteocytes needs further investigation.

CONCLUDING REMARKS

Osteoclasts are key players in osteolytic diseases, such as osteoporosis, RA, and periodontitis. Upon infection, the activation of TLRs induces osteoclast differentiation, leading to bone and joint destruction. Many efforts have been made to understand TLR-mediated osteoclastogenesis using *in vitro* and *in vivo* osteolytic disease models. Various studies suggest that targeting the mechanism by which TLRs mediate osteoclast differentiation could be a promising therapeutic strategy for the development of novel anti-osteolytic drugs. Given that there are limited options for patients suffering from inflammatory osteolytic diseases, targeting TLR-mediated signaling pathways would provide additional opportunities for effective treatment of these diseases. Currently, several modulators of TLRs are under clinical evaluation for various diseases.[84] The elucidation of the regulatory mechanism of TLRs specific for osteoclast-mediated osteolysis would be helpful to develop new drugs for precision medicine.

DECLARATIONS

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Ethics approval and consent to participate

Not applicable.

Conflict of interest

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

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