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Targeting signaling factors for degradation, an emerging mechanism for TRAF functions

Xiao-Dong Yang¹ and Shao-Cong Sun^{2,3}

¹Shanghai Institute of Immunology, Shanghai Jiao Tong University School of Medicine, Shanghai, China

²Department of Immunology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

³The University of Texas Graduate School of Biomedical Sciences at Houston, Houston, Texas, USA

Summary

Tumor necrosis factor receptor (TNFR)-associated factors (TRAFs) form a family of proteins that are best known as signaling adapters of TNFRs. However, emerging evidence suggests that TRAF proteins, particularly TRAF2 and TRAF3, also regulate signal transduction by controlling the fate of intracellular signaling factors. A well-recognized function of TRAF2 and TRAF3 in this aspect is to mediate ubiquitin-dependent degradation of NF- κ B-inducing kinase (NIK), an action required for the control of NIK-regulated noncanonical NF- κ B signaling pathway. TRAF2 and TRAF3 form a complex with the E3 ubiquitin ligase cIAP (cIAP1 or cIAP2), in which TRAF3 serves as the NIK-binding adapter. Recent evidence suggests that the cIAP-TRAF2-TRAF3 E3 complex also targets additional signaling factors for ubiquitin-dependent degradation, thereby regulating important aspects of immune and inflammatory responses. This review provides both historical aspects and new insights into the signaling functions of this ubiquitination system.

Keywords

TRAF2; TRAF3; cIAP; NIK; noncanonical NF- κ B; inflammation

Introduction

Tumor necrosis factor receptor (TNFR)-associated factors (TRAFs) form a family of structurally related proteins that mediate signal transduction from receptors of the TNFR superfamily as well as many other receptors, such as Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), NOD-like receptors (NLRs), and different cytokine receptors (1, 2). Since the discovery of TRAF1 and TRAF2 as TNFR2-interacting adapters (3), a total of 7 TRAF members have been characterized. The 6 typical TRAF members (TRAF1-TRAF6) share a

Correspondence to: Shao-Cong Sun, The University of Texas MD Anderson Cancer Center, 7455 Fannin Street, Box 902, Houston TX 77030, USA, Tel: +1 713 563 3218, ssun@mdanderson.org.

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similar modular structure composed of well-defined functional domains, including a C-terminal TRAF domain that mediates TRAF dimerizations and interaction with other proteins as well as a zinc finger domain with a variable number of zinc fingers (1). With the exception of TRAF1, all of the TRAF members also contain a RING finger, a domain known to mediate the catalytic activity of a large family of E3 ubiquitinase ligases (4). Indeed, several TRAF family members, including TRAF2, TRAF3, TRAF5, and TRAF6, have been shown to possess E3 ubiquitin functions (1, 2, 5).

The best-defined function of TRAF proteins is to serve as signaling adapters of TNFRs. Many TNFR members contain TRAF-interacting motifs capable of directly interacting with specific TRAF proteins, whereas other TNFRs lack TRAF-interacting motifs and bind TRAFs indirectly via adapters, such as TRADD (TNFR1-associated death domain protein) (2, 6). Upon receptor crosslinking, TRAF proteins recruit downstream signaling factors to the TNFRs, thereby triggering the activation of downstream signaling events, including activation of canonical NF- κ B and mitogen-activated protein kinase (MAPK) pathways (1, 2). The signaling functions of some TRAF members, particularly TRAF6, requires their E3 ubiquitin ligase activity, which conjugates lysine 63 (K63)-linked ubiquitin chains that facilitate the activation of downstream signaling molecules, such as TAK1 and its target I κ B kinase (IKK) (7).

Recent studies suggest a novel mechanism of TRAF function, which involves ubiquitin-dependent degradation of intracellular signaling proteins. The best-known case is the control of the NF- κ B-inducing kinase (NIK) by TRAF2 and TRAF3. NIK is a kinase that mediates activation of the noncanonical NF- κ B signaling pathway, which is characterized by the inducible processing of the NF- κ B2 precursor protein p100 (8, 9). In contrast to the canonical NF- κ B pathway, which is activated by diverse receptor signals via IKK, the noncanonical NF- κ B pathway responds to a specific subset of TNFR superfamily members, including CD40, BAFF receptor (BAFFR), lymphotoxin β receptor (LT β R), RANK, TNFR2, TWEAK, CD27, etc. (9, 10). A hallmark of the noncanonical NF- κ B signaling is its slow kinetics and dependence on protein synthesis (9). This is at least partially due to the unique mechanism of NIK activation: the steady-state level of NIK is normally low due to its continuous degradation by TRAF2 and TRAF3 and that induction of noncanonical NF- κ B signaling involves signal-induced TRAF3 degradation and NIK accumulation (9). TRAF2 and TRAF3 mediate NIK ubiquitination by forming a complex with the E3 ligase cIAP (cIAP1 or cIAP2). Emerging evidence suggests that the cIAP-TRAF2-TRAF3 complex may function as a novel multi-component E3 ubiquitin ligase that targets additional proteins for degradation, thereby regulating expanding cellular functions.

Personal and historical narrative

Our work on TRAFs originates from the study of noncanonical NF- κ B signaling (9). Following the discovery of NIK as a central player of the noncanonical NF- κ B signaling pathway (8), we made a major effort to screen for NIK-regulatory factors. By yeast two-hybrid screening, TRAF3 was identified as a predominant NIK-interacting protein (11). We demonstrated further that TRAF3 mediates the degradation of NIK and thereby serves as a pivotal negative regulator of noncanonical NF- κ B signaling (11). Co-immunoprecipitation

(IP) analyses revealed that newly synthesized NIK is rapidly bound by TRAF3 and targeted for ubiquitin-dependent degradation, keeping the level of NIK extremely low. These findings established a mechanism by which NIK and the noncanonical NF- κ B signaling pathway are controlled under steady-state conditions.

In search for the mechanism mediating signal-stimulated noncanonical NF- κ B activation, we found that an anti-CD40 agonistic antibody and BAFF induce degradation of TRAF3, coupled with NIK accumulation and p100 processing (11). Similarly, TRAF3 knockdown by RNA interference results in NIK accumulation and p100 processing, further emphasizing TRAF3 degradation as a mechanism mediating noncanonical NF- κ B activation. Additional demonstrated that signal-induced TRAF3 degradation requires its recruitment to the stimulating receptors (12). In particular, TRAF3 binds to an atypical TRAF-binding motif in the cytoplasmic tail of BAFFR, which is required for BAFFR-mediated induction of TRAF3 degradation and noncanonical NF- κ B activation. These studies suggest that noncanonical NF- κ B activation is mediated by a novel mechanism that involves receptor-induced TRAF3 degradation and rescue of NIK from TRAF3-programmed destruction.

Our work was subsequently confirmed by Cheng's laboratory (13) using TRAF3 knockout mice. TRAF3-deficient mouse embryonic fibroblasts (MEFs) and B cells display NIK accumulation and constitutive p100 processing (13). Both our initial study and Dr. Cheng's work demonstrate that TRAF3 is unable to directly ubiquitinate NIK, raising the idea that TRAF3 may function as a critical component of an E3 ubiquitin ligase mediating NIK ubiquitination (11). It is well accepted now that TRAF3 functions as a substrate-binding component in an E3 complex that also contains TRAF2 and cIAP (cIAP1 and cIAP2). As detailed below, our current understanding of this unusual mechanism of TRAF function was contributed by studies from a number of investigators using TRAF2,3 mouse models or small-molecule cIAP antagonists (9). Furthermore, emerging evidence suggests that the cIAP-TRAF2-TRAF3 E3 complex may regulate cell signaling via targeting both NIK and additional substrates for proteolysis (14).

Regulation of noncanonical NF- κ B signaling by TRAF2 and TRAF3

A hallmark of noncanonical NF- κ B activation is its slow kinetics and dependence on protein synthesis. This feature was initially thought to be due to possibly the cotranslational nature of p100 processing, as learned from the constitutive processing of the p100-related protein p105 (15). However, pulse-chase studies suggest that the processing of p100 occurs posttranslationally, thus differing from the processing of p105 (8). It is now clear that induction of NIK requires its *de novo* synthesis and stabilization and that TRAF2 and TRAF3 play a pivotal role in regulating this unusual mechanism of signal transduction.

TRAF3 as a fate-controller of NIK

An initial clue to the mechanism of NIK regulation came from a yeast two-hybrid screening study, which led to the identification of TRAF3 as a major NIK-binding protein (11). Upon *de novo* synthesis, NIK is rapidly bound by TRAF3 and targeted for ubiquitination and proteasomal degradation, a mechanism that keeps the steady level of NIK extremely low (11). Thus, incubation of cells with a proteasome inhibitor leads to NIK accumulation as a

complex with TRAF3 (11), and TRAF3 knockdown or knockout also elevates the steady-state levels of NIK (11, 13). NIK accumulation in TRAF3-deficient cells is coupled with robust processing of p100, suggesting that the function of NIK is primarily controlled through its degradation by a TRAF3-dependent mechanism. Indeed, induction of p100 processing by CD40 and BAFF receptor involves TRAF3 degradation and NIK accumulation (11).

NIK was originally identified as a kinase that binds to TRAF2 and induces canonical NF- κ B activation under overexpression conditions (16). However, compared to TRAF3, TRAF2 displays much weaker NIK-binding activity even under overexpression conditions (11). Furthermore, while TRAF2 binds to the C-terminal region of NIK, the TRAF3-NIK interaction requires the N-terminal portion of NIK. Deletion of a sequence motif, amino acids 78–84, of NIK largely abolishes its ability to bind TRAF3 (11). This mutant, termed NIK 78–84 or NIK T3 (defective in TRAF3 interaction), is stable when expressed in cell lines or transgenic mice, further emphasizing the importance of TRAF3-NIK interaction in controlling the fate of NIK (11, 17).

The cIAP-TRAF2-TRAF3 ubiquitin ligase complex

Although TRAF3 mediates ubiquitination and proteolysis of NIK in cells, purified TRAF3 fails to directly induce NIK ubiquitination *in vitro*, leading to the hypothesis that TRAF3 may function as a substrate-binding component of a ubiquitin ligase complex mediating NIK ubiquitination *in vivo* (11). As seen with the TRAF3 deficiency, TRAF2 ablation in B cells causes constitutive activation of the noncanonical NF- κ B pathway (18). Comparative studies of mutant mice lacking TRAF2 or TRAF3 in B cells further suggest cooperative functions of these two TRAF members in the control of noncanonical NF- κ B activation (19). Two independent studies (20, 21) reveal that the E3 ubiquitin ligase cIAP mediates degradative ubiquitination of NIK. Treatment of cells with small-molecule cIAP antagonists causes NIK accumulation and NIK-dependent p100 processing. Genetic and biochemical evidence suggests that TRAF2 and TRAF3 form an E3 ubiquitin ligase complex with cIAP, in which TRAF2 and TRAF3 bind to cIAP and NIK, respectively (22, 23). TRAF2 contains a cIAP-binding motif and directly interacts with cIAP (24), whereas TRAF3 recruits NIK to the cIAP-TRAF2 complex via dimerization with TRAF2 (22, 23). This model explains why genetic deficiency in either TRAF2 or TRAF3 leads to NIK accumulation and constitutive activation of noncanonical NF- κ B signaling (11, 13, 18, 19, 25). In contrast to the cooperative mode of TRAF2/TRAF3 function, cIAP1 and cIAP2 are functionally redundant, since simultaneous ablation of cIAP1 and cIAP2 is required for NIK stabilization and noncanonical NF- κ B activation (26). The crucial role of the cIAP-TRAF2-TRAF3 complex in noncanonical NF- κ B regulation has also been demonstrated in human cancer cells carrying loss-of-function mutations in TRAF2, TRAF3, or cIAP1,2 (27–33). As seen in genetically manipulated mouse cells, these human cancer cells have elevated levels of NIK and constitutive processing of p100. A recent study indicates that cIAP may also be directly interacting with NIK (34). The N-terminus of NIK contains a cIAP-binding motif (IBM), which binds to the second baculovirus IAP repeat (BIR2) domain of cIAP1. This direct binding appears to be weak, though, and requires TRAF3-NIK interaction for the assembly of the cIAP-TRAF2-TRAF3-NIK complex (34).

Although TRAF2 and TRAF3 are the major accessory components of the cIAP-TRAF2-TRAF3 E3 complex, recent evidence suggests that TRAF1 may also play a role in modulating the function of this E3 ubiquitin ligase in some cell types (35, 36). Structural analyses reveal that TRAF1 and TRAF2 can form a TRAF1:(TRAF2)₂ heterotrimer that interacts with cIAP2 more strongly than TRAF2 alone and that TRAF1 appears to be a component of the NIK ubiquitin ligase complex (35). In murine CD8⁺ T cells, the noncanonical NF- κ B is activated via stimulation of TCR and the costimulatory receptor 4-1BB, and ablation of TRAF1 allows noncanonical NF- κ B activation in the absence of 4-1BB costimulation (36). Because the expression of TRAF1 is upregulated by many stimuli, TRAF1 may function to enhance the activity of the NIK-destructing cIAP-TRAF2-TRAF3 E3 complex. However, the role of TRAF1 in regulating noncanonical NF- κ B signaling is somewhat controversial, since another study suggests that TRAF1 serves as a positive regulator of the noncanonical NF- κ B pathway (37). Overexpressed TRAF1 induces TRAF3 degradation and p100 processing in transfected 293 cells, whereas TRAF1 knockdown in a Hodgkin's disease cell line (L1236) inhibits p100 processing. It is important to note that the effect of TRAF1 knockdown on p100 processing is not quite clear, since only 2 out of 4 TRAF1 shRNAs inhibited p100 processing and since the effect of TRAF1 shRNA on NIK expression was not analyzed (37). So, at least in CD8⁺ T cells, TRAF1 is involved in negative regulation of NIK and noncanonical NF- κ B.

In addition to the TRAF3-directed function of cIAP in NIK regulation, cIAP has various TRAF3-independent functions. A widely known function of IAP is to mediate activation of canonical NF- κ B and MAPK pathways by the TNF receptor (TNFR) superfamily members and pattern-recognition receptors (PRRs) (38, 39). This function requires the binding of cIAP with TRAF2, the latter of which serves as an adapter to recruit cIAP to receptor complexes (24). Within the receptor signaling complexes, cIAP conjugates K63- and, possibly, K11-linked polyubiquitin chains to signaling adapters, such as TRAF2, RIP1, and NEMO, thereby facilitating the activation of downstream kinases (38, 39). The complex function of cIAP proteins has been most clearly elucidated using mice lacking cIAP1 and cIAP2 in B cells (26). Simultaneous ablation of cIAP1 and cIAP2 causes BAFF-independent B-cell survival, due to activation of the noncanonical NF- κ B pathway, but it compromises germinal center response because of attenuated CD40 signaling. The role of cIAP in mediating germinal center response requires TRAF2 but not TRAF3 (26), further suggesting that TRAF2 is a general adapter of cIAP whereas TRAF3 serves as a specific adapter in the NIK-specific function of cIAP.

TRAF2,3 degradation as a trigger for noncanonical NF- κ B activation

An initial study demonstrated that induction of noncanonical NF- κ B signaling by BAFFR and CD40 is coupled with degradation of TRAF3 and concomitant accumulation of NIK (11). This finding has since been confirmed by many other studies (9). At least in some cases, the activation of noncanonical NF- κ B also involves TRAF2 degradation. Thus, it is generally accepted that inducible degradation of TRAF3 or TRAF2 disrupts the NIK ubiquitin ligase complex, thereby causing NIK accumulation and noncanonical NF- κ B activation. The signal-induced degradation of TRAF2 and TRAF3 appear to involve their recruitment to the inducing receptors. The cytoplasmic domain of CD40 contains a typical

binding motif for TRAF2 and TRAF3, which is essential for the recruitment and degradation of these TRAF members (40, 41). BAFRR lacks a typical TRAF2/TRAF3-binding motif, but it contains an atypical TRAF-binding sequence that predominantly recruits TRAF3 (12). Mutation of this motif in BAFRR abolishes its function in mediating the recruitment and degradation of TRAF3 and the induction of noncanonical NF- κ B activation (12).

Otud7b as a DUB of TRAF3 in noncanonical NF- κ B signaling

Consistent with its pivotal role in triggering noncanonical NF- κ B activation, TRAF3 degradation is subject to tight control. The signal-induced TRAF3 ubiquitination is associated with its physical interaction with a deubiquitinase (DUB), Otud7b (also called Cezanne) (42). Otud7b is a member of the ovarian tumor (OTU) family of DUBs that shares sequence homology with A20 in the OTU domain and zinc fingers (43). Otud7b has been implicated in the regulation of canonical NF- κ B activation via deubiquitination of TRAF6 (44, 45). Interestingly, a gene targeting study reveals an important role for Otud7b in controlling noncanonical NF- κ B activation by CD40 and BAFRR in B cells and by LT β R in MEFs (42). The Otud7b deficiency promotes receptor-mediated degradation of TRAF3 and processing of p100. Upon ligation of these receptors, Otud7b binds to TRAF3, and this inducible interaction requires a ubiquitin-association (UBA) domain present in the N-terminus of Otud7b, suggesting the possibility that ubiquitination of TRAF3 may facilitate its binding by Otud7b (42).

Although the OTU domain of Otud7b preferentially cleaves K11-linked ubiquitin chains *in vitro* (46), the Otud7b deficiency *in vivo* promotes TRAF3 conjugation with K48 ubiquitin chains (42). Overexpressed Otud7b also inhibits K48 as well as K63 ubiquitination of cellular proteins in transfected cells (44). Several possible reasons may contribute to the discrepancy between the *in vitro* and *in vivo* studies. First, the chain-specificity of Otud7b may be modulated *in vivo* by accessory proteins, as proposed for its homologue A20 (47). Second, the full-length Otud7b protein may differ from the OTU domain alone (used in the *in vitro* studies) in ubiquitin chain cleavage. Indeed, the full-length Otud7b is considerably more effective than the protease (OTU) domain alone in cleaving a synthetic ubiquitin chain (48). Third, it remains possible that Otud7b regulates K11 ubiquitination of some yet to be identified substrates. Notwithstanding, Otud7b-mediated TRAF3 deubiquitination serves as a mechanism that controls the magnitude of noncanonical NF- κ B activation.

cIAP as an E3 mediating TRAF3 degradation

TRAF2 and cIAP play dual roles in the regulation of noncanonical NF- κ B signaling. Under steady state levels, they mediate degradation of NIK to prevent basal activation of noncanonical NF- κ B. However, the signal-induced degradation of TRAF3 and, therefore, stabilization of NIK require both TRAF2 and cIAP (22, 49). TRAF2 appears to function by recruiting cIAP proteins to the receptor complex for activation (50), where cIAP may mediate ubiquitination of TRAF3. It has also been suggested that TRAF2 may mediate K63 ubiquitination and catalytic activation of cIAP (22). The question is why cIAP has constitutive E3 ligase activity towards NIK but requires receptor recruitment for mediating TRAF3 ubiquitination. There are two possible answers. First, the constitutive ubiquitination of NIK may require sub-optimal cIAP activity, whereas the inducible degradation of TRAF3

requires stronger cIAP activity. In support of this hypothesis, cIAP also mediates certain levels of TRAF3 constitutive degradation, as revealed by the increase in TRAF3 steady-state level in B cells lacking both cIAP1 and cIAP2 (26). The constitutive degradation of TRAF3 also depends on TRAF2, indicating the involvement of the same mechanism that mediates NIK degradation. Second, the inducible degradation of TRAF3 may involve receptor internalization and lysosomal pathway, as revealed in the LT β R-stimulated cells (51).

Degradation-independent inactivation of cIAP-TRAF2-TRAF3

The induction of noncanonical NF- κ B signaling may not always require degradation of TRAF3 and/or TRAF2. One example is provided by noncanonical NF- κ B activation by the oncoprotein Tio encoded by T-lymphotropic virus *Herpesvirus ateles* (52). Tio is a membrane-bound protein that activates the canonical NF- κ B via interaction with TRAF6 (53). Tio has an atypical TRAF3-binding site, which is required for activation of the noncanonical NF- κ B pathway (52). Tio induces ubiquitin-independent depletion of TRAF3 from the cytosol, instead of TRAF3 degradation, which may serve as a mechanism to disrupt the cIAP-TRAF2-TRAF3 complex. A similar, although not identical, mechanism of noncanonical NF- κ B activation has been shown for TNF-related weak inducer of apoptosis (TWEEK), which acts through its receptor Fn14 (54, 55). TWEEK induces relocalization of TRAF2 from triton X-100-soluble to triton X-100-insoluble compartment without reducing total TRAF2 level (55). It is important to note, though, TWEEK can induce TRAF2 degradation at high strength of receptor ligation. While a FLAG-tagged trimeric variant of TWEEK induces TRAF2 relocalization without causing TRAF2 degradation, a hexameric immunoglobulin-TWEEK fusion protein (Fc-TWEEK) induces lysosomal degradation of TRAF2 as well as cIAP (21, 55, 56). Nevertheless, these findings suggest that induction of NIK and noncanonical NF- κ B signaling by TWEEK can be achieved via either degradation of the cIAP-TRAF2-TRAF3 components or their depletion from the cytosolic compartment. LT β R-induced noncanonical NF- κ B activation may also occur without the requirement of TRAF3 degradation (51, 57). Although LT β R crosslinking triggers the degradation of TRAF2 and TRAF3 via the lysosomal pathway, inhibition of their degradation using bafilomycin A1 or chloroquine does not block the induction of p100 processing (51). It is suggested that LT β R may stabilize NIK by competing with NIK for binding the cIAP-TRAF2-TRAF3 complex, a process that requires LT β R internalization via a dynamin-dependent mechanism (51, 57).

Regulation of B-cell survival and homeostasis by TRAF2,3

In peripheral B cells, noncanonical NF- κ B pathway is stimulated by BAFF and required for the survival and maturation of transitional B cells in the spleen (58, 59). Deregulated activation of noncanonical NF- κ B is associated with B-cell hyperplasia and autoantibody production and has also been linked to human B-cell malignancies (60–62). Moreover, transgenic expression of a NIK mutant lacking the TRAF3-binding sequence (NIK T3) causes BAFF-independent B-cell survival and abnormal expansion (17). Conversely, mice or humans harboring *nfkb2* gene mutations that impair p100 processing show severe defect in B-cell maturation and are associated with primary immunodeficiency syndromes (63–65). Consistent with the crucial role of TRAF3 in controlling the fate of NIK and noncanonical

NF- κ B activation, B cell-specific ablation of TRAF3 causes BAFF-independent B-cell survival and aberrant expansion of marginal zone (MZ) and follicular B cells associated with hyperimmunoglobulinemia (19, 25). These mutant animals also display autoimmune symptoms, characterized by accumulation of autoantibodies against double-stranded DNA. Like TRAF3, TRAF2 plays a crucial role in controlling the survival and homeostasis of B cells, since deletion of TRAF2 in B cells also causes aberrant B-cell survival and maturation (18, 19). As seen in NIK regulation, TRAF2 and TRAF3 function cooperatively to control the survival and maturation of B cells (19). A subsequent study, employing B cell-conditional cIAP1/cIAP2 KO mice, further emphasized the crucial role for cIAP-TRAF2-TRAF3 complex in the control of B-cell survival and homeostasis (26). Simultaneous ablation of cIAP1 and cIAP2 in B cells renders peripheral B cells independent of BAFF signal for survival and maturation. However, unlike the cooperative function of TRAF2 and TRAF3, cIAP1 and cIAP2 are functionally redundant in regulating B-cell survival, consistent with their redundant function in mediating NIK degradation (26).

The function of TRAF3 in B cells has also been studied by using transgenic mice expressing human TRAF3 under the control of immunoglobulin H (IgH) μ -chain enhancer (66). In these animals, the TRAF3 transgene is abundantly expressed in B cells and to a lesser extent in T cells. In agreement with the TRAF3 targeting studies, overexpression of TRAF3 in the transgenic mice led to downregulation of NIK and decreased B-cell number. Surprisingly, however, the TRAF3 transgenic mice develop plasmacytosis and hypergammaglobulinemia as well as autoimmune phenotypes. These unexpected phenotypes may be due to the hyperresponsiveness of the TRAF3 transgenic B cells to TLR stimulation, rather than to BCR or BAFF stimulation (66). These findings suggest that TRAF3 may have paradoxical functions in different stages of B-cell differentiation.

Regulation of T-cell function by TRAF2,3

As seen in B cells, the loss of TRAF2 or TRAF3 causes spontaneous processing of p100 and nuclear translocation of p52 and RelB in T cells (19, 67). However, TRAF2 and TRAF3 play distinct roles in regulating T-cell activation and function (62, 67).

Role of noncanonical NF- κ B in T cells

Compared to the well-studied function of noncanonical NF- κ B signaling in B cells, the role of this pathway in T cells is appreciated only recently. A study using NIK KO mice demonstrates that NIK is required for the induction of the T-cell-dependent autoimmunity experimental autoimmune encephalomyelitis (EAE), an animal model of the neuroinflammatory disease multiple sclerosis (68). Consistently, a subsequent study reveals a T-cell-intrinsic requirement for NIK in mediating graft-versus-host disease (69). The T-cell-intrinsic function of NIK has also been demonstrated using a viral infection model, which demonstrates a particular requirement of NIK in mediating memory T-cell responses (70). Although NIK may have functions independent of noncanonical NF- κ B activation (68, 71), the role of NIK in regulating T-cell function is largely dependent on the noncanonical NF- κ B pathway. Like the NIK KO mice, mice expressing a nonprocessable p100, NF- κ B2^{lym1}, are resistant to EAE induction (72). Like NIK, the noncanonical NF- κ B regulates T-cell responses in a cell-intrinsic manner, as demonstrated by mixed bone-marrow adoptive

transfer experiments. Noncanonical NF- κ B is dispensable for the initial activation of naive T cells, but it is required for the inflammatory effector function of Th17 cells. NF- κ B2 p52, in cooperation with the canonical NF- κ B member c-Rel, mediates TCR-stimulated expression of GM-CSF and the IL-23 receptor in Th17 cells (72).

NIK also plays an important role in dendritic cell (DC) maturation and function, particularly cross-priming of soluble antigens (73–76). The DC-specific function of NIK may indirectly impact on T-cell responses. Nevertheless, it is increasingly clear that NIK and noncanonical NF- κ B have a T-cell-intrinsic role in regulating T-cell function and autoimmunity. Some controversies in the literature are at least partially due to the use of different mouse models and experimental approaches. Precautions should be taken in drawing conclusions based on the use of *nfkb2* knockout (KO) and *Relb* KO mice. The noncanonical NF- κ B signaling is defined as a mechanism that activates NF- κ B via inducible processing of p100 (9). While the generation of p52 is stringently controlled by the noncanonical NF- κ B signaling, the activation of RelB may also be induced by canonical NF- κ B inducers, such as TNF- α (77–80). Furthermore, the p100 processing not only generates p52 but also inactivates the I κ B-like function of p100. Since the p100 processing/degradation also contributes to the activation of other NF- κ B members, such as RelA (63, 81, 82), neither *nfkb2* KO nor *Relb* KO mice are perfect for the study of noncanonical NF- κ B signaling. An ideal model is mice expressing a p100 mutant defective in inducible processing, such as NF- κ B2^{lym1} (63). This idea is further supported by the recent finding that p100 processing but not RelB expression has a T cell-intrinsic role in mediating EAE induction (72).

How the noncanonical NF- κ B pathway is activated in T cells is incompletely understood. Crosslinking TCR and CD28 induces the processing of p100 in T cells in a mechanism that is dependent on NIK and C-terminal phosphorylation of p100 (72). Since activated T cells express TNFR members and their ligands (83, 84), one possibility is that noncanonical NF- κ B is indirectly activated via specific TNFRs. Indeed, some TNFR superfamily members, including OX40 and 4-1BB, have been shown to induce noncanonical NF- κ B signaling in T cells (36, 69). However, since TCR/CD28-stimulated activation of p52 can be detected as early as 4 h (72), the possibility for TCR/CD28 signals to directly stimulate noncanonical NF- κ B activation cannot be excluded. It has recently been shown that the T-cell activation signals induce the generation of an alternatively spliced form of TRAF3 lacking its exon 8, TRAF3DE8 (85). This TRAF3 mutant binds TRAF2 but not NIK and, thereby, dissociates NIK from the cIAP-TRAF2-TRAF3 E3 ligase complex. The induction of TRAF3DE8 may partially contribute to the activation of noncanonical NF- κ B.

Regulation of T-cell function by TRAF2

Ablation of TRAF2 in T cells causes spontaneous activation of noncanonical NF- κ B, although the functional consequence of this signaling perturbation has not been carefully examined in the T-cell-conditional TRAF2 KO mice (19). Conventional TRAF2 KO mice are postnatal lethal, which could be rescued after crossing with TNF- α KO mice (86). Interestingly, the TRAF2/TNF- α double KO (DKO) mice have spontaneous autoimmunity, characterized by perturbed T-cell homeostasis, organ infiltration of activated T cells, and autoantibody accumulation (67). Bone marrow adoptive transfer studies suggest a role for T

cells in the induction of autoimmunity in the TRAF2/TNF- α DKO mice, since transfer of bone marrows from the DKO but not DKO/TCR $\beta^{-/-}$ mice to RAG1 $^{-/-}$ mice induces autoimmunity (67). The autoimmunity associated with TRAF2 deficiency could be corrected by ablation of one allele of NIK gene, suggesting the involvement of the noncanonical NF- κ B activation. Since the method used in this study cannot exclude the contribution of non-T cells (although T cells are required), it will be important to examine whether T-cell-specific TRAF2 ablation is sufficient for triggering the spontaneous autoimmunity. It also remains to be investigated whether TRAF2 regulates T-cell homeostasis and autoimmunity via a cell-intrinsic mechanism or acts through mediating the immunosuppressive function of Treg cells.

Regulation of T-cell function by TRAF3

A role for TRAF3 in regulating T-cell functions was initially suggested by a study employing chimeric mice reconstituted with TRAF3-deficient fetal liver cells (87). The recent study of T-cell-conditional TRAF3 KO (TRAF3-TKO) mice further emphasizes a cell-intrinsic role for TRAF3 in regulating TCR signaling and T-cell activation (88). Although the TRAF3-TKO mice have normal development of conventional T cells, these animals display impaired T-cell responses to *Listeria monocytogenes* as well as reduced IgG1 production in response to a T-dependent antigen (88). The TRAF3-deficient T cells also show reduced cytokine production, proliferation, and survival ability in response to *in vitro* stimulation through TCR and CD28. TRAF3 appears to facilitate TCR-proximal signaling events, since the TRAF3 deficiency partially inhibits TCR/CD28-stimulated phosphorylation of several signaling factors, including the tyrosine kinase ZAP70, the adapter protein LAT, and phospholipase C γ 1 (PLC γ 1) (88). Among the downstream signaling events, the activation of ERK is severely attenuated, whereas the activation of IKK appears to be competent in the TRAF3-deficient T cells. Although precisely how TRAF3 regulates TCR signaling is unknown, this function of TRAF3 seems to be independent of the noncanonical NF- κ B pathway. Interestingly, TRAF3 is physically recruited to the TCR/CD28 complex upon *in vitro* T-cell activation (88). Identification of the direct target of TRAF3 in the TCR signaling complex will be crucial for further elucidating the mechanism underlying TRAF3 function in T cells.

Ablation of TRAF3 in T cells (using CD4-Cre) does not appreciably alter the profile of thymocyte populations or the frequency of peripheral CD4 $^{+}$ and CD8 $^{+}$ conventional T cells (88). However, the TRAF3-TKO mice have a substantial reduction in the frequency of invariant natural killer T (iNKT) cells (88, 89). The development of iNKT cells occurs in the thymus from CD4 $^{+}$ CD8 $^{+}$ DP thymocytes with rearranged semi-invariant TCR that recognize glycolipids presented by the MHC-like molecule CD1d (90). Following positive selection, immature iNKT cells mature through several stages (stage 1, 2, 3) defined based on surface expression of CD44 and NK1.1 markers (91, 92). The TRAF3 ablation using CD4-Cre, which occurs from the late stage of double negative thymocytes, causes a block of iNKT maturation from stage 1 to the later stages. TRAF3 appears to mediate IL-15-induced expression of the transcription factor T-bet as well as the proliferation and survival of iNKT cells (89). This function is consistent with the requirement of TRAF3 in the central memory CD8 $^{+}$ T-cell homeostasis, a function that also involves regulation of IL-15 signaling (93).

Regulation of Treg development and function by TRAF3

In contrast to the defective iNKT development, the generation of Foxp3⁺ Treg cells is enhanced in the TRAF3-TKO mice (88, 94). It is generally believed that thymic Treg development involves two major steps. Initially, positively selected thymocytes are instructed by the TCR and CD28 signals to become a population of CD25⁺Foxp3⁻ Treg precursors, which subsequently develop into CD25⁺Foxp3⁺ Treg cells in an IL-2-dependent manner (95, 96). TRAF3 ablation in the T cell compartment does not alter thymocyte selection or Treg precursor generation, but it promotes the conversion of Treg precursors to mature Treg cells (94). TRAF3 appears to inhibit IL-2 receptor (IL-2R) signaling by facilitating the recruitment of a protein tyrosine phosphatase, TCPTP, to the IL-2R complex.

Despite the elevated production of Treg cells, the TRAF3 TKO mice have an increased frequency of CD4⁺ effector/memory T cells (93). These seemingly contradictory phenotypes can be explained by a recent study demonstrating a positive role for TRAF3 in regulating the effector function of Treg cells (97). Treg-specific deletion of TRAF3 in TRAF3^{fl/fl}Foxp3⁻ Cre mice results in accumulation of effector/memory T cells. The attenuated function of the TRAF3-deficient Treg cells may be partially attributed by the activation of noncanonical NF- κ B, since transgenic expression of a stable form of NIK (NIK^{T3}) in Treg cells also leads to perturbed CD4⁺ T-cell homeostasis (97). The effect of overexpressed NIK on Treg function has also been observed in mice overexpressing NIK in total T cells (69). However, TRAF3 clearly has some NIK-independent function in Treg cells. A major phenotype of the TRAF3-deficient Treg cells, which is not seen in NIK^{T3}-expressing Treg cells, is attenuated production of follicular Treg (Tfr) cells, a specific subset of Treg cells that control the formation of germinal centers and antibody responses (97). Consistently, the TRAF3 Treg-KO mice display heightened responses to antigen stimulation in the formation of germinal centers and production of high-affinity antibodies. TRAF3 appears to regulate TFR development by promoting the expression of the costimulatory molecule ICOS on Treg cells, which in turn involves the activation of the ERK MAPK signaling pathway (97).

TRAF2,3 as negative regulators of inflammation

TRAF2 has been extensively studied for its role in mediating signaling from different members of the TNFR superfamily, in which it mediates activation of JNK and NF- κ B (98). TRAF3 plays an important role in mediating CD40-stimulated JNK activation. However, emerging evidence suggests that these TRAF family members are important anti-inflammatory factors in myeloid cells. An initial study suggests that TRAF3 negatively regulates proinflammatory gene induction by the MyD88-dependent TLRs, such as TLR4 (99). Subsequently, *in vivo* anti-inflammatory function of TRAF3 was demonstrated using a central nervous system (CNS) inflammation model, in which TRAF3 negatively regulates the induction of EAE (100, 101). Transgenic expression of TRAF3 ameliorates EAE induction, whereas ablation of TRAF3 in myeloid cells promotes EAE induction in mice.

It has been shown that the TLR4 ligand LPS stimulates TRAF3 degradation in a macrophage cell line (Raw264.7) and primary microglia (99, 100), although this signaling event is weak in bone marrow-derived macrophages (100). Microglia are resident macrophages of the central nervous system (CNS) that play an important role in mediating

innate immunity and inflammation (102). The degradation of TRAF3 also occurs along with EAE induction and contributes to EAE pathogenesis (100). TLR4-stimulated TRAF3 degradation involves K48 ubiquitination of TRAF3 by the ubiquitin ligase cIAP (99). At least in microglia, the E3 ubiquitin ligase Peli1 is also required for TRAF3 degradation. It appears that Peli1 regulates the catalytic activation of cIAP, since the Peli1 deficiency attenuated LPS-stimulated cIAP2 ubiquitination (100). It is currently unclear whether Peli1 also mediates TLR-stimulated TRAF3 degradation in other cell types. A unique feature of microglia is the predominant expression of Peli1 over its homologues, Peli2 and Peli3, whereas many other cell types express at least two of the three Peli members and may have functional redundancy (100). Genetic evidence suggests that Peli1-mediated TRAF3 degradation is crucial for the induction of CNS inflammation.

The anti-inflammatory function of TRAF3 has been further elucidated in two recent studies using myeloid conditional TRAF3 KO (MKO) mice (14, 103). The TRAF3 MKO mice have normal frequency of macrophages and only a slightly higher frequency of neutrophils. Interestingly, the TRAF3-deficient BMDMs are hyper-responsive to stimulation by both LPS and poly(I:C) in the expression of a number of proinflammatory cytokines. These findings suggest that TRAF3 is a negative regulator of proinflammatory cytokine induction in both the MyD88- and TRIF-dependent pathways. Although young TRAF3-MKO mice do not display spontaneous inflammation, they have exacerbated colon inflammation in a colitis model induced by dextran sodium sulfate (DSS) (14). At older ages (15–22 months), about 68% of the TRAF3-MKO mice display spontaneous inflammation in multiple organs (103).

Like the TRAF3-MKO mice, the TRAF2-MKO mice develop exacerbated colon inflammation in the DSS-model of colitis (14). Similarly, the TRAF2-deficient macrophages have greatly enhanced induction of proinflammatory cytokine genes by both LPS and poly(I:C) as well as the IL-1 β (14). A potential role for TRAF2 and TRAF3 in regulating human IBD is indicated by intestinal protein expression profile analyses (104, 105). In addition to its anti-inflammatory role in myeloid cells, TRAF2 may suppress colitis via mediating survival of epithelial cells. Mice with global TRAF2 deficiency (Traf2^{-/-} mice) spontaneously develop colitis, which involves enhanced apoptosis of colonic epithelial cells (106). This latter function is consistent with the function of TRAF2 in mediating the survival signaling of TNFR1, since ablation of TNFR1 prevents epithelial cell apoptosis and ameliorates colitis in the Traf2^{-/-} mice (106). Thus, it seems that TRAF2 restrains colon inflammation by two different mechanisms: inhibition of TLR-stimulated pro-inflammatory cytokine production in myeloid cells and maintenance of colon epithelial integrity by mediating TNFR1 survival signal (Fig. 1).

Degradation of c-Rel and IRF5 by cIAP-TRAF2-TRAF3

The signaling mechanism underlying the anti-inflammatory functions of TRAF2 and TRAF3 is just beginning to be understood. TRAF3 has been implicated in the regulation of MyD88 signaling complex (5, 99). Upon stimulation by LPS, TLR4 recruits MyD88 and its association with a large signaling complex, composed of TRAF6, TAK1, IKK γ , Ubc13, cIAP, and TRAF3. It is thought that within this complex, cIAP becomes activated and mediates ubiquitin-dependent degradation of TRAF3, causing cytoplasmic translocation of

the MyD88-associated signaling complex; this in turn triggers the activation of TAK1 and its downstream MAPKs p38 and JNK (5). However, it is likely that additional mechanisms are involved in the anti-inflammatory function of TRAF3, since two recent studies reveal that TRAF3-deficient macrophages do not show substantially enhanced MAPK activation (14, 103). Similarly, loss of TRAF2 in macrophages does not promote TLR-stimulated activation of MAPKs (14). The ablation of TRAF2 or TRAF3 in macrophages causes NIK accumulation and constitutive activation of noncanonical NF- κ B (14, 103). The TRAF2,3 deficiency also results in moderately increased steady-state phosphorylation of I κ B α and NF- κ B p65, apparently due to NIK accumulation (14). However, the deregulated NIK accumulation only has a minor contribution to the hyper-induction of proinflammatory cytokines in the TRAF3- or TRAF3-deficient macrophages.

Analysis of downstream signaling factors led to the identification of two transcription factors, c-Rel and IRF5, which are regulated by TRAF2 and TRAF3. Under normal conditions, c-Rel and IRF5 undergo ubiquitin-dependent degradation in macrophages, and loss of either TRAF2 or TRAF3 inhibits ubiquitination and degradation of c-Rel and IRF5 (14). The ubiquitin-dependent degradation of c-Rel and IRF5 appears to also involve cIAP, since this proteolytic event can be inhibited by a cIAP antagonist, Smac mimetic. These findings suggest that c-Rel and IRF5 are new substrates of the cIAP-TRAF2-TRAF3 ubiquitin ligase complex. Given the critical role of c-Rel and IRF5 in mediating proinflammatory cytokine induction (107–111), controlling the fate of these transcription factors likely serves as a major mechanism by which TRAF2 and TRAF3 restrain inflammatory responses in myeloid cells (Fig. 2). These findings also raise the question of whether the cIAP-TRAF2-TRAF3 ubiquitin ligase complex has additional substrates.

TRAF2,3 in regulating tumorigenesis

Emerging evidence suggests that TRAF2 and TRAF3 play an important role in the regulation of tumorigenesis; however, the underlying mechanism appears to be complex, since both deficiency and overexpression of these TRAF molecules have been linked to oncogenesis. In line with the role of TRAF2 and TRAF3 in the negative regulation of B-cell survival, loss-of-function mutations of TRAF2, TRAF3, or cIAP1 and cIAP2 are associated with human B-cell malignancies, including multiple myeloma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, splenic marginal zone lymphoma, and other subtypes of B-cell lymphoma (27–33). These findings are corroborated by studies using mouse models. At older ages (9–18 months), a high percentage of the B-cell-conditional TRAF3 KO (BKO) develops B-cell lymphoma (112). Although tumorigenesis has not been reported for TRAF2 BKO mice, transgenic mice expressing a dominant-negative TRAF2 (TRAF2DN) mutant in B cells are predisposed to B-cell malignancies (66). When crossed with the B-cell-specific Bcl-2 transgenic mice, the TRAF2DN transgenic mice develop chronic lymphocytic leukemia and small B-cell lymphoma and CLL-like leukemia with high incidence (>85%). Notably, the B-cell-specific TRAF2DN transgenic mice resemble TRAF2-BKO mice, since B cells from the TRAF2DN transgenic mice express negligible levels of endogenous TRAF2 protein and TRAF2DN, apparently due to TRAF2 degradation in the proteasome (66). As seen with the TRAF2-deficient B cells, the TRAF2DN B cells display constitutive p100 processing and BAFF-independent B-cell survival. These findings suggest that

deregulated noncanonical NF- κ B activation, as a result of disrupted cIAP-TRAF2-TRAF3 E3 complex function, contributes to aberrant B-cell survival and development of B-cell malignancies.

In contrast to the tumor-suppressive role of TRAF3 described above, another study suggests an oncogenic function of TRAF3 (66). Transgenic expression of TRAF3 using the B-cell-specific immunoglobulin H μ -chain enhancer induces the formation of tumors, particularly squamous cell carcinomas of the tongue and salivary gland tumors (66). These transgenic animals display systemic inflammation, characterized by high levels of inflammatory cytokines in the serum and immune cell infiltration into tissues. The inflammatory conditions may contribute to the development of tumors in the TRAF3 transgenic mice. However, since the transgenic approach used in this study involves random transgene insertion, one other possibility is the possible effect of the transgene integration on the expression of endogenous genes involved in tumorigenesis. Nevertheless, an oncogenic role has also been implicated for TRAF2 (113). The TRAF2 gene is recurrently amplified and rearranged in 15% of human epithelial cancers (113). Suppression of TRAF2 expression in these cancer cells inhibits their proliferation and anchorage-independent growth and tumorigenesis. The oncogenic function of TRAF2 appears to be linked to its role in mediating activation of canonical NF- κ B (113). Future studies should examine whether the role of TRAF2 and TRAF3 in regulating tumorigenesis is cell-type specific.

TRAF2,3 in macrophage polarization and anti-tumor immunity

Macrophages are among the major types of immune cells present in tumor microenvironment and known to play an important role in regulating tumor growth and metastasis (114). Based on their cytokine production profile, tumor-associated macrophages (TAMs) can be classified into two polarized subsets: the classically activated (M1) macrophages and alternatively activated (M2) macrophages (115). The M1 macrophages have strong tumoricidal activity and produce inflammatory cytokines, including IL-12, which promote Th1 type of anti-tumor immune T-cell responses and the cytotoxic activity of NK cells (115–117). In contrast, M2 macrophages have low tumoricidal activity and suppress anti-tumor immunity by producing high levels of immunosuppressive cytokines, such as IL-10, thereby promoting tumor growth and progression (118). The M2-like TAMs, which are typically seen in tumor microenvironment, also stimulate tissue remodeling and angiogenesis. However, TAMs are plastic and can be ‘re-educated’ to acquire M1 functions, a potential approach that has been implicated in cancer immunotherapy (119–121). Interestingly, IKK β has been shown to promote macrophage polarization to the immunosuppressive M2 subset (120, 122). IKK β ablation in macrophages causes these cells to switch to the classically activated M1 phenotype, coupled with enhanced anti-tumor and anti-infection immunity (120, 122). The IKK β deficiency elevates the activation of STAT1 in macrophages cocultured with tumor cells or stimulated with a bacterial pathogen, group B streptococcus (120, 122). It is currently unclear how IKK β negatively regulates STAT1 activation.

A recent study suggests a potential role for TRAF2 and TRAF3 in regulating macrophage polarization (14). When stimulated with LPS *in vitro*, both the TRAF2- and TRAF3-

deficient BMDMs display an M1-like phenotype, characterized by hyper-expression of proinflammatory cytokines and the iNOS gene. In a B16 melanoma model, the myeloid cell-specific TRAF2 ablation greatly promotes anti-tumor immunity, as shown by increased tumor infiltration with IFN γ -producing CD4⁺ and CD8⁺ effector T cells and increased tumor-rejection ability in the *Traf2*-MKO mice (14). The TAMs isolated from the *Traf2*-MKO mice resemble M1 macrophages, exhibiting enhanced expression of proinflammatory genes, particularly those encoding IL-12 family of cytokines and iNOS. These findings suggest that TRAF2, and possibly TRAF3, play an important role in regulating macrophage function in tumor environment. Given the crucial role of IRF5 in the regulation of macrophage polarization (108, 111), deregulated expression of this proinflammatory transcription factor may contribute to the M1-like phenotype of the TRAF2- and TRAF3-deficient macrophages.

TRAF3 in antiviral response

TRAF proteins are also linked to type I interferon (IFN-I) induction in antiviral innate immunity (5, 123, 124). In particular, TRAF3 has been shown to have a positive role for mediating IFN-I induction by ligands for different TLRs and RLRs (125–127). TRAF3-deficient MEFs and bone marrow-derived macrophages are severely attenuated in PRR-stimulated IFN-I induction and resistance to infection by the vesicular stomatitis virus (VSV) (125, 126). TRAF3 appears to mediate IFN-I induction by facilitating phosphorylation of the IFN-I transcription factor IRF3. TRAF3 interacts with the signaling adapters of TLRs and RLRs, including MyD88, TRIF, and MAVS, and promote the recruitment IRF3 and its kinases TBK1 and IKK ϵ to the signaling complexes for IRF3 phosphorylation (123).

How TRAF3 regulates IFN-I induction is still elusive and somewhat controversial. An autosomal dominant human TRAF3 deficiency has been found in a young adult with a history of herpes simplex virus-1 encephalitis (128). Epithelial cells derived from this patient have no detectable TRAF3 protein expression and are defective in TLR-stimulated expression of IFN-I as well as the proinflammatory cytokines IL-6 and IL-12 (128). Interestingly, in these human cells, the loss of TRAF3 severely attenuated poly(I:C)-stimulated RelA nuclear expression but only partially inhibited the dimerization of IRF3. Whether this reflects a difference between human and mouse cells remains to be further examined. The role of TRAF3 in regulating IFN-I induction may also vary in different cell types and PRR pathways. Recent studies suggest that TRAF3 is not essential for MAVS signaling, since the TRAF3-deficient MEFs are largely competent in IRF3 activation by RNA viruses (124, 129). It appears that several TRAF members, TRAF2, TRAF5, and TRAF6, function redundantly in the regulation of IRF3 activation by the MAVS pathway (129).

The role of TRAF3 in regulating IFN-I is further complicated by the recent finding that noncanonical NF- κ B pathway negatively regulates IFN-I induction by TLR ligands, such as LPS and poly(I:C), and the RNA viruses VSV and SeV (130). The noncanonical NF- κ B is activated along with infection by several RNA viruses, including respiratory syncytial virus, human immunodeficiency virus, vesicular stomatitis virus, and Sendai virus (130–134), and

both transfected poly(I:C) and RNA viruses induce the degradation of TRAF3 (and TRAF2) and NIK accumulation (130). The noncanonical NF- κ B pathway is also activated along with macrophage differentiation by macrophage colony-stimulating factor macrophage growth factor (M-CSF), a developmental program that regulates the responses of macrophages to IFN-I induction (130). Genetic ablation of either NIK or noncanonical NF- κ B members greatly potentiates the induction of IFN-I. Conversely, macrophages expressing a stable form of NIK (NIK4T3) suppress IFN-I induction. The noncanonical NF- κ B seems to compete with the canonical NF- κ B RelA for binding to the IFN β enhancer and inhibits the histone modification and transcription activation of the IFN β gene. These studies suggest that the reduced IFN-I induction in TRAF3-deficient cells may involve both an adapter function of TRAF3 and accumulation of the noncanonical NF- κ B members (Fig. 3).

Perspectives: conclusions and outstanding questions

Although TRAF proteins are best known as signaling adapters, accumulating evidence has now established TRAF2 and TRAF3 as major components of an E3 ubiquitin ligase complex, cIAP-TRAF2-TRAF3, which regulates signal transduction by targeting intracellular signaling factors for degradation. These findings suggest the intriguing possibility that cIAP-TRAF2-TRAF3 may represent a novel multi-component E3 ubiquitin ligase that targets both NIK and additional signaling components, thereby regulating diverse biological functions, ranging from B-cell survival to inflammatory TLR signaling. These findings shed new light onto the mechanism of TRAF function and provide valuable information for designing therapeutic approaches in the treatment of inflammation and cancer. However, a number of outstanding questions need to be addressed in future studies. For example, it remains to be examined whether the cIAP-TRAF2-TRAF3 E3 complex targets additional cellular substrates and regulates additional physiological or pathophysiological processes. Another question is regarding the cell-specific function of TRAF members. It is intriguing that TRAF2 functions as an anti-inflammatory factor in myeloid cells, despite its role in mediating TNFR signaling. It is important to examine whether the TRAF2 deficiency promotes inflammatory TLR signaling in additional cell types. The mechanism mediating the cell-type specific function of TRAF3 is also enigmatic and warrants further investigation. The functions of TRAF3 in regulating T-cell activation and IFN-I production are particularly complex. It is important to determine whether these different functions are dependent on or independent of cIAP. Finally, the biochemical mechanism underlying the E3 ligase function of the cIAP-TRAF2-TRAF3 is far from clear and needs to be further examined.

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Fig. 1. Regulation of colitis by TRAF2

TRAF2 controls inflammation in the intestine with two different mechanisms. The first is to negatively regulate TLR-stimulated proinflammatory cytokine production by macrophages, thus preventing aberrant inflammatory responses from innate immune cells. The second mechanism is to suppress TNF α -induced apoptosis of intestinal epithelial cells (IECs), which involves activation of canonical NF- κ B. Since IEC destruction causes bacterial invasion, impaired IEC survival mechanism often causes unresolved inflammation.

Fig. 2. Regulation of proinflammatory TLR signaling by the cIAP-TRAF2-TRAF3 E3 ubiquitin ligase complex

TLR4 stimulation by LPS stimulates cascades of signaling events, leading to activation of several transcription factors, including AP1, IRF5, and the NF- κ B members RelA and c-Rel. The steady-state level of both c-Rel and IRF5 is controlled via ubiquitin-dependent degradation by the cIAP-TRAF2-TRAF3 E3 ubiquitin ligase. Thus, deficiencies in the components of this E3 complex promotes the induction of proinflammatory cytokines due to accumulation of c-Rel and IRF5.

Fig. 3. Negative regulation of IFN β induction by noncanonical NF- κ B

(A) RNA viruses, such as VSV and Sendai virus, and transfected poly(I:C) stimulate TRAF3 degradation, likely via RIG-I and MAVS, thereby causing activation of noncanonical NF- κ B. RIG-I and MAVS also activate the canonical NF- κ B and the IRF3 pathways. Noncanonical NF- κ B may negatively regulate the induction of *Ifnb* gene expression by competing with RelA for binding to the *Ifnb* promoter and inhibiting transcriptionally active histone modification. (B) Noncanonical NF- κ B pathway is also activated by M-CSF along with macrophage development, which negatively regulates *Ifnb* gene induction by TLR4.