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Roles of tumor necrosis factor receptor associated factor 3 (TRAF3) and TRAF5 in immune cell functions

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Summary

A large and diverse group of receptors utilizes the family of cytoplasmic signaling proteins known as tumor necrosis factor receptor (TNFR)-associated factors (TRAFs). In recent years, there has been a resurgence of interest and exploration of the roles played by TRAF3 and TRAF5 in cellular regulation, particularly in cells of the immune system, the cell types of focus in this review. This work has revealed that TRAF3 and TRAF5 can play diverse roles for different receptors even in the same cell type, as well as distinct roles in different cell types. Evidence indicates that TRAF3 and TRAF5 play important roles beyond the TNFR-superfamily (SF) and viral mimics of its members, mediating certain innate immune receptor and cytokine receptor signals, and most recently, signals delivered by the T-cell receptor (TCR) signaling complex. Additionally, much research has demonstrated the importance of TRAF3-mediated cellular regulation via its cytoplasmic interactions with additional signaling proteins. In particular, we discuss below evidence for the participation by TRAF3 in a number of the regulatory post-translational modifications involving ubiquitin that are important in various signaling pathways.

Keywords

signal transduction; lymphocyte activation; tumor necrosis factor receptor; tumor necrosis factor receptor associated factor

Introduction

Tumor necrosis factor receptor (TNFR)-associated factor 3 (TRAF3) was one of the first identified TRAFs, initially isolated by virtue of its binding to the cytoplasmic domain of TNFR-superfamily (TNFR-SF) member CD40 (1, 2). In the following year, identification of TRAF5 was reported (3, 4). Interestingly, however, investigation and understanding of these two TRAFs lagged considerably behind that of TRAF2 and TRAF6 for the subsequent decade. A major factor in this may have been the most popular model approach used in investigations of TRAF signaling, the exogenous overexpression of transfected, epitope-tagged TRAFs with various combinations of transfected reporter genes, receptors, and other

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signaling molecules in non-immune transformed cell lines, such as HEK293. In this system, overexpressed TRAF2 or TRAF6 robustly activate the easily detectable canonical nuclear factor κ B (NF- κ B) and c-Jun kinase (JNK) pathways (5, 6), while TRAF3 does not. The overlapping nature of the binding sites on many TRAF3 and TRAF5-binding receptors (7) also complicates interpretation of the results of experiments using overexpression of wildtype (WT) or mutant TRAFs, as well as approaches that mutate this overlapping binding motif. The varying quality of available antibodies to endogenous TRAF molecules adds to the technical challenges of studying endogenous TRAFs in primary cells, where detection is much more difficult than more artificial systems. Finally, TRAF3 'knockout' mice have a lethal phenotype in the neonatal period (8), complicating use of this *in vivo* model to clearly delineate the roles of TRAF3 in the functions of specific cell types. While TRAF5-deficient mice are viable, the initial report of their somewhat modest phenotype may have contributed to a relatively lower number of studies specifically focusing upon this TRAF (9).

The summation of this work from many laboratories emphasizes the critical importance of studying and validating TRAF functions through careful investigation of models measuring endogenous signaling in normal primary cell types. Such research has shown that, while exogenous overexpression experiments may provide initial helpful hints, their results cannot be safely extrapolated without verification.

Investigation of TRAF5 has also moved beyond *in vitro* overexpression studies in more recent years, and TRAF5 has been shown to contribute to important signals in cells of the immune system, generated by a greater number and variety of receptors than initially appreciated (10–14). This work is discussed in detail below; building upon these findings should lead to a much-enhanced understanding of the biological roles played by TRAF5 in the mammalian immune response.

TRAF-TRAF interactions provide another regulatory mechanism allowing diversity of function of the TRAF family of proteins. In this regard, TRAF3 and TRAF5 can associate *in vitro* (7, 15). It was recently shown that TRAF5 plays a critical role in *in vitro* signaling and *in vivo* function in primary B cells by the Epstein-Barr virus (EBV) encoded mimic of CD40, latent membrane protein 1 (LMP1) (13), and that TRAF5 requires TRAF3 to associate with endogenous LMP1 in B cells (16). Additional roles for TRAF3/5 association are likely to be forthcoming.

Roles of TRAF3 in B lymphocytes

B-cell survival and homeostasis

Understanding the physiologic roles played by TRAF3 in B cells is especially complex, because in addition to its contributions to B-cell signaling by various receptors, TRAF3 plays an important role in negative regulation of homeostasis of B cells from the transitional through mature stages. TRAF3-deficient mouse B cells display prolonged and BAFF-independent survival (17, 18). B cells isolated from both reported strains of B-cell-specific TRAF3^{-/-} mice have increased nuclear levels of the non-canonical NF- κ B2 subunits p52 and RelB (17, 18). TRAF3^{-/-} B cells also show decreased nuclear protein kinase C δ (PKC δ) (17). Both these pathways have been associated with BAFF-induced survival signals in B cells (19, 20). In the absence of TRAF3, B cells become independent of BAFF-mediated survival (17). This finding suggests that TRAF3 does not serve as a negative regulator of BAFF receptor (BAFF-R) signaling *per se* but rather that BAFF signaling overcomes a receptor-independent role for TRAF3 that restrains B-cell survival. The precise mechanism of this role in B cells has been challenging to elucidate, in part because many of the experiments addressing the function of TRAF3 in cell survival have been performed in other cell types, most frequently mouse embryonic fibroblasts (MEFs) and the transformed

kidney epithelial cell line 293T. This is not a trivial distinction, because it is now clear that TRAFs, in particular TRAF3, can be highly context-dependent in their function, playing distinct functional roles not only for different receptors (21) but also in different cell types (22). Also, while TRAF3^{-/-} MEFs, T cells, and dendritic cells (DCs) also show elevated basal NF- κ B2 activation, unlike in B cells, TRAF3 deficiency does not result in enhanced survival in these other cell types (23, 24). Thus, the link between TRAF3, NF- κ B2, and elevated survival needs to be unraveled in B cells themselves to gain an accurate understanding of how TRAF3 uniquely promotes extended survival in this cell type. This role for TRAF3 may be relevant to B-cell malignancies. Several different mutations in TRAF3 are associated with human multiple myeloma (25, 26) and Waldenström's macroglobulinemia (27). As elevated NF- κ B activation is associated with a variety of different types of cancer (28), it has been assumed that the elevated NF- κ B2 activity associated with decreased TRAF3 is responsible for enhanced B-cell survival that increases propensity for malignant transformation.

It has been posited that the NF- κ B-inducing kinase (NIK) (29) is key to the effect of TRAF3 deficiency on B-cell survival. A role for TRAF3 in restraint of NIK function was suggested by the finding that the early lethality of mice lacking TRAF3 in all cells (8) can be rescued by a concomitant deficiency in NIK (23). The survival of these mice can also be rescued by a deficiency in the p100 protein that is processed to p52 in the NF- κ B2 pathway (30). However, studying the interaction between TRAF3 and NIK in B cells has been challenging, because B cells typically express barely to undetectable levels of NIK. It has been suggested that this is because TRAF3 association promotes NIK degradation, which is countered by CD40 and BAFF-R-induced TRAF3 degradation (23). When released from TRAF3, NIK is then proposed to induce NF- κ B2 activation, which enhances B-cell survival (reviewed in 31). To date, much of the evidence supporting this model comes from experiments performed *in vitro* in non-B cells. However, B cells from a mouse expressing a mutant form of NIK lacking the TRAF3-binding domain show detectable levels of this mutant NIK protein (32), consistent with the model. Interestingly, B cells from two different strains of mice completely lacking detectable amounts of TRAF3 still do not have detectable levels of NIK (17, 18). It is also curious that deficiency in NIK does not preclude the presence of B cells in NIK^{-/-} mice, and these B cells activate NF- κ B in response to a CD40 signal, although MEF responses to lymphotoxin β receptor (LT β R) are markedly defective (33). In contrast to the variety of human TRAF3 mutations in B-cell malignancies discussed above, no human mutations in NIK, including mutations specific to tumors, have been reported.

Strong evidence thus supports an important role for TRAF3 in restraining normal B-cell survival, a role that appears to be unique to B cells. While this role in B cells correlates well with the constitutive presence of nuclear p52 and RelB, in other cell types the same constitutive NF- κ B2 activation does not enhance cell survival, indicating that other B-cell-specific mechanisms contribute to this role for TRAF3. Finally, there is evidence that TRAF3 mediates its B-cell-specific inhibition of survival in part via regulation of NIK levels, but the relationship between B cell TRAF3 and NIK is complex, and it seems likely that other factors and mechanisms also contribute to this TRAF3 function. Further studies of the TRAF3-NIK connection conducted in both mouse and human B cells would be valuable in enhancing understanding of this important pathway.

TRAF3 in B-cell signaling by members of the TNFR-SF and the CD40 mimic LMP1

TRAF3 was discovered via its association with the important immune activator and TNFR-SF receptor CD40, and its initial designation was 'CD40-binding protein' (1). Understanding the specific roles of TRAF3 in CD40 signaling has proven a complex undertaking. The early lethality of globally TRAF3-deficient mice and their multiple developmental abnormalities (8) precluded use of this strain to clearly address the specific

role of TRAF3 in B-cell CD40 signaling. Additionally, the canonical TRAF binding site PXQXT in the cytoplasmic domain of CD40 is important for its association with TRAF1, TRAF2, TRAF3, and TRAF5 (7), not just TRAF3. Thus, mutations in this site can affect the binding of any or all of these TRAFs, and overexpression of WT or mutant versions of any of these TRAFs can indirectly affect the functions of any of the others, by changing the balance of distinct TRAF molecules in the signaling complex. This considerably complicated interpretation of data in initial reports exploring how TRAF3 impacts CD40 signaling. Mutation in the PXQXT motif reduces CD40 signals to B cells, both *in vitro* (34, 35) and in CD40^{-/-} mice expressing CD40 mutant transgenes (36–38). However, assignment of these functions to a specific type of TRAF binding to this motif was not possible.

An early study using a ‘dominant negative’ TRAF3 mutant [lacking the really interesting new gene (RING) domain] concluded that TRAF3 is a necessary positive mediator of CD40 signals, because the presence of this mutant leads to decreased CD40 signaling in a B-cell line (39). However, transfection of B-cell lines with full-length WT TRAF3 also inhibits CD40 signaling (40), re-casting TRAF3 as a negative regulator of CD40 B-cell signals. TRAF3 appears to fulfill its inhibitory role in part by competing with TRAF2 for binding to CD40 (41–43), which is consistent with the ability of non-functional mutant TRAF3 molecules to inhibit CD40-mediated B-cell activation and the dominant effect of reduced TRAF2 binding on the functional defects observed with CD40 molecules with mutations in the overlapping TRAF1/2/3/5 binding site.

CD40 signals to B cells recruit TRAF2 and TRAF3 to membrane lipid rafts and are associated with the K48-mediated degradation of both these TRAFs (44–46), likely via TRAF2 recruitment of the E3 ubiquitin ligases cIAP1 and cIAP2 (47). While degradation of TRAF2 inhibits CD40 signals to B cells (46, 48, 49), loss of TRAF3 enhances CD40 signaling, as discussed above. In addition to competition with TRAF2 for CD40 association, TRAF3 negatively regulates the non-canonical NF-κB2 pathway, both basally and that induced by CD40 (17, 18, 50).

TRAF3 in contrast plays an important positive role in signaling to B cells by the EBV-encoded CD40 mimic LMP1. Because LMP1 is a potent mimic of CD40 in B cells and like CD40, LMP1 binds TRAF1, TRAF2, TRAF3, TRAF5, and TRAF6 (reviewed in 21), it was initially assumed that CD40 and LMP1 employ these TRAFs for the same purposes. This assumption was challenged when a method was developed to produce cell lines completely deficient in specific gene products, using gene targeting via homologous recombination. This approach allowed the production of B-cell lines lacking only TRAF2 (51) or TRAF3 (52). TRAF-deficient B cells revealed that while TRAF2 is an important positive mediator of CD40 signals to B cells (51), in contrast, B-cell activation by the cytoplasmic tail of LMP1 is TRAF2 independent but TRAF3 dependent (52). A B-cell-specific TRAF3^{-/-} mouse model also confirmed the previous inhibitory role of TRAF3 in CD40-induced NF-κB2 signals, which are elevated in its absence (52) but show no effect of TRAF3’s absence on NF-κB1 and mitogen-activated protein kinase (MAPK) signaling pathways (17). The sharp difference between how CD40 and its viral mimic use TRAF3 may be related to binding differences. As mentioned above, the cytoplasmic domain of CD40 associates with TRAF3 via the ‘canonical’ TRAF-binding motif PVQETL. The cytoplasmic tail of LMP1 instead binds TRAF3 via a ‘non-canonical’ motif, PQQATD (53). When hybrid molecules of CD40 and LMP1 which swapped these motifs were analyzed, it was found that these binding site differences account for the tighter binding of TRAF2 to CD40 but do not explain the tighter association of TRAF3 with LMP1 (54). The latter appears to be also impacted by additional contacts in the LMP1 binding pocket for TRAF3, possibly by cooperative interactions between the two C-terminal activating regions in LMP1 (55, 56).

A major mechanism by which TRAF3 acts as an important activator of LMP1 signaling may be to recruit TRAF5 to the LMP1 signaling complex. TRAF3 binds CD40 independently of TRAF5, and CD40 signals to B cells are only modestly impacted in TRAF5^{-/-} mice (9). In contrast, both *in vitro* and *in vivo* functions of LMP1 are markedly diminished in the absence of TRAF5 (13), and it was shown recently that TRAF3 is necessary to recruit TRAF5 to LMP1 in B cells (16). Further studies will be needed to determine whether there are important TRAF5-independent roles for TRAF3 in LMP1 functions, and if so, what these roles entail.

An interesting human polymorphism in the CD40 cytoplasmic domain (P227A) was recently described that results in enhanced antibody and proinflammatory cytokine production, preceded by elevated phosphorylation of the transcription factor c-Jun (57). Of particular biological interest, hCD40P227A is found in nearly 30% of persons of native Mexican and South/Central American ancestry but at < 1% frequency in all other populations (57). This observation correlates with the reported tendency to enhanced severity of certain autoimmune diseases in native Hispanic populations (58). Although the single amino acid change in hCD40P227A lies outside all known TRAF binding sites on CD40, hCD40P227A shows markedly enhanced TRAF3 binding (59). Most striking is the observation that hCD40P227A uses TRAF3 as a positive mediator of activation signals, as does LMP1 (52), in sharp contrast to CD40, as discussed above.

TRAF3 in BAFF receptor (BAFF-R) signaling

Initial exogenous overexpression of both BAFF-R and various TRAFs in the transformed epithelial cell line 293 indicated that BAFF-R can bind TRAF3 but no other TRAFs (60). This assumption influenced interpretation of the crystal structure of the BAFF-R binding crevice for TRAF3, which was cited as demonstrating why BAFF-R binds only to this TRAF (61). However, it was recently shown that endogenous BAFF-R in normal B cells associates not only with TRAF3 but also TRAF2 and TRAF6 and requires TRAF6 to transmit activating signals (62). These findings illustrate the importance of examining TRAF associations with receptors and other signaling proteins in physiologically relevant settings, prior to drawing conclusions. The functional significance of the TRAF3-BAFF-R association must now be reconsidered in light of the ability of BAFF-R in B cells to associate with additional TRAFs, particularly the requirement for TRAF6 in BAFF-R signaling. Previous studies found that exogenously overexpressed TRAF3 inhibits the activity of an NF- κ B reporter gene in unstimulated cells of the Bjab B-cell line and IL-10 production by the RPMI 8226 cell line, implying that TRAF3 negatively regulates BAFF-R signaling (60). However, the potential role(s) of TRAF3 in BAFF-R function have since been revealed as more complex. The BAFF-R TRAF-binding motif PVPAT preferentially induces noncanonical NF- κ B2 activation compared to the PVQET motif of CD40 and LT β R (63), consistent with the more robust activation of the canonical NF- κ B1 pathway in B cells by CD40 (64). Interestingly, mutation of the BAFF-R motif to PVQET increases BAFF-R-mediated NF- κ B1 activation but also increases association with TRAF3 (63), complicating the earlier view of TRAF3 as strictly a negative regulator of BAFF-R signaling. A human BAFF-R mutation associated with B-cell lymphoma and displaying a gain-of-function phenotype also shows enhanced association with TRAF3, as well as TRAF2 and TRAF6 (62). In B cells deficient in TRAF3, rather than enhanced responsiveness to BAFF-R, a complete independence from BAFF-R signaling is seen for B-cell survival and NF- κ B2 activation, which is constitutively enhanced (17). As discussed above, this effect has been attributed to constitutive activity of NIK, which in the absence of TRAF3 avoids the K48 E3 ubiquitin ligase activity of cIAP1/2 (32). Evidence for and complexities associated with this model were covered above. Thus, there may yet be additional pathways and complexities to be discovered in the role of TRAF3 in BAFF-R signaling.

B-cell signaling by innate immune receptors

For most of the years since its discovery, TRAF3 has been considered primarily if not exclusively as a signaling protein of the TNFR-SF, as were all members of the TRAF family. Over a dozen years ago, it was reported that TRAF6 is also an important participant in signaling by the innate immune Toll-like receptors (TLRs) (65). However, for another ~8 years, this function was considered to be unique to TRAF6. It is now clear that TRAF3 makes important contributions to signaling by innate immune receptors stimulated by viral nucleic acids and bacterial lipopolysaccharide (LPS) in cells of the myeloid lineage (66–69); this role will be discussed in detail below. Detailed explanations of how TRAF3 plays this role have relied heavily on *in vitro* experiments performed in fibroblasts and/or by overexpression studies in transformed fibroblast or epithelial cell lines. As the theme of this review emphasizes, extrapolation about TRAF3 functions from one cell type to another is not usually wise. This is true even for the same receptors when expressed by different cell types. Innate immune receptors are critical for myeloid cell function and regulation and are thus most intensively studied in this cell type. However, B cells also express most innate receptors, and are highly responsive to their signals (70), which can interact with signals from adaptive immune receptors such as the B-cell antigen receptor (BCR) (71–73) and CD40 (74–76). Thus, an important knowledge gap is whether TRAF3 plays similar or distinct roles in regulating innate immune receptors of B cells, compared to myeloid cells.

An unusual mouse strain expressing a transgene encoding human TRAF3 behind an immunoglobulin (Ig) gene promoter and enhancer was recently described (77). The human TRAF3 transgene in this strain is markedly overexpressed compared to endogenous TRAF3, in B lymphocytes and a population of T lymphocytes. As TRAF3 appears to play sharply contrasting roles in these two cell types (17, 24), this is a complicating factor in interpretation of the complex phenotype of this interesting strain. Pertinent to the role of TRAF3 in B-cell TLR responses, the human TRAF3 transgenic mice show elevated production of both IgG1 and IgG2b in response to ligands for TLR4 and TLR9 (77). However, explanation of the phenotype of the highly overexpressing TRAF3-transgenic mice awaits detailed information on how overexpressed human TRAF3 impacts various TRAF3 binding proteins in both the cytoplasm and at the membrane of B and T cells, and how different signaling pathways are subsequently affected. Recent studies on the TLR responses of mature B cells from TRAF3-deficient mice indicate that these B cells have enhanced cytokine production and Ig isotype switching in response to TLR3, TLR4, TLR7, and TLR9, associated with increased activation of the canonical NF- κ B1 pathway (78). The latter and early cytokine responses were seen well before any detectable survival advantage conferred by TRAF3 depletion. This finding suggests that endogenous levels of TRAF3 normally restrain TLR-mediated B-cell activation, and that in contrast to the restraint of B-cell homeostasis influenced by the noncanonical NF- κ B2 pathway, the effect on TLR responses involves the rapid NF- κ B1 response.

Roles of TRAF3 in myeloid cells

Because of the early post-natal mortality of TRAF3^{-/-} mice (8), most of our current understanding about the roles played by TRAF3 in myeloid cells is from *in vitro* studies. These studies have revealed a critical role for TRAF3 in TLR signaling pathway in myeloid cells. By employing *in vitro* cultured myeloid cells generated from irradiated C57BL/6 mice reconstituted with TRAF3^{-/-} bone marrow or fetal liver cells, it has been shown that TRAF3 is required for TLR-mediated type I interferon (IFN) production by myeloid cells (66, 67). Furthermore, TRAF3 deficiency results in defective type I IFN production in response to *in vitro* vesicular stomatitis virus (VSV) infection by both plasmacytoid dendritic cells (pDCs) and murine embryonic fibroblasts (MEFs) (67). While type I IFN production in response to VSV infection in pDCs is thought to be dependent on the TLR7

signaling pathway (79), cytoplasmic receptors such as RIG-I and PKR are responsible for type I IFN production in response to VSV infection in MEFs (80–82). Thus, TRAF3 plays roles in both TLR-dependent and TLR-independent signaling pathways involved in type I IFN production. Interestingly, TRAF3 deficiency has also resulted in enhanced IL-12 and IL-6 production by myeloid cells in response to TLR4 and TLR9 stimulation (66, 67), and the enhanced proinflammatory cytokine production by TRAF3^{-/-} bone marrow derived macrophages (BMDMs) is thought to be due to defective IL-10 production by these cells (66).

How may TRAF3 regulate TLR-mediated type I IFN production in myeloid cells? It has been shown that TRAF3 is recruited to TLR adapters, MyD88 and TRIF, in the RAW 264.7 macrophage cell line (66) and associates with IRF3/7 kinases, TBK-1 and IKK- ϵ , and IRAK1 when these proteins are over expressed in HEK-293T transformed epithelial cells (67)(Table 2). Thus, during TLR signaling, TRAF3 may serve as a cytoplasmic adapter and transmit upstream signals to downstream kinases involved in type I IFN production. However, this possibility has not been verified in DCs.

The molecular basis for the differential regulation of type I IFNs versus proinflammatory cytokines by TRAF3 has been investigated in murine macrophages (83) and is thought to be due to differences in ubiquitination status of TRAF3 (Fig. 1). Thus, K63-linked ubiquitination of TRAF3 following TRIF-dependent signaling may result in activation of IRF3/7 kinases and ultimately in type I IFN production. In contrast, cIAP-1 and cIAP-2 dependent degradative ubiquitination of TRAF3 following MyD88 dependent signaling leads to proinflammatory cytokine production.

A new mouse model to study TRAF3 functions in DCs

As mentioned above, most of our current understanding about the roles played by TRAF3 in DC function is from studies conducted using *in vitro* cultured cells. However, DCs are a heterogeneous population of cells consisting of various subsets that differ in their anatomic location, phenotype, and function (84). DCs utilize a wide variety of innate immune receptors, especially those belonging to the TLR and RIG-like receptor (RLR) families (RIG-I and MDA5) to detect molecular patterns unique to or enriched in microbial pathogens (85–87). Upon recognition of microbial ligands in tissue microenvironments, DCs undergo maturation, a process characterized by upregulation of costimulatory molecules such as CD40, CD70, CD80, and CD86 and production of various cytokines and chemokines, and migrate to the draining lymph node where they activate antigen specific T cells. Depending on the type of DC and its activation state, interaction between DCs and T cells can either lead to development of an immune response necessary to eradicate the invading pathogen or can result in T-cell anergy (88, 89). Considering the cell type and receptor specific roles played by TRAF3, it is important to study the roles played by TRAF3 in different DC subsets.

To address the roles played by TRAF3 in DC function, we created a DC-specific TRAF3^{-/-} mouse (DC-TRAF3^{-/-}) by crossing the TRAF3^{lox/lox} mouse (17) with a CD11c-Cre transgenic mouse (90). DC-TRAF3^{-/-} mice survive and reproduce normally. Consistent with previously published results (66, 67), bone marrow-derived dendritic cells (BMDCs) from TRAF3^{-/-} mice produced higher amounts of IL-12 and reduced amounts of IL-10 in response to TLR4, TLR7, and TLR 9 stimulation (Poovassery JS and Bishop GA, unpublished data). pDCs purified from the spleen of DC-TRAF3^{-/-} mice produced very little or no IFN- α in response to TLR7 and TLR 9 ligands. Although MAPK activation was not different between DCs from DC-TRAF3^{-/-} and littermate controls, I κ B α phosphorylation was enhanced in TRAF3^{-/-} DCs in response to TLR stimulation. Consistent with the proposed negative regulatory role of TRAF3 in the non-canonical NF-

κ B2 pathway (23, 30), TRAF3^{-/-} DCs also exhibited constitutive processing of p100 to p52 in the nucleus. However, unlike TRAF3^{-/-} B cells, this did not result in enhanced DC survival. Although, pDCs are the major source of type I IFN *in vivo* during viral infections (91), conventional DCs (cDCs) can also produce type I IFN. However, in contrast to pDCs, type I IFN production in response to viral infection in cDCs depends on the RLR pathway (86). While TRAF3 has been shown to play critical roles in RLR dependent type I IFN production in MEFs (67, 82), its role in cDCs has not been explored. Additionally, how TRAF3 may regulate the functions of different DC subsets *in vivo* during an infection is also not clear. These important aspects are currently under investigation in our laboratory.

TRAF3 in human DC function

Only limited information is available about the roles played by TRAF3 in immune responses against microbial pathogens in humans. Recently, a defect in TLR3 signaling, attributed to a loss-of-function, dominant-negative mutation in TRAF3, has been shown to contribute to herpes simplex virus-induced encephalitis in a human patient (69). As in the case of mouse BMDCs, monocyte derived DCs from this patient exhibit defective type I IFN in response to TLR4 and TLR7 stimulation. Interestingly, type I IFN production by PBMCs from this patient is similar to controls in response to a number of viruses tested. In addition to myeloid cells, B cells are also known to express almost all known TLRs and respond robustly to various TLR ligands. We have shown recently that, in contrast to DCs, type I IFN production is actually enhanced in B cells in the absence of TRAF3 in response to TLR4, TLR7, and TLR9 stimulation (78). Thus, it is quite possible that B cells from this patient may be responding differently to TLR stimulation. However, it is also possible that different myeloid cell subsets may be responding differently to viral infections *in vivo*, further confirming the need to study how TRAF3 regulates the functions of various myeloid cell subsets *in vivo*.

Roles of TRAF3 in T lymphocytes

Although the role of TRAF3 in antigen-presenting cells (APCs) has been extensively investigated and increasingly refined, until recently it was unclear whether TRAF3 plays important role(s) during T-cell-mediated immune responses. Our recent work strongly supports earlier suggestions that TRAF3 is a critical regulatory factor in T-cell functioning, signaling and the development of T-cell subsets.

TRAF3 and T-cell function

Because of the early lethality of TRAF3 knockout mice, Xu *et al.* (8) reconstituted the mouse immune system by transfer of TRAF3-deficient liver stem cells into irradiated mice and found that T-cell-dependent (TD) immune responses are impaired in TRAF3-deficient chimeric mice, but T-cell-independent immune responses are normal. This result suggests that T cells deficient in TRAF3 might be dysfunctional. Additional experiments indicated that although TRAF3^{-/-} lymph node T cells proliferate normally in response to anti-CD3 antibody, antigen-primed TRAF3^{-/-} T cells are defective in their proliferative response to recall antigen *in vitro*. However, this study cannot exclude the effect of APCs during T-cell priming *in vivo*, because some of the APCs in this model also lack TRAF3. Therefore, generation of a T-cell-specific TRAF3-deficient mouse would be a useful tool to specifically explore the role of TRAF3 in T-cell immunology. In 2008, Gardam *et al.* (18) reported that T-cell-specific TRAF3-deficient mice exhibit normal T-cell numbers. Although the alternative NF- κ B2 pathway is constitutively activated in the absence of TRAF3 in these T cells, TRAF3^{-/-} T cells do not show enhanced survival compared to littermate controls (17, 18). Importantly, these results indicate that although TRAF3 suppresses the alternative NF- κ B2 pathway in multiple cell types, suppression of cellular survival pathways only affects B

cells. Interestingly, B TRAF3^{-/-} mice have enhanced responses to TD antigens (17), suggesting that the defective response seen by Xu *et al.* (8) might be due to the absence of TRAF3 in T cells, APCs, or both.

The studies mentioned above implicate TRAF3 in the TD immune response, but further experiments were needed to clarify the exact role of TRAF3 during T-cell-mediated immune responses and how TRAF3 affects T-cell function. To address these issues, we generated T-cell-specific TRAF3-deficient mice by crossing CD4-Cre mice with TRAF3^{flox/flox} mice, so that TRAF3 is depleted from both CD4⁺ and CD8⁺ T cells (24). Consistent with studies reported in (18), we showed that deficiency of TRAF3 in T cells does not affect the number and ratio of T-cell subsets and the survival of T cells *in vitro*, although the alternative NF- κ B2 pathway is activated constitutively (24). However, we also found that TRAF3-deficient mice exhibit a twofold increase of CD4⁺CD25⁺Foxp3⁺ regulatory T cells (Tregs). We also extended the previous finding that TRAF3 is required for TD antibody responses by showing that TRAF3 in T cells is particularly critical for the production of IgG1 (24). We further delineated the immune response of CD4⁺ and CD8⁺ T cells. Surprisingly, antigen-specific CD8⁺ and CD4⁺ T-cell responses to infection with the intracellular pathogen *Listeria monocytogenes* are strikingly impaired. Collectively, our data demonstrate that TRAF3 is required for optimal T-cell function *in vivo*.

We initially hypothesized that this impairment might be the combined result of an increase in Tregs and reduced activation by costimulatory receptors of the TNFR-SF that bind TRAF3 (see below). However, *in vitro* stimulation of purified Treg-depleted T cells with anti-CD3⁺anti-CD28 antibodies stimulates less proliferation but more apoptosis, and cytokine production is markedly decreased (24). These findings indicated that TRAF3 impacts signaling by the TCR+CD28 complex.

TRAF3 in early T-cell signaling

Subsequent to our results described above, we found that TCR/CD28-mediated early signaling events, including phosphorylation of the early TCR signaling molecules ZAP70, LAT, PLC- γ , and ERK, are impaired by TRAF3 deficiency (24). Additionally, TRAF3 is recruited to the TCR and CD28 signaling complex upon costimulation with anti-CD3 and anti-CD28 antibodies but not with either antibody alone. These data strongly support the conclusion that recruitment of TRAF3 to the TCR complex upon stimulation is CD28 dependent. Future work will focus on whether and in what manner TRAF3 directly binds CD28, and the molecular mechanisms by which TRAF3 affects TCR signaling.

TNFR-SF members and T-cell TRAF3

T cells express most TNFR-SF receptors either constitutively or inducibly, and TRAF3 directly binds to almost all the receptors of the TNFR-SF that do not contain death domains. Exogenous overexpression of TRAF3 in some cell lines can inhibit NF- κ B activation induced by OX40 (CD134), CD27, 4-1BB (CD137), CD30, GITR, CD40, and HVEM (15, 92–95) (Table 1). In 293T transformed epithelial cells exogenously over-expressing each of these receptors and TRAFs, Hauer *et al.* (15) showed that NF- κ B2 p100 processing is downregulated by co-expression of TRAF3. In contrast to the conclusions of these *in vitro* studies, suggesting a negative role for TRAF3 in TNFR-SF receptor-mediated NF- κ B activation, findings in primary T cells indicate an important positive role for TRAF3 in a variety of T-cell functions (24). In primary TRAF3-deficient T cells, canonical NF- κ B activation is not significantly affected by the absence of TRAF3 (24). Thus, conclusions based upon the effect of TRAF3 deficiency on this pathway in cell lines did not yield a true picture of the role(s) played by TRAF3 in T cells.

4-1BB (CD137) is a TNFR-SF member upregulated on T cells within 12–24 h after stimulation through the TCR (96). TRAF1, TRAF2, and TRAF3 have the potential to interact with human 4-1BB as demonstrated using GST fusion proteins (97). 4-1BB signaling appears to be involved in T-cell survival or expansion, particularly in CD8⁺ T cells (96). TRAF1 has been implicated in Bcl1 upregulation upon signaling through 4-1BB, and TRAF1^{-/-} cells show increased levels of Bim upon 4-1BB signaling (98). TRAF3 effects on 4-1BB signaling in T cells have not been examined.

OX40 (CD134) is a TNFR-SF member that is expressed on T cells after stimulation through TCR+CD28 ligation. OX40 plays a role in costimulation of activated T cells to sustain NF- κ B activation in an antigen-independent manner. Using a human T-cell line overexpressing transfected TRAFs, Kawamata *et al.* (99) showed that TRAF2 and TRAF5 are positive regulators of OX40-mediated NF- κ B reporter gene activity while TRAF3 is not, and TRAF3 appears to downmodulate the TRAF2 and TRAF5-associated responses.

In recent work reported by Croft and colleagues (100), OX40 signal transduction in T cells has been divided into the antigen-dependent phase and the antigen-independent, long term activation of NF- κ B involved in survival (101). The antigen or TCR-dependent phase involves the OX40 ligand-driven movement of OX40 to detergent-insoluble membrane microdomains, and the formation of a signaling complex that includes TRAF2, protein kinase B and PI3K, with TRAF2 playing a critical role in the recruitment of the kinases. OX40 stimulation by OX40 ligand in a T-cell hybridoma system in the absence of antigen showed that OX40 recruits a signalosome that includes TRAF2, PKC θ , and CARMA1 as well as IKK $\alpha/\beta/\gamma$ (100). Though TRAF3 was not examined in this system, TRAF2 and TRAF3 typically share an overlapping binding site on TNFR-SF members in lymphocytes. TRAF2 and TRAF3 both interact with OX40, and thus a role for TRAF3 in T-cell-associated TNFR-SF member signaling has not been excluded.

Although CD40 is mainly expressed on B cells, DCs, and macrophages, in the past several years the expression of CD40 on a subset of T cells has been described (102–104, reviewed in 105). CD40 is upregulated on a population of T cells in several autoimmune mouse strains, as well as in C57Bl/6 mice with collagen-induced arthritis (102) and cells from diabetes patients (103). As has been shown in B cells, CD40 in T cells associates with endogenous TRAF1, TRAF2, TRAF3, and TRAF6 upon stimulation in both mouse and human T-cell lines (TRAF5 was not examined in this study) (102). There is movement of these TRAFs to the insoluble membrane fraction after stimulation with either anti-CD40 agonistic antibodies or CD154-expressing cells, and the TRAFs can be immunoprecipitated with CD40 (102). CD40 signals cooperate with signals through CD3 to induce JNK phosphorylation and drive AP1 and NF- κ B reporter genes. The question of whether TRAF3 has a positive or negative role in CD40 signal transduction in T cells has not been examined.

T-cell TLRs

Mouse and human T cells express low but detectable amounts of several TLRs (reviewed in 106), detected at the mRNA level (107). Furthermore, stimulation through the TCR causes upregulation of TLRs (108). Costimulation of T cells through TLRs and immobilized anti-CD3 antibodies can lead to MyD88-dependent and -independent production of IL-2 and the upregulation of costimulatory molecules such as OX-40 (106, 109). TLR stimulation of Tregs can lead to their proliferation as well as alteration of suppressive activity (110, 111). The specific role of TRAF3 in T-cell TLR signaling remains unexplored.

TRAF3 and IL17R

TRAF3 was recently demonstrated to be involved in IL-17-induced signaling (95). In these studies, TRAF3 was found to be a proximal negative regulator of NF- κ B signaling by IL-17RA, acting by preventing the formation of the IL17R/Act1/TRAF6 complex (95) (Table 2). TRAF3 is recruited upon receptor binding of IL-17. Interestingly, TRAF3 appears to interact with a site distinct from the Act1 binding site, suggesting that direct competition for binding is not the mechanism of TRAF3 interference with Act1. Overexpression of TRAF3 in transgenic mice causes a reduction in the induction of IL-6, keratinocyte chemoattractant (KC), and matrix metalloproteinase3 (MMP3) mRNA levels in the brains and spinal cords of mice in an experimental autoimmune encephalitis model, and the intraventricular delivery of siRNA to TRAF3 increases the levels of spinal cord IL-6, KC, and MMP3 mRNAs (95). One function of IL-17RA identified in T cells is the feedback regulation of IL-17 production (112), raising intriguing questions about IL-17 receptor signaling in TRAF3-deficient T cells that remain to be addressed.

TRAF3 in T-cell development

A particularly interesting facet of the phenotype of the T-cell-specific TRAF3-deficient mouse is that the number and percentage of Foxp3⁺ Treg cells are doubled (24). There is a similar percentage of Ki-67 and BrDU-positive Tregs compared with LMC mice (Z. Yi and G. Bishop, unpublished data), indicating that the higher level of Tregs in T cell-TRAF3-deficient mice is not derived from inducible Tregs in the periphery. Intracellular staining for the transcription factor Helios, a marker for natural Tregs (113), also supports the conclusion that higher levels of Tregs can be attributed to natural Tregs from the thymus. We are currently investigating how TRAF3 affects Treg development.

It is now apparent that TRAF3 plays multiple important roles in T-cell development, biology, and activation, via multiple types and families of receptors. It is also clear that many questions remain about the mechanisms by which TRAF3 impacts T-cell regulation, so much interesting work remains to be done.

Control of TRAF3 function through ubiquitin modification and degradation

While TRAF3 is yet to be proven as a *bone fide* direct E3 ubiquitin conjugating enzyme in a closed *in vitro* system, TRAF3 has been implicated as an important player in a diversity of pathways leading to both degradative K48-linked ubiquitination and noncanonical K63-linked ubiquitination of several different proteins. TRAF3 itself is also subjected to a stochastic interplay of ubiquitination and de-ubiquitination, which modulate its role in a variety of signaling contexts, several of which offer tantalizing targets for therapeutic intervention in auto-inflammatory disease.

K63 'on' K48 'off' circuits of TRAF3 modulation

Ubiquitin modification of TRAF3 follows a similar biphasic pattern following activation of several different receptor types. In the first step, TRAF3 is recruited to the proximal receptor complex where it undergoes initial K63 ubiquitination. This modification facilitates the recruitment and activation of effector kinases. Resultant signaling cascades lead to the expression of TRAF3 de-ubiquitinases and TRAF3 K48 E3 ligases after several hours, which work in a negative feedback loop to halt signal transduction, removing K63 links from TRAF3 and targeting it for proteasomal destruction respectively (Fig. 1).

Following TLR4 activation in macrophages, TRAF3 undergoes K63-linked ubiquitination. This modification of TRAF3 is dependent on TRIF, the formation of endosomes, and the RING finger domain of TRAF3 (83). The latter feature points to TRAF3 self-ubiquitination

in this case, akin to that observed for TRAF6 (114), though this has not been shown in an isolated system *in vitro*. In contrast, Mao *et al.* (115) demonstrate that Sendai virus-induced K63-induced ubiquitination of TRAF3 (occurring proximal to activated TLR3 and RIG-1-Cardif) requires the E3 ubiquitin ligase activity of cIAP1/2. Despite different utilization of E3 ligases by TLR4, TLR3, and RIG-1-Cardif receptors, K63 modification of TRAF3 occurs in each case and is essential for the recruitment and activation of downstream kinases TBK1 and IKK ϵ , and subsequently IRF3, to facilitate IFN induction.

TRAF3-dependent IFN production is halted several hours following IFN production by second phase K63 de-ubiquitination and K48 ubiquitination events. De-ubiquitinating enzyme A (DUBA), which selectively cleaves K63-linked ubiquitin chains on TRAF3, is upregulated in TLR and IL1-R1 stimulated cells. This causes TRAF3 to dissociate from TBK1, disabling type 1 IFN production in TLR-stimulated cells (116, 117). Two close relatives of DUBA, OTUB1 and OTUB2, were shown to reduce TRAF3 ubiquitination coupled with decreased anti-viral signaling responses in Sendai virus-infected HEK 293T cells (118). MCP-induced protein 1 (MCPIP1) is likewise an important effector of TRAF3 de-ubiquitination, with MCPIP1 deficiency leading to fatal inflammatory disorders in a mouse model (119). In the case of RIG-1/Cardif activation, IFN-mediated induction of de-ubiquitinases is also coupled with induction of the K48 - E3 ubiquitin ligase TRIAD domain containing protein 3a (TRIAD3A). TRIAD3A dependent TRAF3 proteasomal destruction, like TRAF3 de-ubiquitination by DUBA, halts the activation of TBK1 and IKK ϵ and subsequent IFN production (68).

K48-ubiquitin-mediated destruction of TRAF3 as a positive regulator of signaling

While TRAF3 is known as an important positive regulator of IFN production in myeloid cells and fibroblasts, its negative regulation of pro-inflammatory p38 and JNK signaling cascades and pro-survival NF- κ B pathways is well known. TRAF3 acts in this negative regulatory capacity in both innate and adaptive immune signaling pathways, with the cIAP1/2-dependent K48-mediated degradation of TRAF3 forming a rate limiting step to signal transduction (Fig. 1A,B).

In the case of TLR4 stimulation, K48 ubiquitination and degradation of TRAF3 promotes the cytosolic translocation of the MyD88-containing signaling complex, which is a necessary step in the induction of pro-inflammatory MAPK signaling pathways (83). A similar release of a MEKK1 containing complex is also required for JNK and p38 activation by CD40 (50)(Fig. 1C).

TRAF3's negative regulation of the alternative NF- κ B2 pathway has led to its designation as a 'tumor suppressor' (25), a function that centers on both its ability to mediate the K48 ubiquitin decoration of the pro-survival NF- κ B-inducing kinase (NIK) and its ability to undergo K48 ubiquitin decoration itself. It is believed that TRAF3 works to constitutively suppress levels of NIK by sequestering newly formed NIK into a complex consisting of TRAF2 and cIAP1/2, with the later tagging NIK with K48 ubiquitin for degradation. Following activation of CD40, LT β R, and BAFF-R activation by their ligands, this NIK-suppressing complex is recruited by the receptors and K63 ubiquitin modification of TRAF2 and cIAP1/2 occurs. This modification causes cIAP1/2 to switch K48 ubiquitin decoration from NIK to TRAF3, causing the degradation of the later and restoration of cellular NIK levels (23, 63, 120, 121)(Fig. 1D). NIK homodimerization and autophosphorylation is thought to be the key initiator of the prosurvival alternative NF- κ B2 pathway, dysregulation of which is a hallmark of several cellular malignancies (122). However, as discussed above, it is now clear that while constitutive activation of the NF- κ B2 pathway is a consistent feature of TRAF3 deficiency in multiple cell types, this correlates with enhanced survival only in B lymphocytes, again emphasizing the context specificity of TRAF3 functions.

TRAF5 in immune cell regulation

TRAF3 is most homologous to TRAF5, with which it shares a common ancestral gene (3, 4, 123). TRAF5 consists of 557 amino acids in humans, 558 in mice, and is located on chromosome 1 in both species. Like TRAF3, TRAF5 is comprised of a C-terminal receptor-binding domain (TRAF-C), a coiled-coil, leucine-zipper domain (TRAF-N), five zinc fingers, and an N-terminal RING finger domain (4, 124). TRAF5 can form homotypic multimers or heterotypic multimers with TRAF3 via interactions through the TRAF-N domain, and this interaction was recently shown to be biologically important in TRAF5 recruitment to certain receptors (7, 16, 125) (Table 2). Although TRAF5 is most structurally similar to TRAF3, it has been suggested to be most functionally similar to TRAF2, as both are putative positive regulators of a number of TNFR-SF members, and they share an overlapping binding site on these receptors (3, 4, 124, 126).

TRAF5 was initially identified by two separate groups as a putative signal transducer for CD40 and LT β R, positively regulating activation of the canonical NF- κ B pathway (3, 4). Additionally, TRAF5 has been implicated in activation of the noncanonical NF- κ B pathway, JNK, and IRF3/IRF7 (13–15, 126–130). TRAF5 interacts with CD30, HVEM, LMP1, OX40, CD27, RANK, TROY, BCMA, TWEAKR, 4-1BB, TACI, p75TNFR, GITR, and MAVS, although an important caveat is that the majority of these interactions were characterized using either yeast two-hybrid screening or transiently overexpressed TRAF5 in transformed, nonimmune cell lines (3, 11, 14, 15, 92, 99, 124, 126, 127, 131–140). Recent studies have demonstrated that TRAF proteins can function in both cell type- and receptor-specific fashions. For example, truncated TRAF5 inhibits TNF α -induced NF- κ B activation in HEK293 cells but not in TRAF5-deficient primary thymocytes or embryonic fibroblasts (9). Thus, validation of TRAF5 interactions between endogenous proteins in immune cell types remains an important knowledge gap.

TRAF5-deficient mice

Two separate groups have developed TRAF5^{-/-} mice by removing exons coding for the RING finger domain essential for TRAF5 function (4, 9, 11, 128). In contrast to TRAF2-deficient mice, which are runted, exhibit profound immune defects, and die prematurely, TRAF5-deficient mice exhibit a milder phenotype (141). Initial studies show viable, normal-appearing mice in terms of breeding, lymphocyte composition and lymphoid architecture in naive mice, with more subtle immune dysregulation as described below (9).

T cells

While naive TRAF5^{-/-} mice have normal lymphocyte composition, upon infection with an intracellular bacterial pathogen, CD8⁺ T cells have a defect in expansion. This defect leads to lower levels of antigen-specific CD8⁺ T cells in both primary and secondary expansion and results in decreased bacterial clearance from the liver during secondary challenge. This defect is T-cell intrinsic and results from an increased susceptibility to apoptosis. While naive TRAF5^{-/-} CD8⁺ T cells proliferate normally, they display increased activity of caspase-3 and decreased survival following activation through the TCR+CD27, suggesting that TRAF5 is involved in prevention of apoptosis and may be important in pro-survival signals via CD27. Despite their decreased survival, TRAF5-deficient CD8⁺ T cells do not have defects in upregulation of surface molecules or in NF κ B, JNK, ERK, or p38 activation and produce normal levels of IL-2 and TNF α . Interestingly, total numbers of CD4⁺ T cells are also decreased after infection, but the frequency of memory CD4⁺ T cells is unaffected (12). These defects likely occur as a result of TRAF5-dependent signaling downstream of T-cell costimulatory receptors, such as CD27.

TRAF5 is a positive regulator of signaling downstream from several receptors that serve as costimulatory molecules on T cells. The initial report characterizing the TRAF5^{-/-} mouse described a defect in CD27-mediated costimulation, as proliferation is significantly impaired in TRAF5-deficient thymocytes after stimulation through CD3/CD27 (9). However, a more recent study, with TRAF5^{-/-} mice fully backcrossed to C57Bl/6, shows normal proliferation but increased apoptosis of CD8⁺ T cells after CD3/CD27 stimulation (12). Despite this potential defect, activation of NF- κ B and JNK is not impaired in thymocytes or CD8⁺ T cells after CD27 stimulation (9, 12). Similarly, although TRAF5 is reported to associate with CD30, CD30-mediated NF- κ B, JNK, and p38 activation are unaffected by TRAF5 deficiency (124, 142, 143).

While TRAF5 is not necessary for all aspects of CD27 and CD30 signaling, it is critical for downstream signaling from the T-cell costimulatory molecules GITR and OX40. After stimulation through CD3 and GITR, TRAF5-deficient T cells have significant defects in proliferation, IL-2 production, and NF- κ B, p38, and ERK1/2 activation. Stimulation through CD28, which does not utilize TRAF5 for downstream signaling, results in normal T-cell proliferation (11). Unlike stimulation through GITR, OX40-stimulated TRAF5^{-/-} CD4⁺ T cells proliferate and produce IL-2 normally. However, the addition of agonistic α -OX40 antibody to α -CD3/ α -CD8 antibody stimulation *in vitro* results in significantly greater production of the Th2-type cytokines IL-4, IL-5, and IL-13. Additionally, *in vivo* immunizations containing a strong Th1-skewing adjuvant and agonistic α -OX40 antibody lead to greater IL-4 and IL-5 production from TRAF5^{-/-} CD4⁺ T cells both in primary and secondary responses. Similar *in vivo* Th2 skewing is seen in an OVA-sensitization model of murine asthma where Th2 cells are a critical mediator of lung inflammation. OVA-sensitized TRAF5^{-/-} mice have significantly greater lung inflammation characterized by eosinophilia, airway hyperreactivity, and higher levels of Th2 cytokines and IgE in plasma and bronchoalveolar lavage fluid (10). As OX40/OX40L interactions are crucial for Th2 priming, these data suggest that TRAF5 is involved in limiting Th2 skewing *in vivo*.

B cells

B lymphocytes from TRAF5^{-/-} mice have defects in CD40-mediated proliferation and upregulation of surface molecules including CD23, CD54, CD80, CD86, and Fas. Additionally, TRAF5^{-/-} B cells have dysregulated antibody production. After *in vitro* stimulation with α -CD40 agonistic antibody and IL-4, TRAF5^{-/-} B cells produce less IgM and IgG1 antibodies, suggesting that TRAF5 may be involved in optimal CD40-mediated immunoglobulin production and class-switching (9). However, in an OVA-sensitization model of murine asthma, TRAF5^{-/-} mice had threefold higher levels of OVA-specific IgE in plasma, suggesting that antibody class switching in TRAF5-deficient B cells may be only impaired under certain as yet undefined conditions (10). *In vivo* immunization with a TD antigen results in more antigen-specific IgM in TRAF5^{-/-} mice, but a trend towards decreased total as well as high-affinity IgG1 levels. However, normal humoral responses are seen after immunization with a TI antigen, suggesting that TRAF5 positively regulates humoral responses specifically to TD antigens. Despite these abnormalities in surface molecule upregulation and antibody production, CD40-mediated NF- κ B and JNK activation are not impaired in TRAF5^{-/-} B cells (9).

LMP1, an oncogenic protein encoded by the Epstein-Barr virus described above, also binds TRAF5. Although LMP1 is a functional viral mimic of CD40, the two receptors utilize TRAF5 in B cells in distinct ways. LMP1-transgenic mice have a phenotype consistent with B-cell hyperactivity including splenomegaly, lymphadenopathy, increased serum IL-6, production of anti-dsDNA antibody, and spontaneous germinal center formation (144). When these mice are crossed with TRAF5^{-/-} mice, these *in vivo* effects of LMP1 are ablated, demonstrating that TRAF5 is essential for LMP1 function. Additionally, purified

splenic B cells from LMP1⁺TRAF5^{-/-} mice show significantly decreased JNK activation and Akt phosphorylation and produce less IL-6, TNF α , and IL-17 compared to LMP1⁺TRAF5^{+/+} mice after stimulation through LMP1. However, NF- κ B activation and the production of both IL-10 and IL-12 are not affected by TRAF5 deficiency in LMP1-transgenic B cells. These data together demonstrate that TRAF5 is essential for many but not all of the effects of LMP1-mediated signaling (13).

Monocytes, macrophages, and dendritic cells

Unlike TRAF5^{-/-} B cells, TRAF5^{-/-} BMDCs do not have defects in CD40-mediated upregulation of OX40L, CD70, and CD86, while TRAF5 deficiency in mouse peritoneal macrophages does not affect CD40L- or TNF α -inducible IL-6 expression (12, 145). Similarly, bone marrow-derived macrophages (BMMs) from TRAF5^{-/-} mice have normal NF- κ B and JNK activation after either RANKL or TNF α stimulation. However, when TRAF5^{-/-} BMMs are used *in vitro* as osteoclast progenitors, both RANKL and TNF α fail to effectively stimulate osteoclast differentiation, suggesting that TRAF5 is important for RANK- and TNFR-mediated signaling in these cells. Despite this, TRAF5^{-/-} mice have normal bone development and function, suggesting that other pathways can compensate for the loss of TRAF5 *in vivo* (146).

In a mouse model of atherosclerosis, BMMs from TRAF5^{-/-} mice crossed with low-density lipoprotein receptor (LDLR)^{-/-} mice express higher basal levels of scavenger receptor CD36, have increased JNK activation and CXCL1 production after TNF α stimulation, and internalize more LDL than BMMs from TRAF5-sufficient LDLR^{-/-} mice. Monocytes from these mice express higher levels of ABCA1, a lipid transporter, and CD49d, a component of the integrin VLA-4. Additionally, peritoneal macrophages from these mice form significantly more 'raffles' in a cell-spreading assay, suggesting that TRAF5 deficiency promotes actin polymerization in macrophages. These irregularities contribute to accelerated atherogenesis in TRAF5^{-/-}LDLR^{-/-} mice, corroborating recent data showing decreased TRAF5 mRNA in the blood of human patients with coronary heart disease (129).

TRAF5, for years an under-studied signaling mediator in comparison to other TRAFs, is rapidly emerging as an important regulator of many immune cell functions. Its ability to form complexes with TRAF3 also increases the variety and complexity of the signaling mechanisms available to TRAF3 (Table 2).

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References

1. Hu HM, O'Rourke K, Boguski MS, Dixit VM. A novel RING finger protein interacts with the cytoplasmic domain of CD40. *J Biol Chem.* 1994; 269:30069–30072. [PubMed: 7527023]
2. Cheng G, Cleary AM, Ye Z, Hong DI, Lederman S, Baltimore D. Involvement of CRAF1, a relative of TRAF, in CD40 signaling. *Science.* 1995; 267:1494–1498. [PubMed: 7533327]
3. Ishida T, et al. TRAF5, a novel TNF-R-associated factor family protein, mediates CD40 signaling. *Proc Natl Acad Sci USA.* 1996; 93:9437–9442. [PubMed: 8790348]
4. Nakano H, et al. TRAF5, an activator of NF- κ B and putative signal transducer for the LT- β receptor. *J Biol Chem.* 1996; 271:14661–14664. [PubMed: 8663299]

5. Rothe M, Sarma V, Dixit VM, Goeddel DV. TRAF2-mediated activation of NF- κ B by TNF receptor 2 and CD40. *Science*. 1995; 269:1424–1427. [PubMed: 7544915]
6. Ishida T, et al. Identification of TRAF6, a novel TRAF protein that mediates signaling from an amino-terminal domain of the CD40 cytoplasmic region. *J Biol Chem*. 1996; 271:28745–28748. [PubMed: 8910514]
7. Pullen SS, Miller HG, Everdeen DS, Dang TTA, Crute JJ, Kehry MR. CD40-TRAF interactions: Regulation of CD40 signaling through multiple TRAF binding sites and TRAF hetero-oligomerization. *Biochem*. 1998; 37:11836–11845. [PubMed: 9718306]
8. Xu Y, Cheng G, Baltimore D. Targeted disruption of TRAF3 leads to postnatal lethality and defective T-dependent immune responses. *Immunity*. 1996; 5:407–415. [PubMed: 8934568]
9. Nakano H, et al. Targeted disruption of Traf5 gene causes defects in CD40 and CD27-mediated lymphocyte activation. *Proc Natl Acad Sci USA*. 1999; 96:9803–9808. [PubMed: 10449775]
10. So T, Salek-Ardakani S, Nakano H, Ware C, Croft M. TRAF5 limits the induction of Th2 immune responses. *J Immunol*. 2004; 172:4292–4297. [PubMed: 15034043]
11. Esparza EM, Lindsten T, Stockhausen JM, Arch RH. TRAF5 is a critical intermediate of costimulatory signaling pathways triggered by GITR in T cells. *J Biol Chem*. 2006; 281:8559–8564. [PubMed: 16452475]
12. Kraus ZJ, Haring JS, Bishop GA. TRAF5 is required for optimal T cell expansion and survival in response to infection. *J Immunol*. 2008; 181:7800–7809. [PubMed: 19017969]
13. Kraus ZJ, Nakano H, Bishop GA. TRAF5 is a critical mediator of in vitro signals and in vivo functions of LMP1, the viral oncogenic mimic of CD40. *Proc Natl Acad Sci USA*. 2009; 106:17140–17145. [PubMed: 19805155]
14. Tang ED, Wang CY. TRAF5 is a downstream target of MAVS in antiviral innate immune signaling. *PloS One*. 2010; 5:e9172. [PubMed: 20161788]
15. Hauer J, et al. TRAF3 serves as an inhibitor of TRAF2/5-mediated activation of the noncanonical NF- κ B pathway by TRAF-binding TNFRs. *Proc Natl Acad Sci USA*. 2005; 102:2874–2879. [PubMed: 15708970]
16. Arcipowski KM, Stunz LL, Graham JP, Kraus ZJ, Vanden Bush TJ, Bishop GA. Molecular mechanisms of TNFR-associated factor 6 (TRAF6) utilization by the oncogenic viral mimic of CD40, latent membrane protein 1 (LMP1). *J Biol Chem*. 2011; 286:9948–9955. [PubMed: 21262968]
17. Xie P, Stunz LL, Larison KD, Yang B, Bishop GA. TRAF3 is a critical regulator of B cell homeostasis in secondary lymphoid organs. *Immunity*. 2007; 27:253–267. [PubMed: 17723217]
18. Gardam S, Sierro S, Basten A, Mackay F, Brink R. TRAF2 and TRAF3 signal adapters act cooperatively to control the maturation and survival signals delivered to B cells by the BAFF receptor. *Immunity*. 2008; 28:391–401. [PubMed: 18313334]
19. Claudio E, Brown K, Park S, Wang H, Siebenlist U. BAFF-induced NEMO-independent processing of NF- κ B2 in maturing B cells. *Nat Immunol*. 2002; 3:958–965. [PubMed: 12352969]
20. Mecklenbräuker I, Kalled SL, Leitges M, Mackay CR, Tarakhovsky A. Regulation of B cell survival by BAFF-dependent PKC δ -mediated nuclear signaling. *Nature*. 2004; 431:456–461. [PubMed: 15361883]
21. Graham JP, Arcipowski KM, Bishop GA. Differential B-lymphocyte regulation by CD40 and its viral mimic, latent membrane protein 1. *Immunol Rev*. 2010; 237:226–248. [PubMed: 20727039]
22. Bishop GA, Xie P. Multiple roles of TRAF3 signaling in lymphocyte function. *Immunol Res*. 2007; 39:22–32. [PubMed: 17917053]
23. Vallabhapurapu S, et al. Nonredundant and complementary functions of TRAF2 and TRAF3 in a ubiquitination cascade that activates NIK-dependent alternative NF- κ B signaling. *Nat Immunol*. 2008; 9:1364–1370. [PubMed: 18997792]
24. Xie P, Kraus ZJ, Stunz LL, Liu Y, Bishop GA. TNF receptor-associated factor 3 is required for T cell-mediated immunity and TCR/CD28 signaling. *J Immunol*. 2011; 186:143–155. [PubMed: 21084666]
25. Keats JJ, et al. Promiscuous mutations activate the noncanonical NF- κ B pathway in multiple myeloma. *Cancer Cell*. 2007; 12:131–144. [PubMed: 17692805]

26. Annunziata CM, et al. Frequent engagement of the classical and alternative NF- κ B pathways by diverse genetic abnormalities in multiple myeloma. *Cancer Cell*. 2007; 12:115–130. [PubMed: 17692804]
27. Braggio E, et al. Identification of copy number abnormalities and inactivating mutations in two negative regulators of nuclear factor- κ B signaling pathways in Waldenstrom's macroglobulinemia. *Cancer Res*. 2009; 69:3579–3588. [PubMed: 19351844]
28. Basseres DS, Baldwin AS. Nuclear factor- κ B and inhibitor of κ B kinase pathways in oncogenic initiation and progression. *Oncogene*. 2006; 25:6817–6830. [PubMed: 17072330]
29. Malinin NL, Boldin MP, Kovalenko AV, Wallach D. MAP3K-related kinase involved in NF- κ B induction by TNF, CD95 and IL-1. *Nature*. 1997; 385:540–544. [PubMed: 9020361]
30. He JQ, et al. Rescue of TRAF3-null mice by p100 NF- κ B deficiency. *J Exp Med*. 2006; 203:2413–2418. [PubMed: 17015635]
31. He JQ, Saha SK, Kang JR, Zarnegar B, Cheng G. Specificity of TRAF3 in its negative regulation of the noncanonical NF- κ B pathway. *J Biol Chem*. 2007; 282:3688–3694. [PubMed: 17158868]
32. Sasaki Y, et al. NIK overexpression amplifies, whereas ablation of its TRAF3-binding domain replaces BAFF-BAFF-R-mediated survival signals in B cells. *Proc Natl Acad Sci USA*. 2008; 105:10883–10888. [PubMed: 18663224]
33. Yin L, et al. Defective LT- β receptor-induced NF- κ B transcriptional activity in NIK-deficient mice. *Science*. 2001; 291:2162–2165. [PubMed: 11251123]
34. Hostager BS, Hsing Y, Harms DE, Bishop GA. Different CD40-mediated signaling events require distinct CD40 structural features. *J Immunol*. 1996; 157:1047–1053. [PubMed: 8757608]
35. Hsing Y, Hostager BS, Bishop GA. Characterization of CD40 signaling determinants regulating NF- κ B activation in lymphocytes. *J Immunol*. 1997; 159:4898–4906. [PubMed: 9366415]
36. Ahonen CL, et al. The CD40-TRAF6 axis controls affinity maturation and the generation of long-lived plasma cells. *Nat Immunol*. 2002; 3:451–456. [PubMed: 11967542]
37. Jabara HH, et al. The binding site for TRAF2 and TRAF3 but not for TRAF6 is essential for CD40-mediated Ig class switching. *Immunity*. 2002; 17:265–276. [PubMed: 12354380]
38. Yasui T, et al. Dissection of B cell differentiation during primary immune responses in mice with altered CD40 signals. *Int Immunol*. 2002; 14:319–329. [PubMed: 11867568]
39. Grammer AC, Swantek JL, McFarland RD, Miura Y, Geppert T, Lipsky PE. TRAF3 signaling mediates activation of p38 and JNK, cytokine secretion, and Ig production following ligation of CD40 on human B cells. *J Immunol*. 1998; 161:1183–1193. [PubMed: 9686578]
40. Hostager BS, Bishop GA. Cutting edge: contrasting roles of TRAF2 and TRAF3 in CD40-mediated B lymphocyte activation. *J Immunol*. 1999; 162:6307–6311. [PubMed: 10352240]
41. Haxhinasto SA, Hostager BS, Bishop GA. Cutting Edge: Molecular mechanisms of synergy between CD40 and the BCR: Role for TRAF2 in receptor interaction. *J Immunol*. 2002; 169:1145–1149. [PubMed: 12133933]
42. Haxhinasto SA, Bishop GA. A novel interaction between PKD and TRAFs regulates BCR-CD40 synergy. *J Immunol*. 2003; 171:4655–4662. [PubMed: 14568940]
43. Haxhinasto SA, Bishop GA. Synergistic B cell activation by CD40 and the BCR: Role of BCR-mediated kinase activation and TRAF regulation. *J Biol Chem*. 2004; 279:2575–2582. [PubMed: 14604983]
44. Hostager BS, Catlett IM, Bishop GA. Recruitment of CD40, TRAF2 and TRAF3 to membrane microdomains during CD40 signaling. *J Biol Chem*. 2000; 275:15392–15398. [PubMed: 10748139]
45. Brown KD, Hostager BS, Bishop GA. Differential signaling and TRAF degradation by CD40 and the EBV oncoprotein LMP1. *J Exp Med*. 2001; 193:943–954. [PubMed: 11304555]
46. Brown KD, Hostager BS, Bishop GA. Regulation of TRAF2 signaling by self-induced degradation. *J Biol Chem*. 2002; 277:19433–19438. [PubMed: 11909853]
47. Li X, Yang Y, Ashwell JD. TNF-RII and c-IAP1 mediate ubiquitination and degradation of TRAF2. *Nature*. 2002; 416:344–349.

48. Gardam S, et al. Deletion of cIAP1 and cIAP2 in murine B lymphocytes constitutively activates cell survival pathways and inactivates the germinal center response. *Blood*. 2011; 117:4041–4051. [PubMed: 21300983]
49. Moore CR, Bishop GA. Differential regulation of CD40-mediated TRAF degradation in B lymphocytes. *J Immunol*. 2005; 175:3780–3789. [PubMed: 16148124]
50. Matsuzawa A, et al. Essential cytoplasmic translocation of a cytokine receptor-assembled signaling complex. *Science*. 2008; 321:663–668. [PubMed: 18635759]
51. Hostager BS, Haxhinasto SA, Rowland SR, Bishop GA. TRAF2-deficient B lymphocytes reveal novel roles for TRAF2 in CD40 signaling. *J Biol Chem*. 2003; 278:45382–45390. [PubMed: 12958312]
52. Xie P, Hostager BS, Bishop GA. Requirement for TRAF3 in signaling by LMP1, but not CD40, in B lymphocytes. *J Exp Med*. 2004; 199:661–671. [PubMed: 14981114]
53. Ye H, Park YC, Kreishman M, Kieff E, Wu H. The structural basis for the recognition of diverse receptor sequences by TRAF2. *Molecular Cell*. 1999; 4:321–330. [PubMed: 10518213]
54. Graham JP, Moore CR, Bishop GA. Roles of the TRAF2/3 binding site in differential B cell signaling by CD40 and its viral oncogenic mimic, LMP1. *J Immunol*. 2009; 183:2966–2973. [PubMed: 19667091]
55. Busch LK, Bishop GA. Multiple carboxyl-terminal regions of the EBV oncoprotein, LMP1, cooperatively regulate signaling to B lymphocytes via TRAF-dependent and TRAF-independent mechanisms. *J Immunol*. 2001; 167:5805–5813. [PubMed: 11698454]
56. Xie P, Bishop GA. Roles of TRAF3 in signaling to B lymphocytes by CTAR regions 1 and 2 of the EBV-encoded oncoprotein LMP1. *J Immunol*. 2004; 173:5546–5555. [PubMed: 15494504]
57. Peters AL, et al. A novel polymorphism of the human CD40 receptor with enhanced function. *Blood*. 2008; 112:1863–1871. [PubMed: 18591382]
58. Fernandez M. A multiethnic, multicenter cohort of patients with SLE as a model for the study of ethnic disparities in SLE. *Arthritis Rheum*. 2007; 57:576–584. [PubMed: 17471524]
59. Peters AL, Bishop GA. Differential TRAF3 utilization by a variant human CD40 receptor with enhanced signaling. *J Immunol*. 2010; 185:6555–6562. [PubMed: 21041727]
60. Xu L-G, Shu H-B. TRAF3 is associated with BAFF-R and negatively regulates BAFF-R-mediated NF- κ B activation and IL-10 production. *J Immunol*. 2002; 169:6883–6889. [PubMed: 12471121]
61. Ni C-Z, et al. Key molecular contacts promote recognition of the BAFF receptor by TRAF3: implications for intracellular signaling regulation. *J Immunol*. 2004; 173:7394–7400. [PubMed: 15585864]
62. Hildebrand JM, et al. A BAFF-R mutation associated with non-Hodgkin lymphoma alters TRAF recruitment and reveals new insights into BAFF-R signaling. *J Exp Med*. 2010; 207:2569–2579. [PubMed: 21041452]
63. Morrison MD, Reiley W, Zhang M, Sun S-C. An atypical TRAF binding motif of BAFFR mediates induction of the noncanonical NF- κ B signaling pathway. *J Biol Chem*. 2005; 280:10018–10024. [PubMed: 15644327]
64. Munroe ME, Bishop GA. Role of TRAF2 in distinct and overlapping CD40 and TNFR2/CD120b-mediated B lymphocyte activation. *J Biol Chem*. 2004; 279:53222–53231. [PubMed: 15485859]
65. Kirschning CJ, Wesche H, Merrill Ayres T, Rothe M. Human toll-like receptor 2 confers responsiveness to bacterial lipopolysaccharide. *J Exp Med*. 1998; 188:2091–2097. [PubMed: 9841923]
66. Häcker H, et al. Specificity in TLR signalling through distinct effector functions of TRAF3 and TRAF6. *Nature*. 2006; 439:204–207. [PubMed: 16306937]
67. Oganessian G, et al. Critical role of TRAF3 in the TLR-dependent and independent antiviral response. *Nature*. 2006; 439:208–211. [PubMed: 16306936]
68. Nakhaei P, et al. The E3 ubiquitin ligase Triad3A negatively regulates the RIG-I/MAVS signaling pathway by targeting TRAF3 for degradation. *PLoS Pathog*. 2009; 5:e1000650. [PubMed: 19893624]
69. Perez de Diego R, et al. Human TRAF3 adaptor molecule deficiency leads to impaired Toll-like receptor 3 response and susceptibility to herpes simplex encephalitis. *Immunity*. 2010; 33:400–411. [PubMed: 20832341]

70. Peng SL. Signaling to B cells by TLRs. *Curr Opin Immunol.* 2005; 17:230–236. [PubMed: 15886111]
71. Krieg AM, et al. CpG motifs in bacterial DNA trigger direct B cell activation. *Nature.* 1995; 374:546–550. [PubMed: 7700380]
72. Poovassery JS, Vanden Bush TJ, Bishop GA. Antigen receptor signals rescue B cells from TLR tolerance. *J Immunol.* 2009; 183:2974–2983. [PubMed: 19648281]
73. Vanden Bush TJ, Buchta CM, Claudio J, Bishop GA. Cutting Edge: Importance of IL-6 and cooperation between innate and adaptive immune receptors in cellular vaccination with B lymphocytes. *J Immunol.* 2009; 183:4833–4837. [PubMed: 19801508]
74. Gantner F, Hermann P, Nakashima K, Matsukawa S, Sakai K, Bacon KB. CD40-dependent and -independent activation of human tonsil B cells by CpG oligodeoxynucleotides. *Eur J Immunol.* 2003; 33:1576–1585. [PubMed: 12778475]
75. Rieger R, Kipps TJ. CpG ODN enhance the capacity of adenovirus-mediated CD154 gene transfer to generate effective B-cell lymphoma vaccines. *Cancer Res.* 2003; 63:4128–4135. [PubMed: 12874017]
76. Vanden Bush T, Bishop GA. TLR7 and CD40 cooperate in IL-6 production via enhanced JNK and AP-1 activation. *Eur J Immunol.* 2008; 38:400–409. [PubMed: 18228247]
77. Zapata JM, Llobet D, Krajewska M, Lefebvre S, Kress CL, Reed JC. Lymphocyte-specific TRAF3 transgenic mice have enhanced humoral responses and develop plasmacytosis, autoimmunity, inflammation, and cancer. *Blood.* 2009; 113:4595–4603. [PubMed: 19074733]
78. Xie P, Brown L, Stunz L, Bishop G. TRAF3 inhibits signaling by Toll-like receptors in B lymphocytes. *FASEB J.* 2008; 22
79. Lund JM, et al. Recognition of ssRNA viruses by TLR7. *Proc Natl Acad Sci USA.* 2004; 101:5598–5603. [PubMed: 15034168]
80. Balachandran S, et al. Essential role for the dsRNA-dependent protein kinase PKR in innate immunity to viral infection. *Immunity.* 2000; 13:129–141. [PubMed: 10933401]
81. Yoneyama M, et al. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. *Nature Immunology.* 2004; 5:730–737. [PubMed: 15208624]
82. Saha SK, et al. Regulation of antiviral responses by a direct and specific interaction between TRAF3 and Cardif. *EMBO J.* 2006; 25:3257–3263. [PubMed: 16858409]
83. Tseng PH, Matsuzawa A, Zhang W, Mino T, Vignali DA, Karin M. Different modes of ubiquitination of the adaptor TRAF3 selectively activate the expression of type I interferons and proinflammatory cytokines. *Nat Immunol.* 2010; 11:70–75. [PubMed: 19898473]
84. Shortman K, Naik SH. Steady-state and inflammatory dendritic-cell development. *Nat Rev Immunol.* 2007; 7:19–30. [PubMed: 17170756]
85. Kaisho T, Akira S. Regulation of dendritic cell function through toll-like receptors. *Curr Mol Med.* 2003; 3:759–771. [PubMed: 14682496]
86. Kato T, et al. Detection of anti-hepatitis C virus effects of interferon and ribavirin by a sensitive replicon system. *J Clin Microbiol.* 2005; 43:5679–5684. [PubMed: 16272504]
87. Meylan E, Tschopp J. Toll-like receptors and RNA helicases: two parallel ways to trigger antiviral responses. *Molecular Cell.* 2006; 22:561–569. [PubMed: 16762830]
88. Lanzavecchia A, Sallusto F. Regulation of T cell immunity by dendritic cells. *Cell.* 2001; 106:263–266. [PubMed: 11509174]
89. Steinman RM, et al. The interaction of immunodeficiency viruses with dendritic cells. *Curr Top Microbiol Immunol.* 2003; 276:1–30. [PubMed: 12797441]
90. Birnberg T, et al. Lack of conventional dendritic cells is compatible with normal development and T cell homeostasis, but causes myeloid proliferative syndrome. *Immunity.* 2008; 29:986–997. [PubMed: 19062318]
91. Siegal FP, et al. The nature of the principal type 1 interferon-producing cells in human blood. *Science.* 1999; 284:1835–1837. [PubMed: 10364556]
92. Kim HH, et al. Receptor activator of NF-kappaB recruits multiple TRAF family adaptors and activates c-Jun N-terminal kinase. *FEBS Lett.* 1999; 443:297–302. [PubMed: 10025951]

93. Duckett CS, Gedrich RW, Gilfillan MC, Thompson CB. Induction of NF- κ B by the CD30 receptor is mediated by TRAF1 and TRAF2. *Mol Cell Biol.* 1997; 17:1535–1542. [PubMed: 9032281]
94. Hirata H, et al. Caspases are activated in a branched protease cascade and control distinct downstream processes in Fas-induced apoptosis. *J Exp Med.* 1998; 187:587–594. [PubMed: 9463409]
95. Zhu S, et al. Modulation of experimental autoimmune encephalomyelitis through TRAF3-mediated suppression of interleukin 17 receptor signaling. *J Exp Med.* 2010; 207:2647–2662. [PubMed: 21078888]
96. Dawicki W, Bertram EM, Sharpe AH, Watts TH. 4-1BB and OX40 act independently to facilitate robust CD8 and CD4 recall responses. *J Immunol.* 2004; 173:5944–5951. [PubMed: 15528328]
97. Jang IK, Lee ZH, Kim YJ, Kim SH, Kwon BS. Human 4-1BB (CD137) signals are mediated by TRAF2 and activate nuclear factor- κ B. *Biochem Biophys Res Comm.* 1998; 242:613–620. [PubMed: 9464265]
98. Sabbagh L, et al. A critical role for TRAF1 and Bim down-regulation in CD8 memory T cell survival. *Proc Natl Acad Sci USA.* 2006; 103:18703–18708. [PubMed: 17116875]
99. Kawamata S, Hori T, Imura A, Takaori-Kondo A, Uchimaya T. Activation of OX40 signal transduction pathways leads to TRAF2 and TRAF5-mediated NF- κ B activation. *J Biol Chem.* 1998; 273:5805–5814.
100. So T, Choi H, Croft M. OX40 complexes with phosphoinositide 3-kinase and protein kinase B (PKB) to augment TCR-dependent PKB signaling. *J Immunol.* 2011; 186:3547–3555. [PubMed: 21289304]
101. So T, Soroosh P, Eun SY, Altman A, Croft M. Antigen-independent signalosome of CARMA1, PKC θ , and TNF receptor-associated factor 2 (TRAF2) determines NF- κ B signaling in T cells. *Proc Natl Acad Sci USA.* 2011; 108:2903–2908. [PubMed: 21282629]
102. Munroe ME, Bishop GA. A costimulatory function for T cell CD40. *J Immunol.* 2007; 178:671–682. [PubMed: 17202327]
103. Waid DM, et al. A unique T cell subset described as CD4^{lo}CD40⁺ T cells (TCD40) in human type 1 diabetes. *Clin Immunol.* 2007; 124:138–148. [PubMed: 17560173]
104. Baker RL, Wagner DH Jr, Haskins K. CD40 on NOD CD4 T cells contributes to their activation and pathogenicity. *J Autoimmun.* 2008; 31:385–392. [PubMed: 18951762]
105. Munroe ME. Functional roles for T cell CD40 in infection and autoimmune disease: the role of CD40 in lymphocyte homeostasis. *Sem Immunol.* 2009; 21:283–288.
106. Rahman AH, Taylor DK, Turka LA. The contribution of direct TLR signaling to T cell responses. *Immunol Res.* 2009; 45:25–36. [PubMed: 19597998]
107. Hornung V, et al. Quantitative expression of TLR1-10 mRNA in cellular subsets of human PBMC and sensitivity to CpG oligodeoxynucleotides. *J Immunol.* 2002; 168:4531–4537. [PubMed: 11970999]
108. Gelman AE, Zhang J, Choi Y, Turka LA. Toll-like receptor ligands directly promote activated CD4⁺ T cell survival. *J Immunol.* 2004; 172:6065–6073. [PubMed: 15128790]
109. Komai-Koma M, Jones L, Ogg GS, Xu D, Liew FY. TLR2 is expressed on activated T cells as a costimulatory receptor. *Proc Natl Acad Sci USA.* 2004; 101:3029–3034. [PubMed: 14981245]
110. Peng G, et al. TLR8-mediated reversal of CD4⁺ regulatory T cell function. *Science.* 2005; 309:1380–1384. [PubMed: 16123302]
- 111.utmüller RP, et al. Toll-like receptor 2 controls expansion and function of regulatory T cells. *The Journal of clinical investigation.* 2006; 116:485–494. [PubMed: 16424940]
112. Smith E, et al. IL-17A inhibits the expansion of IL-17A-producing T cells in mice through "short-loop" inhibition via IL-17 receptor. *J Immunol.* 2008; 181:1357–1364. [PubMed: 18606690]
113. Thornton AM, et al. Expression of Helios, an Ikaros transcription factor family member, differentiates thymic-derived from peripherally induced Foxp3⁺ T regulatory cells. *J Immunol.* 2010; 184:3433–3441. [PubMed: 20181882]
114. Lamothe B, Besse A, Campos AD, Webster WK, Wu H, Darnay BG. Site-specific Lys-63-linked TRAF6 auto-ubiquitination is a critical determinant of I κ B kinase activation. *J Biol Chem.* 2007; 282:4102–4112. [PubMed: 17135271]

115. Mao AP, et al. Virus-triggered ubiquitination of TRAF3/6 by cIAP1/2 is essential for induction of interferon-beta (IFN-beta) and cellular antiviral response. *J Biol Chem.* 2010; 285:9470–9476. [PubMed: 20097753]
116. Kayagaki N, et al. DUBA: A deubiquitinase that regulates Type I interferon production. *Science.* 2007; 318:1628–1632. [PubMed: 17991829]
117. Gonzalez-Navajas JM, et al. Interleukin 1 receptor signaling regulates DUBA expression and facilitates Toll-like receptor 9-driven antiinflammatory cytokine production. *J Exp Med.* 2010; 207:2799–2807. [PubMed: 21115691]
118. Li S, et al. Regulation of virus-triggered signaling by OTUB1- and OTUB2-mediated deubiquitination of TRAF3 and TRAF6. *J Biol Chem.* 2010; 285:4291–4297. [PubMed: 19996094]
119. Liang J, et al. MCP-induced protein 1 deubiquitinates TRAF proteins and negatively regulates JNK and NF-kappaB signaling. *J Exp Med.* 2010; 207:2959–2973. [PubMed: 21115689]
120. Zarnegar BJ, et al. Noncanonical NF-kB activation requires coordinated assembly of a regulatory complex of the adaptors cIAP1, cIAP2, TRAF2 and TRAF3 and the kinase NIK. *Nat Immunol.* 2008; 9:1371–1378. [PubMed: 18997794]
121. Sanjo H, Zajonc DM, Braden R, Norris PS, Ware CF. Allosteric regulation of the ubiquitin:NIK and ubiquitin:TRAF3 E3 ligases by the lymphotoxin-beta receptor. *J Biol Chem.* 2010; 285:17148–17155. [PubMed: 20348096]
122. Baud V, Karin M. Is NF-kappaB a good target for cancer therapy? Hopes and pitfalls. *Nat Rev Drug Discov.* 2009; 8:33–40. [PubMed: 19116625]
123. Grech AP, Quinn R, Srinivasan D, Badoux X, Brink R. Complete structural characterisation of the mammalian and *Drosophila* TRAF genes: implications for TRAF evolution and the role of RING finger splice variants. *Mol Immunol.* 2000; 37:721–734. [PubMed: 11275257]
124. Aizawa S, et al. TRAF5 and TRAF2 are involved in CD30-mediated NF-kB activation. *J Biol Chem.* 1997; 272:2042–2045. [PubMed: 8999898]
125. He L, Grammer AC, Wu X, Lipsky PE. TRAF3 forms heterotrimers with TRAF2 and modulates its ability to mediate NF-kB activation. *J Biol Chem.* 2004; 279:55855–55865. [PubMed: 15383523]
126. Akiba H, et al. CD27, a member of the TNF-R superfamily, activates NF-kB and SAPK/JNK via TRAF2, TRAF5, and NIK. *J Biol Chem.* 1998; 273:13353–13358. [PubMed: 9582383]
127. Marsters SA, Ayres TM, Skubatch M, Gray CL, Rothe M, Ashkenazi A. Herpesvirus entry mediator, a member of the tumor necrosis factor receptor (TNFR) family, interacts with members of the TNFR-associated factor family and activates the transcription factors NF-kappaB and AP-1. *J Biol Chem.* 1997; 272:14029–14032. [PubMed: 9162022]
128. Dadgostar H, Cheng G. An intact zinc ring finger is required for tumor necrosis factor receptor-associated factor-mediated nuclear factor-kappaB activation but is dispensable for c-Jun N-terminal kinase signaling. *J Biol Chem.* 1998; 273:24775–24780. [PubMed: 9733779]
129. Missiou A, et al. TRAF5 deficiency accelerates atherogenesis in mice by increasing inflammatory cell recruitment and foam cell formation. *Circulation Res.* 2010; 107:757–766. [PubMed: 20651286]
130. Tsukamoto N, Kobayashi N, Azuma S, Yamamoto T, Inoue J. Two differently regulated NF-kB activation pathways triggered by the cytoplasmic tail of CD40. *Proc Natl Acad Sci USA.* 1999; 96:1234–1239. [PubMed: 9990007]
131. Hsu H, Solovyyev I, Colombero A, Elliott R, Kelley M, Boyle WJ. ATAR, a novel tumor necrosis factor receptor family member, signals through TRAF2 and TRAF5. *J Biol Chem.* 1997; 272:13471–13474. [PubMed: 9153189]
132. Brodeur SR, Cheng G, Baltimore D, Thorley-Lawson DA. Localization of the major NF-kB activating site and the sole TRAF3 binding site of LMP-1 defines two distinct signaling motifs. *J Biol Chem.* 1997; 272:19777–19784. [PubMed: 9242637]
133. Darnay BG, Haridas V, Ni J, Moore PA, Aggarwal BB. Characterization of the intracellular domain of receptor activator of NF-kappaB (RANK). Interaction with tumor necrosis factor receptor-associated factors and activation of NF-kappaB and c-Jun N-terminal kinase. *J Biol Chem.* 1998; 273:20551–20555. [PubMed: 9685412]

134. Wong BR, Josien R, Lee SY, Vologodskaya M, Steinman RM, Choi Y. The TRAF family of signal transducers mediates NF-kappaB activation by the TRANCE receptor. *J Biol Chem.* 1998; 273:28355–28359. [PubMed: 9774460]
135. Galibert L, Tometsko ME, Anderson DM, Cosman D, Dougall WC. The involvement of multiple TRAFs in the signaling mechanisms of RANK, a member of the TNFR superfamily. *J Biol Chem.* 1998; 273:34120–34127. [PubMed: 9852070]
136. Kojima T, et al. TROY, a newly identified member of the tumor necrosis factor receptor superfamily, exhibits a homology with Edar and is expressed in embryonic skin and hair follicles. *J Biol Chem.* 2000; 275:20742–20747. [PubMed: 10764796]
137. Shu HB, Johnson H. B cell maturation protein is a receptor for the TNF family member TALL-1. *Proc Natl Acad Sci USA.* 2000; 97:9156–9161. [PubMed: 10908663]
138. Brown SA, Richards CM, Hanscom HN, Feng SL, Winkles JA. The Fn14 cytoplasmic tail binds tumour-necrosis-factor-receptor-associated factors 1, 2, 3 and 5 and mediates nuclear factor-kappaB activation. *Biochem J.* 2003; 371:395–403. [PubMed: 12529173]
139. Han S, et al. TNF-related weak inducer of apoptosis receptor, a TNF receptor superfamily member, activates NF-kappa B through TNF receptor-associated factors. *Biochem Biophys Res Comm.* 2003; 305:789–796. [PubMed: 12767899]
140. Tada K, et al. Critical roles of TRAF2 and TRAF5 in tumor necrosis factor-induced NF-kappa B activation and protection from cell death. *J Biol Chem.* 2001; 276:36530–36534. [PubMed: 11479302]
141. Yeh W, et al. Early lethality, functional NF-kB activation, and increased sensitivity to TNF-induced cell death in TRAF2-deficient mice. *Immunity.* 1997; 7:715–725. [PubMed: 9390694]
142. Harlin H, Podack E, Boothby MR, Alegre M-L. TCR-independent CD30 signaling selectively induces IL-13 production via a TRAF/p38 MAPK-dependent mechanism. *J Immunol.* 2002; 169:2451–2460. [PubMed: 12193714]
143. Horie R, et al. A novel domain in the CD30 cytoplasmic tail mediates NFkappaB activation. *Int Immunol.* 1998; 10:203–210. [PubMed: 9533448]
144. Stunz LL, et al. Expression of the LMP1 cytoplasmic tail in mice induces hyperactivation of B lymphocytes and disordered lymphoid architecture. *Immunity.* 2004; 21:255–266. [PubMed: 15308105]
145. Zirlik A, et al. TRAF-1, -2, -3, -5, and -6 are induced in atherosclerotic plaques and differentially mediate proinflammatory functions of CD40L in endothelial cells. *Arterioscler Thromb Vasc Biol.* 2007; 27:1101–1107. [PubMed: 17332487]
146. Kanazawa K, Azuma Y, Nakano H, Kudo A. TRAF5 functions in both RANKL- and TNFalpha-induced osteoclastogenesis. *J Bone Miner Res.* 2003; 18:443–450. [PubMed: 12619928]
147. Devergne O, et al. Association of TRAF1, TRAF2, and TRAF3 with an Epstein-Barr virus LMP1 domain important for B-lymphocyte transformation: role in NF-kappaB activation. *Mol Cell Biol.* 1996; 16:7098–7108. [PubMed: 8943365]
148. Qian Y, et al. Act1, a negative regulator in CD40 and BAFF-mediated B cell survival. *Immunity.* 2004; 21:575–587. [PubMed: 15485634]
149. Xu LG, Wu M, Hu J, Zhai Z, Shu HB. Identification of downstream genes up-regulated by the tumor necrosis factor family member TALL-1. *J Leukoc Biol.* 2002; 72:410–416. [PubMed: 12149433]
150. Hatzoglou A, et al. TNF receptor family member BCMA (B cell maturation) associates with TNF receptor-associated factor (TRAF) 1, TRAF2, and TRAF3 and activates NF-kappa B, elk-1, c-Jun N-terminal kinase, and p38 mitogen-activated protein kinase. *J Immunol.* 2000; 165:1322–1330. [PubMed: 10903733]
151. Van Arsdale TL, et al. LTb receptor signaling complex: Role of TRAF3 recruitment in cell death and activation of NF-kB. *Proc Natl Acad Sci USA.* 1997; 94:2460–2465. [PubMed: 9122217]
152. Bista P, Zeng W, Ryan S, Bailly V, Browning JL, Lukashev ME. TRAF3 controls activation of the canonical and alternative NFkappaB by the lymphotoxin beta receptor. *J Biol Chem.* 2010; 285:12971–12978. [PubMed: 20185819]

153. Force WR, Cheung TC, Ware CF. Dominant negative mutants of TRAF3 reveal an important role for the coiled coil domains in cell death signaling by the lymphotoxin-b receptor. *J Biol Chem.* 1997; 272:30835–30840. [PubMed: 9388227]
154. Feltham R, et al. Tumor necrosis factor (TNF) signaling, but not TWEAK (TNF-like weak inducer of apoptosis)-triggered cIAP1 (cellular inhibitor of apoptosis protein 1) degradation, requires cIAP1 RING dimerization and E2 binding. *J Biol Chem.* 2010; 285:17525–17536. [PubMed: 20356846]
155. Sinha SK, Zachariah S, Quiñones HI, Shindo M, Chaudhary PM. Role of TRAF3 and 6 in the activation of the NF- κ B and JNK pathways by X-linked ectodermal dysplasia receptor. *J Biol Chem.* 2002; 277:44953–44961. [PubMed: 12270937]
156. Takaori-Kondo A, Hori T, Fukunaga K, Morita R, Kawamata S, Uchiyama T. Both amino- and carboxyl-terminal domains of TRAF3 negatively regulate NF- κ B activation induced by OX40 signaling. *Biochem Biophys Res Comm.* 2000; 272:856–863. [PubMed: 10860842]
157. Arch RH, Thompson CB. 4-1BB and Ox40 are members of a TNF-R subfamily that bind TRAFs and activate NF- κ B. *Mol Cell Biol.* 1998; 18:558–565. [PubMed: 9418902]
158. Yamamoto H, Kishimoto T, Minamoto S. NF- κ B activation in CD27 signaling: Involvement of TRAFs in its signaling and identification of functional region of CD27. *J Immunol.* 1998; 161:4753–4759. [PubMed: 9794406]
159. Boucher LM, Marengere LE, Lu Y, Thukral S, Mak TW. Binding sites of cytoplasmic effectors TRAF1, 2, and 3 on CD30 and other members of the TNF receptor superfamily. *Biochem Biophys Res Commun.* 1997; 233:592–600. [PubMed: 9168896]
160. Yao Z, Xing L, Boyce BF. NF- κ B p100 limits TNF-induced bone resorption in mice by a TRAF3-dependent mechanism. *J Clin Invest.* 2009; 119:3024–3034. [PubMed: 19770515]
161. Kwon B, et al. Identification of a novel activation-inducible protein of the tumor necrosis factor receptor superfamily and its ligand. *J Biol Chem.* 1999; 274:6056–6061. [PubMed: 10037686]
162. Sasai M, et al. Direct binding of TRAF2 and TRAF6 to TICAM-1/TRIF adaptor participates in activation of the Toll-like receptor 3/4 pathway. *Mol Immunol.* 2010; 47:1283–1291. [PubMed: 20047764]
163. Gatot JS, et al. Lipopolysaccharide-mediated interferon regulatory factor activation involves TBK1-IKK ϵ -dependent Lys(63)-linked polyubiquitination and phosphorylation of TANK/I-TRAF. *J Biol Chem.* 2007; 282:31131–31146. [PubMed: 17823124]
164. Paz S, et al. A functional C-terminal TRAF3-binding site in MAVS participates in positive and negative regulation of the IFN antiviral response. *Cell Res.* 2011
165. Michallet MC, et al. TRADD protein is an essential component of the RIG-like helicase antiviral pathway. *Immunity.* 2008; 28:651–661. [PubMed: 18439848]
166. Cheng G, Baltimore D. TANK, a co-inducer with TRAF2 of TNF- and CD40L-mediated NF- κ B activation. *Genes Dev.* 1996; 10:963–973. [PubMed: 8608943]
167. Johnsen IB, Nguyen TT, Bergstroem B, Fitzgerald KA, Anthonsen MW. The tyrosine kinase c-Src enhances RIG-I (retinoic acid-inducible gene I)-elicited antiviral signaling. *J Biol Chem.* 2009; 284:19122–19131. [PubMed: 19419966]
168. Mankouri J, et al. Optineurin negatively regulates the induction of IFN β in response to RNA virus infection. *PLoS Pathog.* 2010; 6:e1000778. [PubMed: 20174559]
169. Sanada T, Takaesu G, Mashima R, Yoshida R, Kobayashi T, Yoshimura A. FLN29 deficiency reveals its negative regulatory role in the Toll-like receptor (TLR) and retinoic acid-inducible gene I (RIG-I)-like helicase signaling pathway. *J Biol Chem.* 2008; 283:33858–33864. [PubMed: 18849341]
170. Watanabe T, et al. NOD1 contributes to mouse host defense against *Helicobacter pylori* via induction of type I IFN and activation of the ISGF3 signaling pathway. *J Clin Invest.* 2010; 120:1645–1662. [PubMed: 20389019]
171. Qian Y, et al. The adaptor Act1 is required for interleukin 17-dependent signaling associated with autoimmune and inflammatory disease. *Nat Immunol.* 2007; 8:247–256. [PubMed: 17277779]
172. Leo E, et al. Differential requirements for TRAF family proteins in CD40-mediated induction of NF- κ B and JNK activation. *J Biol Chem.* 1999; 274:22414–22422. [PubMed: 10428814]

173. Liao G, Zhang M, Harhaj EW, Sun S-C. Regulation of NIK by TRAF3-induced degradation. *J Biol Chem*. 2004; 279:26243–26250. [PubMed: 15084608]
174. Tusche MW, et al. Differential requirement of MALT1 for BAFF-induced outcomes in B cell subsets. *J Exp Med*. 2009; 206:2671–2683. [PubMed: 19917778]
175. Chen MC, et al. The role of apoptosis signal-regulating kinase 1 in lymphotoxin-beta receptor-mediated cell death. *The Journal of biological chemistry*. 2003; 278:16073–16081. [PubMed: 12566458]
176. Nishitoh H, et al. ASK1 is essential for JNK/SAPK activation by TRAF2. *Mol Cell*. 1998; 2:389–395. [PubMed: 9774977]
177. Ling L, Goeddel DV. MIP-T3, a novel protein linking tumor necrosis factor receptor-associated factor 3 to the microtubule network. *J Biol Chem*. 2000; 275:23852–23860. [PubMed: 10791955]
178. Gamper C, van Eyndhoven WG, Schweiger E, Mossbacher M, Koo B, Lederman S. TRAF-3 interacts with p62 nucleoporin, a component of the nuclear pore central plug that binds classical NLS-containing import complexes. *Mol Immunol*. 2000; 37:73–84. [PubMed: 10781837]
179. Ha YJ, Lee JR. Role of TRAF3 in the CD40 signaling by production of reactive oxygen species through association with p40^{phox}, a cytosolic subunit of NADPH oxidase. *J Immunol*. 2004; 172:231–239. [PubMed: 14688330]
180. Meylan E, Martinon F, Thome M, Gschwendt M, Tschopp J. RIP4 (DIK/PKK), a novel member of the RIP kinase family, activates NF-kappa B and is processed during apoptosis. *EMBO Rep*. 2002; 3:1201–1208. [PubMed: 12446564]
181. Dadgostar H, Doyle SE, Shahangian A, Garcia DE, Cheng G. T3JAM, a novel protein that specifically interacts with TRAF3 and promotes the activation of JNK(1). *FEBS Lett*. 2003; 553:403–407. [PubMed: 14572659]
182. Hu WH, Mo XM, Walters WM, Brambilla R, Bethea JR. TNAP, a novel repressor of NF-kappaB-inducing kinase, suppresses NF-kappaB activation. *J Biol Chem*. 2004; 279:35975–35983. [PubMed: 15208311]
183. Pype S, et al. TTRAP, a novel protein that associates with CD40, tumor necrosis factor (TNF) receptor-75 and TNF receptor-associated factors (TRAFs), and that inhibits nuclear factor-kappa B activation. *J Biol Chem*. 2000; 275:18586–18593. [PubMed: 10764746]
184. Ikeda O, et al. BS69 cooperates with TRAF3 in the regulation of Epstein-Barr virus-derived LMP1/CTAR1-induced NF-kappaB activation. *FEBS Lett*. 2010; 584:865–872. [PubMed: 20138174]
185. Zhang C, Xie L, Cheng H, Wang Y. TRAF3 interacts with Smac/DIABLO and enhances the proapoptotic effect of Smac/DIABLO in cytoplasm. *Acta Biochim Biophys Sin (Shanghai)*. 2007; 39:108–116. [PubMed: 17277885]

Fig. 1. Different modes of TRAF3 ubiquitination lead to diverse signaling outcomes

Controlled TRAF3-interferon production following TLR and RLR activation occurs in two phases. (A) Soon after receptor activation, TRAF3 undergoes K63 linked ubiquitination and facilitates the recruitment and activity of downstream kinases including IRF3. (B) After several hours, the production of K63 de-ubiquitinases and K48 E3 ubiquitin ligases lead to TRAF3 complex dissolution and TRAF3 destruction, halting further interferon production. (C) TLR stimulation and CD40 activation also facilitate pro-inflammatory cytokine production, a process that requires rapid TRAF3 recruitment and K48 mediated proteasomal degradation for the liberation of cytoplasmic MAPK-activating complexes. (D) TRAF3's role in constitutive suppression of NF- κ B2 through NIK degradation ends following recruitment to some TNFR-SF members. cIAP1/2 mediated K48 ubiquitination of TRAF3 leads to NIK stabilization and NF- κ B2 activation.

Table 1

TNF Receptor superfamily members that interact with TRAF3

Receptor	Experimental system/ cell type	Interacting portion of TRAF3 / partner	Functional role	Citation
CD40	Mouse and human B cell lines and primary cells. Mouse primary T cells, mouse and human T cell lines	TRAF3 portion of TRAF3, PVQET motif of CD40	TRAF3 recruitment to CD40 leads to TRAF2-cIAP dependent TRAF3 degradation and stabilization of NIK for subsequent activation of the NF- κ B2 pathway. In T cells, TRAF3 moves to membrane rafts upon stimulation of CD40	(1, 2, 40–43, 56, 102)
LMP1 (viral CD40 mimic)	Mouse and human B cell lines and primary cells	TRAF3 portion of TRAF3, CTAR1 of LMP1	TRAF3 binding to LMP1 is required for optimal LMP1-mediated signaling to B cells. TRAF3 is necessary for the recruitment of TRAF5 to LMP1	(13, 52, 147)
BAFF-R	M12 mouse B cell line. Human BJAB and IM9 B cell lines. Mouse primary B cells.	TRAF3 portion. Key residues defined by X-ray crystallography. C terminal PVPAT motif of BAFF-R.	TRAF3 recruitment to BAFF-R leads to TRAF2-cIAP dependent TRAF3 degradation and stabilization of NIK for subsequent activation of the NF- κ B2 pathway	(60–63, 148)
TACI	BJAB and A20 B cell line, primary mouse B cells	Not defined	TRAF3 blocks TACI induced NF- κ B activation	(15, 62, 149)
BCMA	COS7 cells	Region within aa 119–143 of BCMA	TRAF3 blocks BCMA induced NF- κ B activation	(15, 150)
LT β R	HT29.14S human colon adenocarcinoma, HEK293T cells	Coil 3 of TRAF3, PEEGDPG motif of LT β R	Induction of JNK, induction of apoptosis, inhibition of NF- κ B1 and regulation of NF- κ B2 via NIK	(121, 151–153)
FN14	Yeast Two Hybrid	PIEE motif of FN14	Not known, NIK stabilization occurs independent of cIAPs	(138, 154)
XEDAR	HEK293T cells	PTQES motif of XEDAR	TRAF3 is required for XEDAR induced JNK activity	(155)
OX40/CD134	Yeast Two Hybrid, HEK293T cells, HSB-2 T cell line	TRAF3 domain of TRAF3, aa 256–263 of OX40	TRAF3 blocks OX40 induced NF- κ B activation	(15, 99, 156, 157)
CD27	HEK293T cells	aa 238–250 of CD27	TRAF3 negatively regulates CD27 induced NF- κ B activity	(15, 158)
CD30	GST-binding assay, COS cells	558-PHYPEQET-565 motif of CD30	TRAF3 negatively regulates CD30 induced NF- κ B activity	(15, 159)
RANK/TRANCE-R	HEK293T cells	aa 571–573 of RANK	TRAF3 inhibits RANK induced NIK and NF- κ B2 activity. Reduced NF- κ B2 can inhibit osteoclastogenesis and bone resorption	(134, 135, 160)
4-1BB/CD137	Yeast Two Hybrid, GST-binding assay, HEK293T cells	aa 237–239 and 248–250	4-1BB induced NF- κ B activity is inhibited by TRAF3	(15, 97, 157)
GITR	HEK293T cells	Not defined	TRAF3 blocks GITR induced NF- κ B activation	(15, 161)
HVEM/TNFRSF14	HEK293T cells	Not defined	TRAF3 blocks HVEM induced NF- κ B activation	(15, 127)
TNFR2/CD120b	293T cells, M12 B cells	Not defined	TRAF3 inhibits non-canonical NF- κ B2 activation by p75TNFR	(15, 64)

Table 2

Other proteins that interact with TRAF3

Protein	Experimental system/ cell type	Interacting portion of TRAF3 / partner	Functional role	Citation
<i>TLR Signaling components</i>				
TLR4	Primary mouse BMDMs, RAW264.7 cell line	Not defined	TRAF3 degradation is required for TLR4 induced MAPK activation. TRAF3 is a positive regulator of TLR4 induced IFN production.	(83)
MyD88	RAW264.7 cells	Not defined	TRAF3 is recruited to MyD88 following TLR stimulation and undergoes TRAF6 dependent degradation to facilitate MAPK activation.	(66, 83)
TRIF/TICAM-1	RAW264.7 cells HEK293T cells, HeLa cells	TRAF3 does not utilize TRAF2/6 binding sites on TRIF	TRAF3 is recruited to TRIF following TLR stimulation and undergoes K63 ubiquitination to facilitate IFN production	(66, 67, 83, 162)
IKKε	HEK293T cells	Not defined	TRAF3 synergizes with IKKε to enhance phosphorylation of IRF3	(67)
TBK1	HEK293T cells, RAW264.7 cells	Not defined	TRAF3 synergizes with TBK1 to enhance phosphorylation of IRF3 leading to IFN production	(66, 67, 163)
IRAK1	HEK293T cells	Not defined	TRAF3 synergizes with IRAK1 to induce IRF7 dependent IFN production	(67)
<i>TLR independent viral response</i>				
Cardif (MAVS, IPS-1, VISA)	HEK293T cells, MEFs	TRAF domain of TRAF3 and TIM (TRAF interaction-motif aa 455–460) of Cardif	Direct recruitment of TRAF3 to the CARDIF-RIG-1 complex is a necessary step in the assembly of the signaling components required for eventual IFN production	(82, 164)
PKR	HEK293T cells	Not defined	MEFs, which detect viral infection via PKR and RIG-1, show defective IFN production in the absence of TRAF3.	(67)
TRIAD3A	HEK293T cells, A549 cells	Y440A and Q442 of TRAF3, TRIAD3A (aa 316–320)	TRIAD3A negatively regulates RIG-1 induced IFN production by acting as a K48 ubiquitin E3 ligase for TRAF3 degradation.	(68)
TRADD	HEK293T cells	Not defined	TRAF3 interacts with TRADD as part of the RIG –like Helicase pathway for the induction of IFN and NF-κB responses to viral infection	(165)
TANK	Yeast Two hybrid, HEK293T cells, RAW 264.7	aa 170 –191 of TANK, TRAF3 domain of TRAF3	Association with TRAF3 is required TANK phosphorylation and ubiquitination, necessary steps in LPS and CPG induced IFN induction.	(163, 166)
c-Src	HEK293T cells	TRAF3 RING domain	c-Src augments TRAF3 dependent IRF3 activation downstream of Cardif/MAVs	(167)
Optineurin	HEK293T cells	Not defined	Optineurin associates with TBK1 and TRAF3 to negatively regulate IFN production.	(168)
FLN29	HEK293T cells	Not defined	FLN29 negatively regulates the RIG –like Helicase pathway possibly by interfering with TRAF3-Cardif association	(169)
<i>NOD1 signaling pathway</i>				

Protein	Experimental system/ cell type	Interacting portion of TRAF3 / partner	Functional role	Citation
RICK	HEK293T cells, HT-29 cells	Not defined	RICK-TRAF3 interaction required for NOD1 induced IFN production in epithelial cells.	(170)
FLN29	HEK293T cells	Not defined	FLN29 negatively regulates TLR4 and RIG-1 induced pathways	(169)
<i>IL Receptors</i>				
IL-17R	HeLa cells, HEK293 cells. Following stimulation with IL-17.	TRAF domain. PXEE motif in distal domain of IL-17R.	TRAF3 binding to IL-17R competitively inhibits the formation of the IL-17R-Act1-TRAF6 signal activation complex, inhibiting NF- κ B1 activation and expression of inflammatory cytokine and chemokine genes.	(95, 171)
<i>Ubiquitin modifying enzymes</i>				
DUBA	HEK293T cells	Not defined	DUBA promotes the removal of Lys-63 linked poly-ubiquitin chains from TRAF3, which result in its dissociation from TAK1 containing complexes. Shown to be an important negative regulator of IFN-1 production	(116)
MCPIP1	HEK293T cells, mouse splenocytes	Not defined	Basal and LPS induced TRAF3 poly-ubiquitination is enhanced in a MCPIP1 deficient mouse strain. TRAF3 de-ubiquitination may form part of the important anti-inflammatory role played by MCPIP1.	(119)
OTUB1/2	HEK293T cells	Not defined	OTUB1/2 catalyze de-ubiquitination of RIG1/Cardif associated TRAF3, preventing downstream IFN induction	(118)
<i>TCR complex</i>				
CD28	Mouse primary T cells; Jurkat human T cell line	Not defined	TRAF3 ^{-/-} T cells show impaired proximal signaling, cytokine production and proliferation with stimulation through CD3+28. Response to Listeria infection and T dependent antibody response is decreased.	(24)
<i>Other Intracellular adaptors</i>				
TRAF2	Sf21 insect cells, Ramos human B cell line	TRAF domain of TRAF3, TRAFN and Zn4, Zn5 of TRAF2	TRAFs 2 and 3 a complex whereby cIAP1/2 ubiquitinates NIK or TRAF3 for degradation.	(7, 23, 120, 125)
TRAF5	Sf21 insect cells, Hela cells (FRET)	Isoleucine zipper domains of both TRAFs 3 and 5.	TRAF3 is required for TRAF5 recruitment to CD40 and LMP1. TRAF5 is required for LMP1-mediated B cell hyperactivity	(7, 13, 125, 172)
NIK	Primary mouse B cells, MEFs, HEK293 cells	TRAF domain, ⁷⁸ ISIIAQA ⁸⁴ (N terminus of NIK)	NIK phosphorylates with IKK α to facilitate the processing of p100 to p52.	(23, 120, 173)
Act1	Human IM9 B cell line.	Not defined	Act1 is required for IL-17 and IL-25 mediated cytokine production, negative regulator of CD40 and BAFF-R signaling in B cells.	(148)
Malt1	Human BJAB cell line, primary mouse B cells	Not defined	Required for maximal BAFF induced NF κ B2 activation in B cells, possibly by facilitating cIAP dependent TRAF3 degradation.	(174)

Protein	Experimental system/ cell type	Interacting portion of TRAF3 / partner	Functional role	Citation
ASK1	HEK293 cells	Not defined	TRAF3 is required for ASK1 activity in the context of LT β R induced cell death	(175, 176)
MIP-T3	HEK293T cells, Hela cells	Coiled coil TRAF N domain of TRAF3, C terminal coiled coil domain of MIP-T3	MIP-T3 recruits TRAF3 to microtubules and actin filaments	(177)
p62 nucleoporin	Yeast Two Hybrid, HEK293T cells.	N terminal coiled coil of p62. Zinc finger 5 and coiled coil of TRAF3.	p62 may be required for the localization of TRAF3 to the nuclear pore, the role of TRAF3 here has not been examined.	(178)
PI-3K p85 subunit	HEK293T cells	Not defined	TRAF3 recruits p85 to CD40. PI-3K produces phosphoinositides that regulate NADPH oxidase and other signaling cascades.	(179)
P40 ^{phox}	HEK293T cells	Not defined	TRAF3 is required for CD40 induced production of reactive oxygen species (ROS)	(179)
RIP4	HEK293T cells	Not defined	TRAF3 is required for RIP4 NF- κ B activation	(180)
T3JAM	HEK293T cells	Isoleucine zipper of TRAF3 and the coiled coil domain of T3JAM	TRAF3 and T3JAM work synergistically to activate the JNK signaling cascade	(181)
TNAP	HEK293T cells	Not defined	TNAP suppresses the function of NIK, and the phosphorylation of I κ B α and p65. It is not clear how interaction with TRAF3 contributes to this	(182)
TTRAP/TDP2	HEK293T cells	Not defined	TTRAP overexpression suppresses TNFR and TRAF induced NF- κ B activity	(183)
BS69	HEK293T cells	aa 368-421 of TRAF3	TRAF3 is involved in the BS69-mediated suppression of LMP1/CTAR1-induced NF- κ B activation	(184)
Smac/DIABLO	Yeast Two hybrid, HepG2 cells, GST pull down assay, HEK293T cells	TRAF N and C domains of TRAF3,	Overexpression of TRAF3 promotes Smac/DIABLO induced apoptosis in HEK293T cells	(185)