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TRAF3, ubiquitination, and B-lymphocyte regulation

Wai W. Lin¹, Bruce S. Hostager², and Gail A. Bishop^{1,3,4,5}

¹The Graduate Program in Immunology, Iowa City, IA, USA

²Dept. of Pediatrics, Iowa City, IA, USA

³Dept. of Microbiology, Iowa City, IA, USA

⁴Dept. of Internal Medicine, Iowa City, IA, USA

⁵The University of Iowa and the VA Medical Center, Iowa City, IA, USA

Summary

The signaling adapter protein tumor necrosis factor receptor (TNFR)-associated factor 3 (TRAF3) is both modified by and contributes to several types of ubiquitination events. TRAF3 plays a variety of context-dependent regulatory roles in all types of immune cells. In B lymphocytes, TRAF3 contributes to regulation of signaling by members of both the TNFR superfamily and innate immune receptors. TRAF3 also plays a unique cell-type-specific and critical role in the restraint of B-cell homeostatic survival, a role with important implications for both B-cell differentiation and the pathogenesis of B-cell malignancies. This review focuses upon the relationship between ubiquitin and TRAF3, and how this contributes to multiple functions of TRAF3 in the regulation of signal transduction, transcriptional activation, and effector functions of B lymphocytes.

Keywords

TRAF3; B cell; ubiquitin

Introduction

The post-translational modification called ubiquitination/ubiquitylation is a frequent and versatile mechanism by which proteins are tagged for intracellular degradation or redistribution (1). The adapter protein tumor necrosis factor receptor (TNFR)-associated protein 3 (TRAF3) is expressed by many cell types, including all nucleated immune cells, where it plays many and highly variable roles in regulating immune functions (2, 3). However, the ubiquitination of TRAF3 and TRAF3-facilitated ubiquitination of other proteins appear particularly important in B lymphocytes. B-cell TRAF3 serves not only as a regulator of receptor-mediated signaling but also plays an important B-cell-specific role in restraining homeostatic survival of cells in secondary lymphoid organs and tissues (4, 5). It

Correspondence to: Gail A. Bishop, Department of Microbiology, University of Iowa, Iowa City, IA 52242, Tel.: +1 319 335 7945, gail-bishop@uiowa.edu.

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is thus not surprising that TRAF3 is implicated as a tumor suppressor in several types of B-cell malignancies (discussed below).

Ubiquitin (Ub) proteins are added onto TRAF3 as both K48- and K63-linked chains, depending upon the context (6). K48-linked polyubiquitination results in TRAF3 degradation, which plays important regulatory roles for B cell signals mediated by CD40 (7) and BAFF-R (B-cell activation factor belonging to the TNF family receptor) (8), as well as B-cell survival, as discussed below. However, the relationship between TRAF3 degradation and its regulatory functions in B cells is multi-faceted and complex. Additionally, it is not clear that all B-cell TRAF3 actions involve ubiquitination. This review discusses both the association between ubiquitin modification and B-cell TRAF3, as well as the varied regulatory roles played by TRAF3 in B lymphocytes.

TRAF3 ubiquitination in B lymphocytes

The role of TRAF3 in signaling is closely tied to ubiquitination events, although as will be discussed below, not all its biological functions are directly attributable to this modification. Since its discovery (9-11), TRAF3 has been identified as a target of ubiquitination in cells activated through various receptors, including members of the TNFR and Toll-like receptor (TLR) superfamilies (3). As a close relative of TRAF2 and TRAF6, which have E3 Ub ligase activity (12), TRAF3 may also have the ability to induce ubiquitination of itself or other proteins, but to date this has not been demonstrated for endogenous TRAF3 in physiologic circumstances.

Not long after its discovery, TRAF3 was found to undergo activation-induced degradation (13). CD40-mediated B-cell activation also induces the appearance of high-molecular weight forms of TRAF3 (14). Both observations are attributable to activation-induced ubiquitination (2, 15). Two forms of ubiquitination of TRAF3 have been documented. The covalent polyubiquitination of TRAF3 with K48-linked Ub chains (in which Ub molecules are covalently attached via their C-terminal glycine residues to lysine 48 of preceding Ubs in a chain) targets TRAF3 for proteasomal degradation. Numerous receptors on B cells induce K48-linked ubiquitination, followed by proteasome-mediated degradation of TRAF3, including CD40, TNFR2, BAFF-R, and TLRs 2, 4, 7, and 9 (reviewed in 3). In the case of CD40 signaling to B cells, TRAF3 degradation is dependent upon the really interesting new gene (RING) domain of TRAF2, with which it forms heteromultimers (16). The degradation of TRAF3 induced by signaling via these receptors appears to be aimed at disrupting the ability of TRAF3 to negatively regulate the activation of the non-canonical NF- κ B2 and mitogen-activated protein kinase (MAPK) pathways, as discussed below and elsewhere in this issue. In contrast, K63-linked polyubiquitination of TRAF3 is not associated with TRAF3 degradation (at least not directly) and may be mediated by TRAF3 itself or other proteins.

K48-linked polyubiquitination of TRAF3

TRAF3 plays critical roles in B-cell development as well as in the activation of mature B cells in an immune response. During B-cell maturation, BAFF-R signaling induces activation of the NF- κ B2 pathway, which is required for immature B cells transitioning to

K63-linked polyubiquitination of TRAF3

TRAF3 is also subject to post-translational modification with K63-linked polyubiquitin chains. The purpose of this covalent modification is markedly different from that of K48-linked polyubiquitination. K63-linked polyubiquitin appears to offer binding sites for other proteins involved in activation-induced signaling. One potential mediator of this ubiquitination activity is TRAF3 itself. Near its amino terminus, TRAF3 contains a RING motif. This motif confers Ub ligase activity on a variety of proteins, including TRAF2 and TRAF6 (29, 30). Point mutation of key cysteine residues within RING domains abolishes E3 ligase activity (31). One report shows decreased LPS/TLR4-induced K63-linked polyubiquitination of a TRAF3 RING mutant in a mouse macrophage cell line (6). While this suggests that TRAF3 is capable of auto-ubiquitination, it is also possible that native structure of the RING domain is required for recruitment of some other E3 Ub ligase to TRAF3. A second report demonstrates TRAF3 auto-ubiquitination *in vitro*, likely K63-linked, although the linkage was not directly evaluated (32). While it appears likely that TRAF3 possesses E3 Ub ligase activity, other proteins may also mediate K63-linked polyubiquitination of TRAF3. Recently, the E3 Ub ligase neural precursor cell expressed developmentally downregulated protein 4 (NEDD4) was found to associate with CD40 in B cells, and appears to mediate the K63-linked polyubiquitination of TRAF3 during CD40 signaling. This ubiquitination event has been posited to mediate phosphoinositide-3 kinase (PI3K) activation (33).

Deubiquitination of TRAF3

Ubiquitination of TRAF3 is important for activation of downstream signaling pathways in B cells. Deubiquitinase enzymes (DUB) discussed below remove Ub on TRAF3 to regulate this signaling.

A20-mediated TRAF3 modification

A20 is a DUB that contains an ovarian tumor (OTU) domain and negatively regulates multiple signaling pathways by removing K63 linked polyubiquitin, including those mediated by TNFR, TLR4, and CD40 (reviewed in 34). While A20 can remove K63-linked polyubiquitin from TRAF6 (35), NF- κ B essential modulator (NEMO) (36), and receptor-interacting protein kinase 2 (RIP2) (37) to inhibit RIG-I mediated NF- κ B activation, it also possess an E3 Ub ligase activity capable of adding back K48-linked polyubiquitin chains to its targets (38). Although TRAF3 has not been identified as a substrate of A20, overexpression of A20 in mouse embryonic fibroblasts (MEF) disrupts the association of TRAF3 with TANK-binding kinase 1 (TBK1), inhibiting downstream IFN regulatory factor (IRF) activation (39). Additionally, similar to B-cell-specific TRAF3 deletion (4, 5), B-cell-specific deletion of A20 results in expansion of marginal zone B cells, increased spontaneous germinal center B cells, and increased serum antibody titers (40) (Table 1). Like TRAF3^{-/-} B cells (41), A20^{-/-} B cells display enhanced classical NF- κ B activation in response to TLR4 (40). Additionally, the *TNFAIP3* gene, which encodes the A20 protein, is frequently inactivated in B-cell lymphomas (42), and loss-of-function mutations of the human *TRAF3* gene are also associated with B-cell malignancies (see below). These observations suggest that one function of A20 in B cells is to induce K48-linked

polyubiquitination and degradation of TRAF3. However, further study is required to address whether A20 functions as a DUB or E3 Ub ligase of TRAF3 in B cells.

OTUD7B as a TRAF3 regulator

OUT domain-containing DUB 7B (OTUD7B) is a DUB that specifically regulates NF- κ B2 activation (43). OTUD7B removes K48-linked polyubiquitin from TRAF3 to block its degradation. It associates with lymphotoxin- β receptor in MEF and CD40 in B cells (43), two well-characterized receptors that induce TRAF3 degradation (as reviewed in 22). B cells isolated from OTUD7B^{-/-} mice (Table 1) show accumulation of NIK and subsequent activation of NF- κ B2 in response to CD40 and BAFF stimulation (43). In addition, B cells lacking OTUD7B display hyper-proliferation and enhanced antibody production (43). Given that OTUD7B specifically regulates the NF- κ B2 pathway and TRAF3 degradation, it would be of interest to determine if OTUD7B also deubiquitinates NIK.

MCPIP 1 and TRAF3 regulation

Monocyte chemotactic protein-induced protein 1 (MCPIP1) is a recently identified DUB belonging to the CCCH zinc-finger family (44, 45). MCPIP 1-deficient macrophages show hyperactivation in response to LPS stimulation, and MCPIP1-deficient mice die from uncontrolled inflammatory responses (46). MCPIP cleaves both K48- and K63-linked polyubiquitin and can deubiquitinate TRAF2 and TRAF3 (46). Consistent with hyperactivation of the TLR4 pathway, MCPIP^{-/-} mice exhibit increased plasma cell frequencies in the spleen, despite the reduction in B-cell numbers in peripheral lymphoid organs (47).

From the preceding discussion, it is clear that modification of TRAF3 by altering its ubiquitination status can provide a powerful means of regulating its signaling functions. In the subsequent sections, we summarize the nature of the roles played by TRAF3 in B lymphocytes, illustrating both the variety and biological impact of this protein in regulating B-cell biology.

TRAF3 roles in regulation of CD40 and BAFF-R mediated signaling

TRAF3 is well-studied as an adapter protein that binds CD40 and inhibits CD40 mediated NF- κ B2 activation, as well as MAPK pathways (48). TRAF3 competes with TRAFs 1, 2 and 5 for a shared TRAF binding site in the cytoplasmic tail of CD40 (49, 50, 51). In B cells, CD40 signaling is important for antibody production and class-switch recombination (52). TRAF3 competes with TRAF2 for CD40 binding, thereby inhibiting JNK activation and CD40-mediated antibody production (53, 54), as well as synergy between CD40 and the B-cell antigen receptor (BCR) (49). In contrast to its negative regulation of NF- κ B2, TRAF3 does not appear to strongly regulate CD40-induced activation of classical NF- κ B1, the MAPKs p38 or ERK, or PI3K in B cells (4, 54). Consistent with unaltered CD40-induced classical NF- κ B1 activation (in contrast to TLR-mediated activation, see below), production of pro-inflammatory cytokines such as TNF- α and IL-6 is similar between wildtype and TRAF3^{-/-} B cells (54).

BAFF-R, B-cell maturation antigen (BCMA), and transmembrane activator and calcium-modulator and cyclophilin ligand interactor (TACI) are three closely related TNFR superfamily receptors that are important for B-cell maturation, homeostasis, and function (17). Signaling through BAFF-R is essential for B-cell maturation, homeostasis, and survival. Mice deficient either in BAFF-R or its cognate ligand BAFF (Table 1) show severe deficiencies in mature B cells (55, 56). BAFF-transgenic (tg) mice show expansion of mature B-cell populations and increased serum antibody titers (57) (Table 1). BAFF-R activates the PI3K-Akt and NF- κ B2 survival pathways (58-60). Upon activation, BAFF-R recruits TRAF molecules including TRAF2, TRAF3 and TRAF6 to activate NF- κ B1 and NF- κ B2 pathways (5, 18, 61). TRAF2, cIAP1/2, and TRAF3 appear to negatively regulate BAFF-R mediated NF- κ B2 activation (4, 5, 18). Similar to CD40, BAFF-R induces degradation of TRAF3 (8, 62). However, the degradation of TRAF3 is not solely sufficient to activate NF- κ B2 in B cells (23), and other TRAFs may interact with TRAF3 in this role.

In the context of BAFF-R signaling to B cells, TRAF6 is important for activation of the classical NF- κ B1 pathway; in the absence of TRAF6 binding, activation of this pathway is abrogated (61). TRAF6 associates with Act1 in response to IL-17 in epithelial cells (63). Act1^{-/-} B cells show drastic increases in BAFF-mediated NF- κ B1 and to a lesser extent NF- κ B2 activation (64). These observations suggest that Act1 and TRAF6 play opposite roles in regulation of BAFF-mediated NF- κ B1 activation in B cells. In contrast, both the Act1 NF- κ B activating protein and TRAF6 are positive regulators of IL-17 dependent NF- κ B activation (63, 65). The exact relationships between Act1, BAFF-R, TRAF3, and TRAF6 molecules *in vivo* remains unclear.

In contrast to BAFF-R^{-/-} or BAFF^{-/-} mice, mice deficient in TACI have an expansion of B cells and elevated serum antibodies (Table 1), suggesting that BAFF-R and TACI function differently in the maintenance of mature B cells (66, 67). TACI serves as a receptor for BAFF and a proliferation-inducing ligand (APRIL); TACI binds TRAF2, TRAF3, and TRAF6 when overexpressed in epithelial cell lines, but TRAF3 is the only TRAF that associates with TACI in human B-cell lines (8, 68). BCMA is another closely related receptor that binds both BAFF and APRIL. BCMA^{-/-} mice have normal B-cell development but are severely deficient in long-lived plasma cells in the bone marrow (69) (Table 1), consistent with BCMA being primarily expressed in plasma cells. There is no evidence that BCMA recruits TRAF molecules in B cells, but studies from epithelial cells show that BCMA associate with TRAF1, TRAF2, and TRAF3 and activates NF- κ B (70). Further work is required to address the functional roles of TRAF molecules in signaling by TACI or BCMA in B cells.

TRAF3 as a regulator of innate immune receptor signals

TRAF3 in B lymphocytes is best known for its regulation of CD40 and BAFF-R functions, as discussed above. However, while it was initially believed that TRAF6 is the only TRAF family member involved in signaling by innate pattern-recognition sensors, such as TLRs, it became clear over the past decade that TRAF3 also participates in signaling by this group of receptors. Almost all published reports to date on this role for TRAF3 have focused upon myeloid cells (usually macrophages) and/or fibroblasts, with additional data obtained by

exogenous overexpression of TRAF3 in the transformed epithelial cell line, 293T. As the focus of this review is TRAF3's roles in B cells, here we will briefly summarize findings in other cell types and what has been found more recently in studies of innate receptors in B cells.

The first reports of involvement of TRAF3 in TLR signaling were performed in the macrophage cell line RAW264.7 or bone marrow macrophages (BMMs) obtained from mice transplanted with fetal liver cells of mice lacking TRAF3 in all cells and tissues, as the latter have an early-lethal phenotype (71). Transfected, epitope-tagged MyD88 co-immunoprecipitates (co-IPs) with TRAF3 in RAW264.7 cells, following stimulation through TLR9. Additionally, TRAF3 co-IPs with the TLR signaling protein TIR-domain-containing adapter inducing IFN β (TRIF) in these cells following TLR3 or TLR4 stimulation (72). In the same report, BMMs of mice transplanted with TRAF3^{-/-} fetal liver cells show increased production of IL-12 and decreased IFN bioactivity when stimulated via TLR4 or TLR9. In the same year, a study using the same source of BMMs reported defective type I IFN mRNA following TLR3 stimulation, as well as defective IRF3 nuclear translocation. Dendritic cells derived from these mice are defective in IFN α production following TLR7 or TLR9 stimulation (73). This report also included studies in which TRAF3 and several type I IFN pathway mediators were co-overexpressed in 293T cells, showing constitutive associations between TRAF3 and TRIF or the kinase TBK1. Additionally, embryonic fibroblasts from TRAF3^{-/-} mice show higher viral titers following vesicular stomatitis virus infection (73).

The latter finding is relevant to the phenotype of a human patient with a single-allele, potentially 'dominant negative' mutation of TRAF3 (74). This patient came to the attention of researchers after developing a rare Herpes simplex virus-associated encephalitis; patient fibroblasts display a defective type I IFN response to TLR3 stimulation. Unfortunately, no other immune cell types of the patient were studied. A recent analysis of TRAF3-deficient mouse macrophages in viral infection presents an interesting model, whereby it is postulated that TRAF3 serves to enhance antiviral immunity by amplifying TLR2 signals, resulting in 'priming' of subsequent macrophage responses to TLR4 (75). Another role for TRAF3 in macrophage TLR functions was posited in a recent study, indicating that the *Leishmania donovani* parasite inhibits TRAF3 ubiquitination, which in turn inhibits macrophage TLR4 responses (76).

The first published report examining the role of TRAF3 in B-cell TLR signaling presented a complex picture, emerging from studies of a mouse strain expressing transgenically encoded human TRAF3, driven by the Ig gene promoter and enhancer (77). This strain expresses endogenous TRAF3 but also has substantial overexpression of the human TRAF3 molecule both in its B cells and in a proportion of its T cells. The very distinct roles played by TRAF3 in T cells versus B cells (reviewed in 3) thus complicate interpretation of the phenotype of this mouse. In response to TLR4 or TLR9 stimulation, these mice produce increased amounts of IgG1 and IgG2b (Table 1). However, the detailed molecular interactions occurring between the transgenic human TRAF3, endogenous mouse TRAF3, and various receptors and signaling proteins in this mouse are unclear. A subsequent study examined TLR responses of B cells of a mouse in which TRAF3 was specifically deleted only in the B-cell lineage (B-TRAF3^{-/-}, described above). In contrast to TRAF3-deficient myeloid cells,

fibroblasts, and 293T cells, TRAF3-deficient B cells have markedly enhanced production of TNF, IL-6, and type I IFNs in response to *in vitro* stimulation through TLR3, TLR4, TLR7, or TLR9 (41). These B cells also respond to TLR stimulation with increased activation of IRF3, and enhanced *in vivo* production of isotype-switched immunoglobulins, corresponding to increased levels of the enzyme activation-induced deaminase. TRAF3^{-/-} B cells display increased canonical NF-κB1 activation in response to TLR signals but show no detectable elevation in TLR-mediated MAPK activation (41). The latter finding is of particular interest in regards to a recent report on TLR responses of TRAF5-deficient B cells. Unlike TRAF3, whole-mouse deletion of TRAF5 is not lethal, and TRAF5^{-/-} mice have relatively normal immune cell composition (78). However, TRAF5-deficient B cells also show enhanced cytokine production in response to TLR stimulation (79). In contrast to the specific pathways involved in the amplified TLR responses of TRAF3^{-/-} B cells, TRAF5^{-/-} B cells have elevated TLR-induced MAPK phosphorylation but not canonical NF-κB1 activation. TRAF5 may exert these effects at least in part by inhibiting association of the protein TAB2 with TRAF6 following B cell TLR signaling (79). As TRAF3 and TRAF5 are reported to heterotrimerize (50), how the two TRAFs may interact in regulating B-cell TLR signals is an intriguing unexplored question.

TRAF3 as a regulator of B-cell survival

Mice genetically deficient in the TRAF3 protein in all cells and tissues display a complex phenotype and early lethality (71), precluding their usefulness as a model for the normal roles played by TRAF3 in B-cell biology. Although *in vitro* work in B-cell lines found that TRAF3 binding to CD40 inhibits CD40-mediated B-cell activation (47, 51, 54, 80), long-term cell lines by their very nature have aberrant regulation of various homeostatic survival pathways. It was thus not until a conditionally *Traf3*-deleted mouse (*Traf3^{fl/fl}*) was produced that this question could be adequately addressed. When this mouse was bred to the CD19-Cre mouse (81) to produce a strain in which B cells become TRAF3-deficient from the T1 transitional stage of development onward (B-TRAF3^{-/-}), B cells were found to have substantially enhanced homeostatic survival in secondary lymphoid organs (4). The consequences of this abnormally extended B-cell survival include marked increases in the size of lymph nodes and spleen, and infiltration of major organs with B cells, as well as production of autoantibodies and immune complex deposition (4). The increased B-cell survival correlates with constitutive nuclear localization of the NF-κB2 proteins p52 and RelB (4). In the following year, a second group produced an independent strain of CD19-Cre *Traf3^{fl/fl}* mice, which also display the phenotype of increased B-cell survival and constitutive activation of NF-κB2 (5). Interestingly, the initial study showed that TRAF3-deficient B cells have BAFF-independent survival (4), while the second report indicated that such cells have many of the mRNA expression patterns of BAFF-tg mice (5).

It has been assumed that as the NF-κB2 pathway promotes survival (reviewed in 22), constitutive activation of this pathway in TRAF3-deficient B cells is sufficient to explain their increased survival. However, TRAF3-deficient T cells also exhibit elevated nuclear p52 and RelB but do not show enhanced survival (5, 82). Thus, additional B-cell-specific pathways must contribute to TRAF3's regulation of homeostatic survival.

TRAF3 as a B-cell tumor suppressor

In the same month that the initial report of B-cell conditionally TRAF3-deficient mice appeared, several studies reported that loss-of-function *TRAF3* mutations are associated with human multiple myeloma (MM) (83, 84). This strongly suggests that the role of TRAF3 in restraining B-cell survival is highly relevant to the pathogenesis of B-cell malignancies. In addition to MM, *TRAF3* mutations were subsequently found in human B-cell lymphomas (85, 86) and Waldenstrom's macroglobulinemia (87). Consistent with these human findings, ~ 30% of B-TRAF3^{-/-} mice develop B-cell lymphomas as they age (88).

It was a reasonable assumption that the mechanism by which TRAF3 suppresses development of B-cell cancers is by inhibiting NF-κB activation, as the latter is implicated in many types of cancer (89), and particularly those of B cells, which have higher basal NF-κB activation than most cell types (90). The association of signaling complexes containing TRAF3 and NIK with both NIK ubiquitination and degradation and elevated NF-κB2 activation (20) is also consistent with this hypothesis. Indeed, the association of *TRAF3* mutations with MM was discovered as part of a wider picture of multiple MM mutations impacting NF-κB pathways (83, 84), and TRAF3 deficiency leads to the NIK stabilization and constitutive nuclear RelB localization associated with Hodgkin's lymphoma (91). The drug bortezomib is a proteasome inhibitor with multiple effects, one of which is inhibition of NF-κB activation; outcomes of MM treatment with Bortezomib have been correlated with the status of genes known to impact NF-κB activation, including *TRAF3* (92). Thus, taken together, multiple studies provide correlative evidence that TRAF3 prevents B-cell malignancies by inhibiting non-canonical NF-κB2 activation, via promoting the poly-ubiquitination and degradation of NIK.

Studies performed in B lymphocytes, however, reveal that the relationship between TRAF3, NIK, NF-κB2 and receptors such as CD40 and BAFF-R that induce TRAF3 ubiquitination and degradation, is not as simple and linear as it may have initially appeared. This work, discussed above (23) and in a recent review (93), reveals that TRAF3 degradation is neither absolutely necessary nor sufficient to induce NF-κB2 activation. It is thus important to explore other mechanisms by which TRAF3 can regulate the balance between B-cell survival and death. It was recently reported that both primary mouse B cells and human MM cell lines deficient in TRAF3 have elevated expression of the pro-survival kinase Pim-2 (94). Preliminary data from our laboratory also show elevations in the pro-survival Bcl-2 family protein Mcl-1 and the glucose transporter Glut1 in TRAF3^{-/-} B cells, together with increased glucose utilization (N. Mambetsariev, WL, and GB, unpublished data). A novel isoform of the Sox5 protein was also recently reported in cell lines derived from tumors arising in B-TRAF3^{-/-} mice (95). It will be both interesting and important to investigate the various ways in which TRAF3 restrains B-cell survival, including how ubiquitin-related mechanisms might be involved in these pathways.

Mechanisms of functional TRAF3 deficiency in B cells

Studies discussed above indicate that genetic alterations leading to B-cell deficiency in TRAF3 protein result in abnormal B-cell survival. However, the manner in which specific B-cell signaling receptors associate with and modify TRAF3 can also make B cells

functionally deficient in TRAF3 in various intracellular compartments, which could also impact B-cell survival and functions. Sections above discuss the CD40 and BAFF-R-mediated ubiquitination and degradation of TRAF3. This degradation lowers the total cellular content of TRAF3 sufficiently to impact B-cell biological functions (96). Thus, prolonged or abnormal signaling through CD40 or BAFF-R can result in the enhanced survival seen in genetically TRAF3-deficient B cells. In this regard, it is interesting to note that a human CD40 polymorphism resulting in enhanced TRAF3 binding is associated with amplified signaling to B cells (97), and a mutant BAFF-R isolated from a human B-cell lymphoma exhibits both enhanced TRAF3 association and increased B-cell signaling (61).

A related example is provided by the oncogenic Epstein Barr virus-encoded protein, latent membrane protein 1 (LMP1), which mimics CD40 signaling pathways in an amplified manner (reviewed in 98). Although both CD40 and LMP1 bind TRAF3, they utilize TRAF3 in distinct and contrasting ways, that likely contribute to LMP1's implication as playing a pathogenic role in various B-cell malignancies (98). One of the first differences to be observed in B cells was that the CD40-mediated ubiquitination and degradation of TRAF3 is not induced by the binding of TRAF3 to LMP1 (7). However, LMP1 binds TRAF3 with markedly enhanced avidity, compared to CD40, allowing LMP1 to sequester TRAF3 from the B-cell cytoplasm to the plasma membrane (54). Recently, we found that TRAF3-LMP1 binding is associated with increased cellular NIK in B cells (B. Hanson, L. Stunz, P. Bangalore-Prakesh, and GAB, unpublished data). Taken together, these findings suggest that multiple mechanisms can lead to B-cell TRAF3 deficiencies, and that such deficiencies may contribute to many more B-cell malignancies than previously appreciated.

Conclusions and future directions

It is now evident that TRAF3, a pleiotropic signaling protein with multiple context-dependent roles in immune cells, serves as an important inhibitor of both receptor-mediated signals and homeostatic survival in B lymphocytes. The process of post-translational modification by ubiquitination in turn impacts the B-cell functions of TRAF3 in various ways, controlling the total amount of TRAF3 in the cell, and potentially its subcellular localization. Decreases in normal levels of cytoplasmic and/or membrane TRAF3 enhance overall B-cell survival, as well as causing amplification of signals to B cells through TNFR superfamily and innate immune receptors. The modification of TRAF3 can thus profoundly impact B-cell biology.

Many interesting and important questions remain to be answered. TRAF3's restraint of B-cell survival is mediated by both NIK-dependent and NIK-independent pathways; the latter remain to be clearly identified. Additionally, the role of TRAF3 ubiquitination and degradation in these processes needs clarification. The attachment of K48-linked Ub to B-cell TRAF3 clearly contributes to TRAF3 degradation, but the specific biological impact of K63-linked Ub requires further investigation. While the mechanism by which TRAF3 regulates non-canonical NF- κ B2 activation has been intensively studied, how it regulates canonical NF- κ B1 activation, particularly in specific response to signals by TLRs, but not CD40, is less understood. TRAF3's inhibition of and degradation by CD40 and BAFF-R signals in B cells is now well documented, but its potential regulation of other TRAF3-

binding members of the TNFR superfamily expressed by B cells (e.g. TNFR2, TACI, CD30) is not well defined. TRAF3 also serves various innate immune receptors on B cells, which appear to contrast in some cases with TRAF3 roles for such receptors on myeloid cells. This highlights the need to avoid extrapolating findings on TRAF3 functions from one specialized cell type to another, as precedent clearly shows this can lead to erroneous conclusions. There is much yet to learn about this fascinating and multi-faceted immune regulator.

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Table 1
B-cell phenotypes of mice with altered TRAF3 or TRAF3-binding proteins

Strain	B cell phenotype	References
Human TRAF3 tg	Enhanced IgG1 and IgG2b production after TLR4 or TLR9 stimuli	77
Peli1 ^{-/-}	Reduced B-cell activation	25
Peli1 ^{tg}	Increased incidence of B-cell lymphoma	26
B-TRAF2 ^{-/-}	Increased mature splenic B cells; enhanced B-cell survival; constitutive NF-κB2 activation	5
B-TRAF3 ^{-/-}	Increased peripheral B cells; enhanced B-cell survival; constitutive NF-κB2 activation	4,5
B-cIAP1/2 ^{-/-}	Enhanced B-cell survival, constitutive NF-κB2 activation	18
NIK ^{-/-}	Severe impairment in mature B cell numbers; disorganized lymphoid organ structure	99
B-NIK ^{tg}	Increased mature peripheral B cells	100
B-NIK T3 ^{tg}	Increased mature peripheral B cells; hyperactivated NF-κB2 pathway	100
OTUD7B ^{-/-}	Increased B-cell numbers, constitutive NF-κB2 activation	43
B-A20 ^{-/-}	Increased B-cell numbers, hyperactive B-cell responses and increased NF-κB1 activation	40
BAFF-R ^{-/-}	Severe impairment in mature B-cell population	56
BAFF ^{-/-}	Severe impairment in mature B-cell population	55
BAFF ^{tg}	Increased mature B-cell population	57
TACI ^{-/-}	Increased mature B-cell population	66, 67
BCMA ^{-/-}	Lack of long-lived plasma cells	69