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COMMENTARIES

NF-κB2 (p100) Emerges as a Negative Regulator of TNFα-Induced Osteoclastogenesis

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Commentary on: Yao Z, Xing L, Boyce BF. NF-kappaB p100 limits TNF-induced bone resorption in mice by a TRAF3-dependent mechanism. *J Clin Invest.* 2009 Oct;119(10):3024-34.

Osteoclasts are primary cells for physiologic and pathologic bone resorption, which is largely mediated by inflammatory cytokines such as receptor activator of NF-κB ligand (RANKL) and tumor necrosis factor (TNF)α. Yao *et al*. (1) now demonstrate that NF-κB2 (p100) induced by TNFα acts as a negative regulator of osteoclastogenesis. TNFα induced sustained accumulation of p100 in osteoclast precursors, and TNFα-induced osteoclast formation was markedly increased in *Nfkb2*(-/-) mice. Yao *et al*. (1) also found that TNF-receptor associated factor (TRAF)3 is involved in the post-translational regulation of p100 expression. These results suggest that targeting the processing of p100 is a novel strategy to treat TNFα-related bone diseases such as rheumatoid arthritis (RA).

TNFα is an inflammatory cytokine known to be implicated in the pathogenesis of bone loss and inflammation in a variety of bone and joint disorders. The remarkable clinical success of anti-TNFα therapies such as anti-TNFα antibody and soluble TNF receptor has established a critical role for TNFα in inflammatory diseases such as RA (2). Anti-TNFα strategies not only ameliorate inflammation but also markedly suppress bone erosion in RA, indicating an essential role for TNFα in pathologic bone resorption. Accumulating evidence has shown that osteoclasts are primarily responsible for both physiologic and pathologic bone resorption. Osteoclasts originate from hematopoietic stem cells, and the important cytokines regulating osteoclast differentiation are macrophage colony-stimulating factor (M-CSF) and RANKL (3). It appears surprising that the relationship between TNFα and RANKL signaling in osteoclast development is not necessarily clear. The osteoclastogenic effect of TNFα independent of RANKL has been particularly controversial. Although several studies have demonstrated that TNFα directly promotes osteoclast formation *in vitro* in the absence of RANKL (4-6), the ability of TNFα to induce osteoclast formation *in vitro* is limited, and the administration of TNFα does not induce osteoclast formation in *Rank*-deficient mice *in vivo* (7). This may be, at least in part, because RANK, but not TNF receptor 1 or TNF receptor 2, recruits an adaptor molecule, TRAF6, which is essential for osteoclast development. However, the possibility that molecules that are induced by TNFα negatively regulate osteoclast differentiation has not been excluded.

NF-κB is a collective term referring to dimeric transcription factors that belong to the Rel family. NF-κB is composed of five members including RelA (p65), RelB, c-Rel, NF-κB1 (p50 and its precursor p105), and NF-κB2 (p52 and its precursor p100). It is currently well-known that two major signaling pathways lead to NF-κB activation: the canonical or classical pathway and the noncanonical or alternative pathway (8). In the canonical pathway, the RelA/p50 complex is sequestrated with inhibitor of κBs (IκBs) in the cytoplasm. Inflammatory stimuli such as TNFα induce phosphorylation and

subsequent degradation of IκBs, and the released RelA/p50 complex translocates to the nucleus. On the other hand, the noncanonical pathway is induced by CD40 ligand, RANKL, and lymphotoxin (LT)β. p100 retains RelB in the cytoplasm as the RelB/p100 complex. Upon stimulation with these ligands, p100 is processed to p52, and then the RelB/p52 complex translocates to the nucleus. Notably, CD40 ligand, RANKL, and LTβ activate both the canonical and noncanonical pathways, whereas TNFα, IL-1, and LPS activate only the canonical pathway.

The essential role of NF-κB in osteoclast development has been clearly shown in knockout mouse experiments. Although targeted disruption of either *Nfkb1* or *Nfkb2* alone did not affect skeletal development, double knockout of these genes induced osteopetrosis in mice due to a defect in osteoclast differentiation (9;10). These results suggest that there are redundant roles for the canonical and noncanonical NF-κB pathways in osteoclast differentiation. NF-κB-inducing kinase (NIK) is involved in the phosphorylation and subsequent processing of p100 to p52, and Novack and co-workers have shown that deletion of the *Nik* gene resulted in the accumulation of p100 in osteoclast precursors, which caused impaired osteoclast differentiation *in vitro* by retaining the RelB/p100 complex in the cytoplasm (11). They also found that deletion of *Nfkb2* restored impaired osteoclastogenesis in *Nik*(-/-) precursors (12).

Yao *et al*. (1) now provide *in vitro* and *in vivo* evidence that p100 is induced by TNFα in osteoclast precursors and acts as a negative regulator of osteoclastogenesis. They found that TNFα induced sustained accumulation of p100 in osteoclast precursors, while RANKL more efficiently processed p100 to p52 through NIK activation. TNFα-induced osteoclast formation was increased in bone marrow cells from *Nfkb2*(-/-) mice to a level comparable to that induced by RANKL treatment, and bone resorption was significantly increased in *Nfkb2*(-/-) mice compared to WT mice when TNFα was injected over the calvaria. Interestingly, TNFα treatment also upregulated osteoclastogenesis in *Rank(-/-)Nfkb2(-/-)* and *Rankl(-/-)Nfkb2*(-/-) mice both *in vitro* and *in vivo*, confirming that the enhancement of TNFα-induced osteoclast formation in *Nfkb2*(-/-) mice does not depend on secondary production of RANKL by TNFα.

Yao *et al*. crossed *Tnfa*-transgenic mice (*Tnfa*-tg) with *Nfkb2(-/-)* mice to confirm a negative regulatory role of p100 in TNFα-induced osteoclast formation (1). *Tnfa*-tg/*Nfkb2*(-/-) mice spontaneously developed more severe inflammation and joint erosion, along with an increase in osteoclast number, than *Tnfa*-tg/*Nfkb2(+/-)* mice. Collectively, these studies conclude that p100 acts as a negative regulator of TNFα-induced osteoclast formation under pathologic conditions.

Yao *et al*. also present the interesting observation that TRAF3 is involved in the posttranslational regulation of p100 expression (1). They found that TNFα increased the protein level of TRAF3 by suppressing TRAF3 degradation, which was reversed by RANKL. Previous studies have shown that TRAF3 binds to and promotes degradation of NIK by forming a complex with TRAF2 and cellular inhibitor of apoptosis protein (c-IAP)1/2, and therefore, stabilization of TRAF3 by TNFα may decrease the level of NIK, resulting in the upregulation of p100 (13-15). Conversely, knockdown of *Traf3* using siRNA promoted TNFα-induced osteoclast formation through downregulation of p100 (13). Dejardin *et al*. (16) and Novack *et al.* (11) have reported that agonistic antibody against LT-β receptor or TNFα upregulated the expression of p100 in an NF-κB-dependent fashion. Taken together, these studies have shown that the expression level of p100 is regulated transcriptionally by NF-κB and/or posttranslationally by TRAF3, although the detailed molecular mechanisms are not fully elucidated.

In conclusion, Yao *et al*. have convincingly demonstrated that NF-κB2p100 plays a

negative role in suppressing TNFα-induced osteoclast formation under pathologic conditions using various animal models. Therefore, suppression of the processing of p100 might be a novel strategy to treat various bone diseases such as RA, in which TNFα-induced osteoclast formation plays a crucial role in the progression of disease.

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