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Commentary

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NF-κB2 (p100) limits TNF-α–induced osteoclastogenesis

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Bone undergoes a continuous cycle of renewal, and osteoclasts — the cells responsible for bone resorption — play a pivotal role in bone homeostasis. This resorption is largely mediated by inflammatory cytokines such as TNF-α. In this issue of the *JCI*, Yao et al. demonstrate that the NF-κB precursor protein NF-κB2 (p100) acts as a negative regulator of osteoclastogenesis (see the related article beginning on page 3024). TNF-α induced a sustained accumulation of p100 in osteoclast precursors, and TNF-α–induced osteoclast formation was markedly increased in *Nfkb2*^{−/−} mice. They also found that TNF receptor–associated factor 3 (TRAF3) is involved in the posttranslational regulation of p100 expression. These results suggest that blockade of the processing of p100 is a novel strategy to treat TNF-α–related bone diseases such as RA.

Osteoclasts are primarily implicated in physiologic and pathologic bone resorption. They are derived from hematopoietic stem cells, and their differentiation is critically regulated by supporting cells and cytokines. The important cytokines regulating osteoclast differentiation are M-CSF and RANKL (1). In particular, the central role of RANKL, which belongs to the TNF-α superfamily, has been demonstrated by the fact that the targeted disruption of *Rankl* or its receptor *Rank* induced severe osteopetrosis (increased bone density) in mice due to the complete lack of osteoclasts, while knockout of osteoprotegerin, a natural antagonist of RANKL, conversely caused marked bone loss (2).

The remarkable clinical success of anti–TNF-α therapies such as anti–TNF-α antibody and soluble TNF receptor has established a critical role of TNF-α in inflammatory diseases such as RA and Crohn disease (3). Anti–TNF-α strategies not only ameliorate the inflammatory conditions in these disorders but also suppress bone erosion in RA, indicating an essential role of TNF-α in the pathologic bone destruction. However, in spite of such strong clinical evidence, the relationship between TNF-α and RANKL signaling in osteoclast development is not necessarily clear. The osteoclastogenic effect of TNF-α independent of RANKL has been particularly controversial. Although several studies have demonstrated that TNF-α directly promotes osteoclast formation in vitro in the absence of RANKL (4–6), the ability of TNF-α–induced osteoclast formation is limited, and the administration of TNF-α does not induce osteoclast formation in *Rank*-deficient mice in vivo (7). This may be at least partly because RANK, but not TNF receptor 1 or TNF receptor 2, recruits the adaptor molecule TNF receptor–associated factor 6 (TRAF6), which is essential for osteoclast development. However, the possibility that molecule(s) induced by TNF-α negatively regulate osteoclast differentiation has not been excluded.

NF-κB (p100) has emerged as a negative regulator of osteoclast differentiation

NF-κB is a collective term referring to dimeric transcription factors that belong to the Rel family, and NF-κB is involved in various aspects of physiologic and pathologic events. NF-κB is composed of five members: 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, the canonical or classical pathway and the noncanonical or alternative pathway, lead to NF-κB activation (8) (Figure 1). In the canonical pathway, the RelA/p50 complex is sequestered with inhibitors of κB (IκBs) in the cytoplasm. After a challenge with inflammatory stimuli such as TNF-α, IL-1, and LPS, phosphorylation and subsequent degradation of IκBs are rapidly induced, 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 β (LTβ). Upon stimulation with these ligands, NF-κB2 (p100) retains RelB in the cytoplasm as the RelB/p100 complex is processed to p52, then the RelB/p52 complex translocates to the nucleus. Notably, CD40 ligand, RANKL, and LTβ activate both the canonical and noncanonical pathways, whereas TNF-α, IL-1, and LPS solely activate the canonical pathway.

The essential role of NF-κB in osteoclast development was first observed in knockout mice. 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 of the canonical and noncanonical NF-κB pathways in osteoclast differentiation. NF-κB–inducing kinase (NIK) is known to be essential for the phosphorylation and subsequent processing of p100 to p52, and Novack et al. showed 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 the deletion of *Nfkb2* restored the impaired osteoclastogenesis in *Nik*^{−/−} precursors (12).

In this issue of the *JCI*, Yao et al. (13) provided in vitro and in vivo evidence that p100 is induced by TNF-α in osteoclast precursors and acts as a potent negative regulator of TNF-α–induced osteoclast formation (Figure 1). They found that TNF-α induced a sustained accumulation of p100 in osteoclast precursors, while RANKL more efficiently processed p100 to p52 through NIK activation. To analyze the
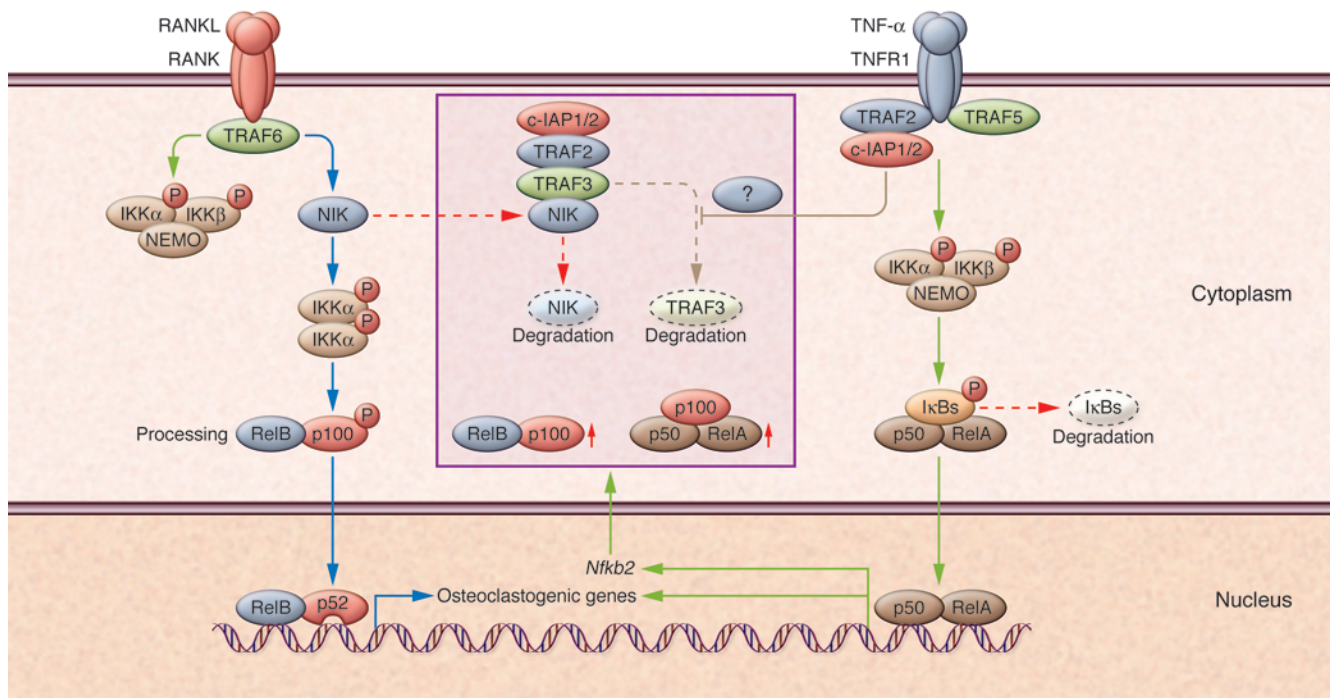
Figure 1
Schematic representation of RANKL and TNF-α–induced signaling in osteoclast precursors. New elements of these signaling pathways, as elucidated by Yao et al. (13) in their study in this issue of the *JCI*, are grouped within the purple box. In unstimulated cells, NIK is constitutively degraded by the TRAF3/TRAF2/c-IAP1/2 complex (red dashed arrows). RANKL activates NIK and induces both the canonical and noncanonical NF-κB pathways, which play critical roles in osteoclastogenesis (blue arrows). On the other hand, TNF-α solely activates the canonical pathways (green arrows) and induces the upregulation of TRAF3 by suppressing TRAF3 degradation (brown dashed arrow and line). TRAF3 in turn binds to and further accelerates the degradation of NIK. A decrease in NIK leads to the accumulation of NF-κB2 (p100) by suppressing its processing to p52 (blue arrows). In addition, TNF-α induces expression of *Nfkb2* in a RelA-dependent fashion. These signaling pathways lead to the accumulation of p100, thereby inhibiting osteoclast differentiation. The mechanism by which TNF-α stabilizes TRAF3 is currently unknown. The proteasome-dependent degradation pathways are indicated by dashed lines.

effect of p100 in osteoclast development, they investigated whether the deletion of *Nfkb2* promotes TNF-α–induced osteoclast formation. TNF-α–induced osteoclast formation was increased in bone marrow cells from *Nfkb2*−/− mice to a level comparable to that induced by RANKL treatment. Consistent with the in vitro observation, bone resorption was significantly increased in *Nfkb2*−/− mice compared with WT mice when TNF-α was injected over the calvaria. Although there is no obvious basal bone phenotype in *Nfkb2*−/− mice (7), the results strongly suggest that TNF-α–dependent upregulation of p100 acts as a negative regulator for osteoclast development under pathologic conditions. TNF-α treatment also upregulated osteoclastogenesis in *Rank*−/−*Nfkb2*−/− and *Rankl*−/−*Nfkb2*−/− mice both in vitro and in vivo, confirming that the enhancement of TNF-α–induced osteoclast formation in *Nfkb2*−/− mice does not depend on secondary production of RANKL by TNF-α.

To further substantiate a negative regulatory role of p100 in TNF-α–induced osteoclast formation under RA-like inflammatory conditions, Yao et al. (13) crossed *Tnfa*-Tg mice with *Nfkb2*−/− mice. *Tnfa*-Tg/*Nfkb2*−/− mice spontaneously developed more severe inflammation and joint erosion along with an increase in osteoclast number compared with *Tnfa*-Tg/*Nfkb2*+/− mice. Collectively, these studies showed that p100 acts as a negative regulator for TNF-α–induced osteoclast formation under pathologic conditions.

Finally, Yao et al. (13) presented an interesting observation that TRAF3 is involved in the posttranslational regulation of p100 expression. They found that TNF-α 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 1/2 (c-IAP1/2), and therefore,

stabilization of TRAF3 by TNF-α may decrease the level of NIK, resulting in the upregulation of p100 (14–16). Conversely, knockdown of *Traf3* using siRNA promoted TNF-α–induced osteoclast formation through downregulation of p100 (13). Dejardin et al. (17) and Novack et al. (11) reported that an agonistic antibody against LTβ receptor or TNF-α 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 (Figure 1).

TNF: The missing link is Now Found, or not yet?
There still remain many questions to be answered. First, how does TNF-α cause pathologic bone resorption in vivo even though it induces a negative regulator for osteoclastogenesis, i.e., p100? Is it because

TNF-α–induced upregulation of p100 is somehow blocked under inflammatory conditions, such as in that in RA synovial joints, or do pathologic conditions induce signaling molecule(s) that suppress the inhibitory effect of p100? Second, while p100 acts as an IκB-like molecule that binds to RelB, p100 also binds to the RelA/p50 dimer (11, 18). Given that *Rela*−/− or *Relb*−/− single-knockout mice do not show an altered bone phenotype, p100 may suppress TNF-α–induced osteoclast formation by retaining both RelA/p50 complex and RelB in the cytoplasm. Thus, it will be interesting to investigate the bone phenotype of *Rela*−/−*Relb*−/− mice. Third, the mechanism of how TNF-α suppresses the degradation of TRAF3 has not been clarified. It will also be intriguing to test whether TNF-α–induced osteoclast formation is enhanced in *Traf3*−/− mice. Given that *Traf3*−/− mice die soon after birth (14), transfer of *Traf3*−/− bone marrow cells to WT mice would be a feasible way to investigate the role of TRAF3 in TNF-α–induced osteoclast formation in vivo. Finally, what is the role of bone-forming osteoblasts in TNF-α–induced pathologic bone resorption, since TNF-α is known to induce RANKL expression in osteoblasts? These intriguing questions remain for future study.

In conclusion, Yao et al. (13) have convincingly demonstrated that NF-κB2p100 plays a negative role in suppressing TNF-α–induced osteoclast formation under pathologic conditions using various animal models. Thus, blockade of the processing of p100 might be a novel strategy to treat various bone diseases such as RA, in which TNF-α–induced osteoclast formation plays a crucial role in the progression of diseases.

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Pathogenic antibodies are active participants in spinal cord injury

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The role of B cells and autoimmunity as contributing factors to poor neurological outcomes following spinal cord injury (SCI) is poorly understood. The study by Ankeny et al., in this issue of the *JCI*, identifies a new immunopathological mechanism arising after SCI in mice (see the related article beginning on page 2990). The study shows that B cells produce pathogenic antibodies that impair lesion repair, resulting in worse neurological outcome. This new understanding of SCI disease pathogenesis, if confirmed in humans, reveals potential avenues for the development of novel neuroprotective immunotherapies.

Primary trauma to the CNS initiates a series of interrelated responses, including edema, excitotoxicity, and inflammation, that lead to secondary injury, resulting in further expansion of the initial lesion and additional loss of neurological function. The treatment of neuroinflammation in the context of both traumatic brain injury and spinal cord injury (SCI) still lacks a standard, universally accepted therapy that leads to improved neurological outcomes. There exists a clear, unmet medical need for an effective antiinflammatory treatment for the acute and chronic stages of traumatic brain injury and SCI arising in general civilian as well in injured military personnel populations. Currently, an important area of SCI research focuses on