Two types of TRAF6-dependent TAK1 activation in the IL-1 signaling pathway

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Interleukin-1 (IL-1) is a proinflammatory cytokine that plays a central role in the immune response during infection and chronic inflammation (1). IL-1α is constitutively expressed in the epithelial layers of the gastrointestinal tract, lung, liver, kidney, endothelial cells, and astrocytes, whereas IL-1β is mainly produced by activated macrophages and dendritic cells. Both IL-1α and IL-1β transduce their signals via the same receptor complex composed of type 1 IL-1 receptor (IL-1RI) and IL-1R accessory protein (IL-1RAcP), and exert similar biological effect. The IL-1R complex is expressed on virtually all cells and tissues. Upon IL-1 binding to the IL-1R complex, an adapter protein, myeloid differentiation primary response gene 88 (MyD88), recruits a death domain-containing Ser/Thr kinase IL-1R-associated kinase 4 (IRAK4) to the receptor complex, followed by interaction with IRAK1. IRAK1 activates multiple E3 ubiquitin ligases, including tumor necrosis factor receptor-associated factor 6 (TRAF6), Pellino 1, or Pellino 2. All of these E3 ligases, together with the E2-conjugating enzyme complex termed ubiquitin conjugating 13 (Ubc13)-ubiquitin conjugating enzyme E2 variant-1 (Uev1a), generate Lys63-linked ubiquitin (K63-Ub) chains. IL-1-induced K63-Ub chains activate another Ser/Thr kinase TGF-β-activated kinase 1 (TAK1). TAK1 phosphorylates and activates the IκB kinase (IKK) complex and mitogen-activated protein kinase kinase (MAPKK) family kinases, which leads to the activation of the transcription factors nuclear factor-κB (NF-κB) and activating protein-1 (AP-1), respectively.

Three TAK1-binding proteins, namely, TAB1, TAB2 and TAB3, have been implicated in TAK1 regulation (2-6). Upon IL-1 stimulation, TAB2 and TAB3, two structurally and functionally related Ub-binding adaptor proteins, recruit TAK1 to TRAF6 through the K63-Ub chain. This complex formation leads to the autophosphorylation of TAK1 on Thr187 and its kinase activation. TAB1 is structurally distinct from TAB2/3, but it strongly activates TAK1 when TAB1/TAK1 are simultaneously overexpressed. The molecular mechanism for how endogenous TAB1 regulates IL-1-induced TAK1 activity is not well understood. In a recent study, Cohen and colleagues investigated the roles of TAB proteins in TRAF6-dependent TAK1 activation in the IL-1 signaling pathway (7). To genetically dissect the molecular mechanisms underlying this process, the authors used CRISPR/Cas9 gene editing technology extensively to knock out TAB1/2/3 and TRAF6 in IL-1R* cells, a human embryonic kidney (HEK) 293 cell line stably expressing IL-1R, in which the endogenous signaling pathways are activated in response to IL-1.

TAK1 can form two distinct complexes consisting of TAK1-TAB1-TAB2 or TAK1-TAB1-TAB3 (3). Each TAB protein can directly interact with TAK1. Although TAB1 binds to the N-terminal kinase domain of TAK1, both TAB2 and TAB3 bind to TAK1 via its C-terminal coiled-coil domain. The authors demonstrated that endogenous TAB1 is co-immunoprecipitated with TAK1 in TAB2/3 DKO cells and, conversely, TAB2/3 is co-immunoprecipitated with TAK1 in TAB1 KO cells, providing genetic evidence that TAB1 and TAB2/3 independently interact with TAK1.

It has been shown that TAB2 and TAB3 play redundant roles in TAK1 activation in the IL-1β and tumor necrosis factor (TNF) signaling pathway (2,4). In the current study, stimulation of TAB2/3 DKO cells with IL-1 resulted in TAK1 activation at a nearly normal level for up to 20 min, but TAK1 activity declined sharply thereafter in these cells, suggesting that TAB2/3 is not essential in the immediate early activation of TAK1 but is required for sustained TAK1 activity in the IL-1 signaling pathway. The IL-1-induced expression of two NF-κB-dependent immediate early genes, IκBα and A20, was comparable in control and TAB2/3 DKO cells for up to 45 min. In contrast, the expression and secretion of IL-8 was blunted in TAB2/3 DKO cells. The authors also reported that the IL-1-induced activation of p38γ and JNK1/2 MAPKs were markedly reduced in TAB2/3 DKO cells, indicating the essential role of TAB2/3 in linking TAK1 to p38γ and JNK1/2 activation—this may explain the impaired IL-8 production in TAB2/3 DKO cells. In contrast to these observations, a previous study demonstrated that TAB2 is essential for the downregulation, but not the initial activation, of TAK1 by recruiting protein phosphatase 6 (PP6) in response to TNF-α in mouse dermal fibroblasts (8). In addition, it was reported that TAB2/3 is essential for the activation of MAPKs, but not NF-κB, in B cells (9). Therefore, TAB2 and TAB3 appear to play multiple roles in TAK1 regulation, and their predominant roles may vary depending on cell type and cell context.

The authors demonstrated that TAB1/2/3 TKO cells are unable to activate TAK1 and its downstream signaling pathways upon IL-1 stimulation. These results, together with TAB2/3 DKO data, strongly imply that TAB1/2/3 has redundant and essential roles in IL-1-induced TAK1 activation, and that TAB1 can activate TAK1 in the absence of TAB2/3. Next, they investigated the molecular mechanisms for TAB1-mediated TAK1 activation by analyzing TAB2/3/TRAF6 TKO cells, and found that TRAF6 expression and its E3 ubiquitin ligase activity are essential for TAB1-mediated TAK1 activation in the absence of TAB2/3. Thus, TRAF6 may ubiquitinate itself or other protein(s) to activate the TAB1-TAK1 heterodimer. It is noteworthy that their recent study revealed that two RING domain E3 ubiquitin ligases, Pellino1 and Pellino2, can generate the K63-Ub chain, which compensates for the loss of TRAF6 E3 ligase activity and mediates the IL-1 signaling in TAB2/3-expressing IL-1R* cells (10). These observations raise another possibility that TAB2/3 is critically involved in the Pellino1/2-mediated ubiquitination of TRAF6 that is required for activation of the TAB1-TAK1 heterodimer. This is also supported by the fact that the overexpression of TAB2 induces the ubiquitination of endogenous TRAF6 in HEK293 cells (11).

How exactly TAB1-TAK1 heterodimer is activated still remains unclear. As mentioned, the overexpression of both TAB1 and TAK1 results in strong TAK1 activation. In addition, the incubation of primary keratinocytes with calyculin A, a potent inhibitor of the type 2A family of protein phosphatases, strongly activates endogenous TAK1 even without IL-1 stimulation (12). Intriguingly, the calyculin A-induced activation of TAK1 requires TAB1, but not TAB2. Therefore, as the authors discussed in the article and as suggested by others, the TAB1-TAK1 heterodimer may be intrinsically active, but suppressed by phosphatase(s) in resting cells. It is possible that the TRAF6-generated K63-Ub chain or the K63-Ub chain attached to TRAF6 mediates the IL-1-induced activation of the TAB1-TAK1 heterodimer by inhibiting such phosphatase(s).

It has been reported that epidermis-specific or intestinal epithelium-specific conditional TAK1 knockout mice exhibit severe tissue damage due to increased apoptosis that is rescued by additional deletion of TNF receptor 1 gene (13,14). Mice bearing deletion of either TAB1 or TAB2 in these tissues show no overt abnormalities. The simultaneous deletion of TAB1 and TAB2 in these tissues, however, causes very similar phenotypes as TAK1 deficiency. Furthermore, the steady-state TAK1 activity in epidermal cell extracts is completely diminished in TAB1/TAB2 DKO mice (12). These results indicate the existence of two different mechanisms of TAK1 activation in vivo, one dependent on TAB1 and the other dependent on TAB2. Because TAB1/TAB2 DKO closely mimics TAK1 deficiency, TAB3 does not appear to play a major role in TNF-induced TAK1 activation in these tissues.

The current study provides strong genetic evidence for the roles of TAB proteins and possible molecular mechanisms for IL-1- and TRAF6-dependent TAK1 activation. Even though TAB1/2/3 is not essential for TAK1 activation by itself, it is now evident that these proteins redundantly engage in the TRAF6-dependent activation of TAK1. Many pathogenic bacteria are known to inhibit the TRAF6-NF-κB/MAPK pathway by delivering effector molecules into host cells (15). For example, *Shigella flexneri* effector OspI inhibits TRAF6-mediated K63-Ub generation by targeting Ubc13 (16), and enteropathogenic *Escherichia coli* effector NleE specifically interferes with the association of TAB2/3 and K63-Ub chains by modifying the zinc finger domains of TAB2/3 (17). Therefore, the

functional redundancy among these TAB proteins may serve as a backup mechanism for host defense against pathogens. Future studies are needed to elucidate the roles of TAB1/2/3 in TAK1 regulation in other signaling pathways or cell types and their physiological impact on the immune system, tissue homeostasis, and development.

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Footnote

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