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Role of Toll-like receptor 2 in osteoclastogenesis in chronic bone diseases

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Toll-like receptors essential for an innate immunity

Toll-like receptors (TLRs) are a family of mammalian proteins homologous to *Drosophila* Toll protein. Toll was originally identified as a molecule regulating dorsoventral pattern formation in early *Drosophila* development[1)]. It is established that Toll and its homologs play pivotal roles in host defense against pathogens[2)]. TLRs serve in the early stage of innate immunity, being capable of detecting a broad range of pathogens and endogenous harmful signals. There are at least 10 TLRs in humans, and they selectively recognize highly-conserved structural motifs of microbial pathogens by a process called pattern recognition[3)]. TLRs 1, 2, 4, 5, and 6 are located primarily in the plasma membrane. They bind to lipopolysaccharide (LPS), lipoteichoic acid, and lipoproteins from microbial cell walls, as well as to flagellin, a structural component of bacterial flagella[4)]. In contrast, TLRs 3, 7, 8, and 9 are situated in the membranes of endosomes and lysosomes. TLR3 is activated by double-stranded RNA, whereas TLR7 and 8 are activated by single-stranded RNA expressed by viruses and/or released from necrotic cells. TLR expression is particularly significant in different types of white blood cells : mast cells, monocyte/macrophages, and dendritic cells. These cells move out of the bloodstream to become tissue-resident cells throughout the body, where they can detect pathogens[5)].

Following ligands' binding to TLRs, the cytoplasmic domains of the TLRs recruit the signaling adaptors, MyD88 (myeloid differentiation primary response 88), TIRAP (toll-interleukin 1 receptor [TIR] domain-containing adaptor protein), TRAM (TRIF-related adaptor molecule), and/or TRIF (TIR domain-containing adaptor protein-inducing IFN-ß). Depending on the nature of the adaptor used, various kinases such as IRAK (interleukin-1 receptor-associated kinases), TBK1 (TRAF associated NF-κB activator [TANK]-binding kinase-1) and TRAF6 (TNF receptor associated factor) ubiquitin ligase are recruited and activated, culminating in the engagement of the NF-κB, p38 MAP kinase (MAPK), and JNK/MAPK pathways[6-10)]. Thus, the TLR family comprises an extremely important part of the immune system, whose function, conserved across evolution, is to detect microbial infection and to link innate to adaptive immunity.

Roles of TLR2 in osteoclast formation in chronic inflammatory diseases

Although TLR2 and TLR4 are implicated in the recognition of various bacterial cell wall components, they recognize different bacterial cell wall components *in vivo*, and only TLR2 has been believed to play a major role in Gram-positive bacterial recognition[11)]. Kawai et al have demonstrated that signaling via MyD88 is essential for LPS response, but the inability of MyD88 knockout mice to induce LPS-dependent gene expression cannot simply be attributed to the lack of the activation of MAP kinases and NF-κB[12)].

In chronic inflammatory bone diseases, including rheumatoid arthritis and periodontitis, receptor activator

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of NF-κB ligand (RANKL) and pro-inflammatory cytokines, such as tumor necrosis factor-α (TNFα), interleukin-1β (IL-1β), and interleukin-6 (IL-6) have been shown to be intrinsic for disease progression[13]. Periodontitis is a common bacteria-mediated inflammatory disease in humans, destroying the periodontal connective tissues and causing the alveolar bone loss. The TLR2 and MyD88 signaling pathway appear to be critical for *Porphyromonas gingivalis* (Gram-negative bacteria)-mediated osteoclast differentiation in periodontitis[14]. Kassem *et al* have demonstrated that LPS derived from *P. gingivalis* stimulated RANKL expression in murine parietal osteoblasts in the presence of TLR2, through a MyD88 and NF-κB-mediated mechanism[15]. Likely, the TLR2-agonists, such as Pam2, stimulated RANKL expression in osteoblasts and parietal bone resorption. Combined administration of LPS obtained from P. gingivalis and Pam2 robustly upregulated RANKL gene and the number of osteoclasts and immense bone loss in wild-type mice, but not in TLR2-deficient mice (Fig. 1).

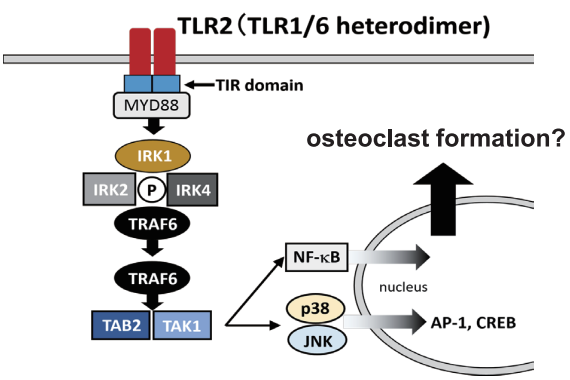

Fig. 1 Signal transduction linked to TLR2 (TLR1/6 heterodimer)

In contrast to TLR2-mediated RANKL signal, Matsumoto *et al* have suggested the prostaglandin E2, rather than RANKL, is more essential for osteoclastogenesis[16]. In their study, the TLR2/6 and TLR2/1 ligands induce the expression of COX-2, PGE synthase-1, and RANKL mRNAs and the production of PGE2 in mouse osteoblasts. The osteoclast formation induced by TLR2/6 and TLR2/1 ligands was completely suppressed by indomethacin. Therefore, they concluded that TLR2 heterodimer signaling may play a key role in PGE-mediated inflammatory bone loss in periodontal disease.

In contrast to the concept that TLR2 promotes osteoclastogenesis, Yang *et al* have reported that lipoteichoic acid derived from *Enterococcus faecalis* (EfLTA) inhibited the RANKL-mediated expression of NFATc1 and c-fos, consequently inhibiting osteoclastogenesis[17]. EfLTA inhibition of osteoclast differentiation from bone marrow-derived macrophages was not observed in TLR2-deficient macrophages. Therefore, it seems likely that EfLTA inhibits the differentiation of macrophages into osteoclasts through TLR2 signaling. Therefore, TLR2 appears to typically stimulate osteoclastogenesis, but sometimes suppress it under a certain circumstance.

The contribution of macrophage-expressed TLRs to osteoclastogenesis is still veiled. Among various TLRs, monocytes/macrophages express both TLR2 and TLR4[18] ; in particular, TLR2 is expressed constitutively in macrophages, dendritic cells, and B cells. Ukai *et al*. have reported a requirement for TLR2 in TNFα-induced osteoclastogenesis in response to exposure to *P. gingivalis*[19]. This activity was dependent on TNFα and occurred independent of RANKL, IL-1β, and IL-6. They concluded that macrophage-dependent TLR2 signaling appears to be crucial for TNFα-dependent/RANKL-independent osteoclastogenesis in response to *P. gingivalis* infection. Thus, many reports suggest that TLR2-mediated RANKL signal is essential for osteoclastogenesis in chronic inflammation; however, some reports insist that TLR-mediated osteoclastogenesis in inflammatory diseases is required for prostaglandin production and/or TNFα-dependent/RANKL-independent signaling pathway.

Considering the reported findings, the involvement of TLR2 in osteoclastogenesis and subsequent acceleration of bone resorption are still to be clearly defined. Further investigation is necessary to clarify the biological role of TLR2 in osteoclastogenesis.

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