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REVIEW

TRAF3 regulation of inhibitory signaling pathways in B and T lymphocytes by kinase and phosphatase localization

Alicia M. Wallis[1] | **Gail A. Bishop**[1,2,3,4]

[1]Graduate Program in Immunology, Iowa City, Iowa, USA

[2]Department of Microbiology & Immunology, The University of Iowa, Iowa City, Iowa, USA

[3]Department of Internal Medicine, The University of Iowa, Iowa City, Iowa, USA

[4]Veterans' Affairs Medical Center, Iowa City, Iowa, USA

Correspondence
Gail A. Bishop, 2296 CBRB, 340 Newton Rd., Dept. of Microbiology & Immunology, The University of Iowa, Iowa City, IA 52242, USA.
Email: gail-bishop@uiowa.edu

Abstract

This brief review presents current understanding of how the signaling adapter protein TRAF3 can both induce and block inhibitory signaling pathways in B and T lymphocytes, via association with kinases and phosphatases, and subsequent regulation of their localization within the cell. In B lymphocytes, signaling through the interleukin 6 receptor (IL-6R) induces association of TRAF3 with IL-6R-associated JAK1, to which TRAF3 recruits the phosphatase PTPN22 (protein tyrosine phosphatase number 22) to dephosphorylate JAK1 and STAT3, inhibiting IL-6R signaling. An important biological consequence of this inhibition is restraining the size of the plasma cell compartment, as their differentiation is IL-6 dependent. Similarly, in T lymphocytes, interleukin 2 receptor (IL-2R) signaling recruits TRAF3, which in turn recruits the phosphatase TCPTP (T cell protein tyrosine phosphatase) to dephosphorylate JAK3. The resulting inhibition of IL-2R signaling limits the IL-2-dependent size of the T regulatory cell (Treg) compartment. TRAF3 also inhibits type 1 IFN receptor (IFNαR) signaling to T cells by this mechanism, restraining expression of IFN-stimulated gene expression. In contrast, TRAF3 association with two inhibitors of TCR signaling, C-terminal Src kinase (Csk) and PTPN22, promotes their localization to the cytoplasm, away from the membrane TCR complex. TRAF3 thus enhances TCR signaling and downstream T cell activation. Implications are discussed for these regulatory roles of TRAF3 in lymphocytes, as well as potential future directions.

KEYWORDS
B cell, signal transduction, T cell, TRAF

1 | TRAF FAMILY MEMBERS

The Tumor-necrosis factor receptor (TNFR) associated factors (TRAF)s are members of a family of adapter proteins, named for their initial identification as associating with TNFR superfamily (TNFRSF) members. TRAF members are characterized as having similar protein structures, which include a TRAF homology domain at the C-terminus and N-terminal really interesting new gene (RING) and zinc (Zn) finger domains.[1–5] There are a few exceptions to this general structural homology; TRAF1 lacks the RING and Zn finger domains, and TRAF7, the most atypical member of the family, lacks the entire TRAFC domain, instead containing a series of WD repeats.[6] It is now known that TRAFs regulate signaling by many other receptor types additional to the TNFRSF, particularly in immune cells. TRAFs also regulate signaling via the T cell antigen receptor (TCR), cytokine receptors, Toll-like receptors (TLR), and other innate immune receptors, such as retinoic acid-inducible gene 1 protein (RIG-I).[7] Owing to the high structural similarity among the TRAF members, as well as their ability to hetero-multimerize, it is common for multiple TRAF proteins to regulate a single signaling pathway. For example, TCR signaling is regulated by both TRAF3 and TRAF6,[6,8] and CD40 signaling is regulated by TRAFs 1, 2, 3, 5 and 6.[9]

Although different TRAF proteins can regulate the same signaling pathway, each can have different roles as an inhibitor or enhancer depending upon their protein-binding partners. The E3 ubiquitin

Abbreviations: Ag, antigen; B-*traf3*$^{-/-}$ mice conditionally deficient in *Traf3* in B, mice conditionally deficient in *Traf3* in B lymphocytes; Csk, C-terminal Src kinase; Erk, extracellular signal-regulated kinase; IFNαR, type 1 IFN receptor; IL-2R, interleukin 2 receptor; IL-6R, interleukin 6 receptor; IRF, IFN response factor; ISGF, IFN stimulated-gene factor; ISRE, IFN-stimulated response element; JAK, janus kinase; LAT, linker of activated T cells; MM, multiple myeloma; NIK, NF-κB inducing kinase; PD-1, programmed cell death protein 1; PTP1B, tyrosine protein phosphatase nonreceptor type 1B; PTPN22, protein tyrosine phosphatase number 22; R, receptor; RING, really interesting new gene; STAT, signal transducer and activator of transcription; T_{con}, conventional T cells; TCPTP, T cell protein tyrosine phosphatase; TNFR, tumor necrosis factor receptor; TNFRSF, TNFR superfamily; TRAF, TNFR associated factor; T_{reg}, T regulatory cell; T-*Traf3*$^{-/-}$, mice conditionally deficient in *Traf3* in T lymphocytes; Tyk2, tyrosine kinase 2; Zap70, zeta chain-associated protein kinase 70.

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ligase capabilities of TRAF proteins suggest additional potential for signaling regulation. Similarly, the function of TRAF proteins is cell type dependent. A striking example is that in B cells, TRAF3 inhibits homeostatic survival, but does not do so in any other immune cell type examined to date, including T cells, dendritic cells, and macrophages.[8,10–12] Each TRAF family member thus has both cell type and receptor-specific roles.

TRAF3 was first discovered in the mid-1990s, when it was identified as a RING and Zn finger-containing protein similar in structure to the already identified TRAF1 and TRAF2 proteins.[13–15] TRAF3 was first shown to associate with the intracellular cytoplasmic domain of CD40, and subsequently with the Epstein Barr virus -encoded latent membrane protein 1 (LMP1), a functional CD40 mimic.[13,14,16,17] A conventional *Traf3*$^{-/-}$ mouse strain developed in the late 1990s displays early neonatal lethality that precluded detailed functional studies. Adoptive transfer experiments using hematopoietic cells from this mouse led to a recipient phenotype of impaired T cell-dependent humoral responses, low blood glucose, and high levels of corticosteroids.[18] While this phenotype suggests role(s) for TRAF3 in neonatal development and in T-dependent B cell responses, early lethality hindered use of *Traf3*$^{-/-}$ mice to further understand TRAF3-regulated immune responses. Further advancements in in vivo models of TRAF3 functions were delayed until the technology became available to delete genes in specific cell types.

To determine the cell type–specific roles of TRAF3 and avoid the early neonatal lethality caused by global lack of TRAF3, conditional gene deletion was used to develop mice in which the *Traf3* gene was flanked by locus of X(cross)-over in P1 (LoxP) sites.[10,19] First produced by the Bishop Lab, this strain was initially bred to mice expressing the *Cre* recombinase gene driven by the B cell-specific *CD19* promoter (B-*traf3*$^{-/-}$).[10] Subsequently, the *Traf3*$^{flox/flox}$ mouse was bred to the CD4-Cre transgenic mouse for the deletion of *Traf3* in T cells (T-*traf3*$^{-/-}$), at the double positive stage in T cell development,[8] to the CD11c-Cre mouse to delete *Traf3* in dendritic cells, and to the Lys-Cre mouse to remove *Traf3* from macrophages.[8,11,12]

One of the most interesting themes to emerge from these studies was the close relationship between TRAF3 and various protein phosphatases in B and T lymphocytes. The following review focuses upon these associations in lymphocytes, their mechanisms, and biological consequences for signaling through both cytokine receptors and the TCR. Future research is aimed to determine the additional regulatory roles of TRAF3 as a promoter of ubiquitination, which is unexplored in the cytokine receptor and TCR-signaling pathways addressed in this review.

An important finding revealed by characterization of B-cell-specific TRAF3-deficient mice was that their B cells exhibit constitutive nuclear localization of the noncanonical NF-κB2 pathway proteins p52 and RelB.[10,19] This results from TRAF3's association with the MAPK NF-κB inducing kinase (NIK) in resting B cells, to which TRAF3 recruits TRAF2 and cellular inhibitor of apoptosis (cIAP). This complex mediates the K48-linked polyubiquitination of NIK, which triggers its proteasomal degradation. The receptors CD40 and BAFF-R recruit TRAFs 2 and 3 to their cytoplasmic domains when ligated, leading to self-ubiquitination and degradation of TRAFs 2 and 3, releasing cytoplasmic NIK to activate noncanonical NF-κB2 signaling. This process has been well-documented over the past decade; for further details, the reader is referred to numerous excellent previous reviews, such as Refs. 20–25. This mechanism does not require TRAF3-mediated alteration of NIK localization, nor interaction with phosphatases, which is the focus of the current review.

While it was initially posited that NIK-mediated NF-κB2 activation completely explains the enhanced survival of TRAF3-deficient B cells, TRAF3-deficient T and myeloid cells also show constitutively activated NF-κB2, but do not exhibit increased viability, proliferation, or survival.[8,11] Thus, TRAF3-mediated regulation of lymphocyte functions must involve pathways additional to NF-κB2. This review focuses upon recent evidence that a key mechanism by which TRAF3 influences negative regulatory signaling pathways is by altering the cellular localization of phosphatases and negative regulatory kinases.

2 | TRAF3-MEDIATED INHIBITION OF IL-6R SIGNALING IN B LYMPHOCYTES

2.1 | Impact of TRAF3 on the plasma cell compartment

B cell-specific TRAF3-deficient mice display enhanced global B cell homeostatic survival, resulting in an increase in B cell numbers and percentages, including a doubling of the plasma cell population.[10] However, there is no apparent additional survival advantage for plasma cells in B-*traf3*$^{-/-}$ mice; crossing B-*traf3*$^{-/-}$ with IL-6-deficient mice decreases normalized plasma cell numbers, but not the increase in total B cells associated with *Traf3* deficiency.[26] These data suggest that interleukin 6 receptor (IL-6R) signaling drives the increased plasma cell phenotype in mice with B cell TRAF3 deficiency, but IL-6 signaling does not play a role in the increased B cell homeostatic survival observed in these mice.[10,26] TRAF3-deficient B cells exposed to LPS in vitro differentiate to plasma cells at a normal rate. However, addition of IL-6 to LPS stimulation recapitulates the in vivo findings of enhanced IL-6-dependent plasma cell differentiation in TRAF3-deficient B cells.[26] These data indicate a regulatory role for TRAF3 in IL-6 signaling, a pathway required for plasma cell differentiation. Interestingly, these findings are also consistent with the loss-of-function mutations in human *TRAF3* often seen in multiple myeloma (MM), a plasma cell malignancy in which IL-6 plays a pathogenic role.[27–29]

2.2 | TRAF3 inhibition of IL-6R signaling to B cells via phosphatase recruitment

Similar to a number of other cytokine receptors, IL-6R signaling proceeds via phosphorylation-induced activation of a Janus family kinase (JAK), and a transcriptional regulator of the Signal Transducer and Activator of Transcription (STAT) family; in the case of IL-6R these are JAK1 and STAT3 (Fig. 1).[30] A previous report, described below, revealed that TRAF3-deficient T cells have enhanced interleukin 2 receptor (IL-2R) signaling because TRAF3 normally recruits T cell protein tyrosine phosphatase (TCPTP) to the IL-2R to reduce JAK3 and STAT5

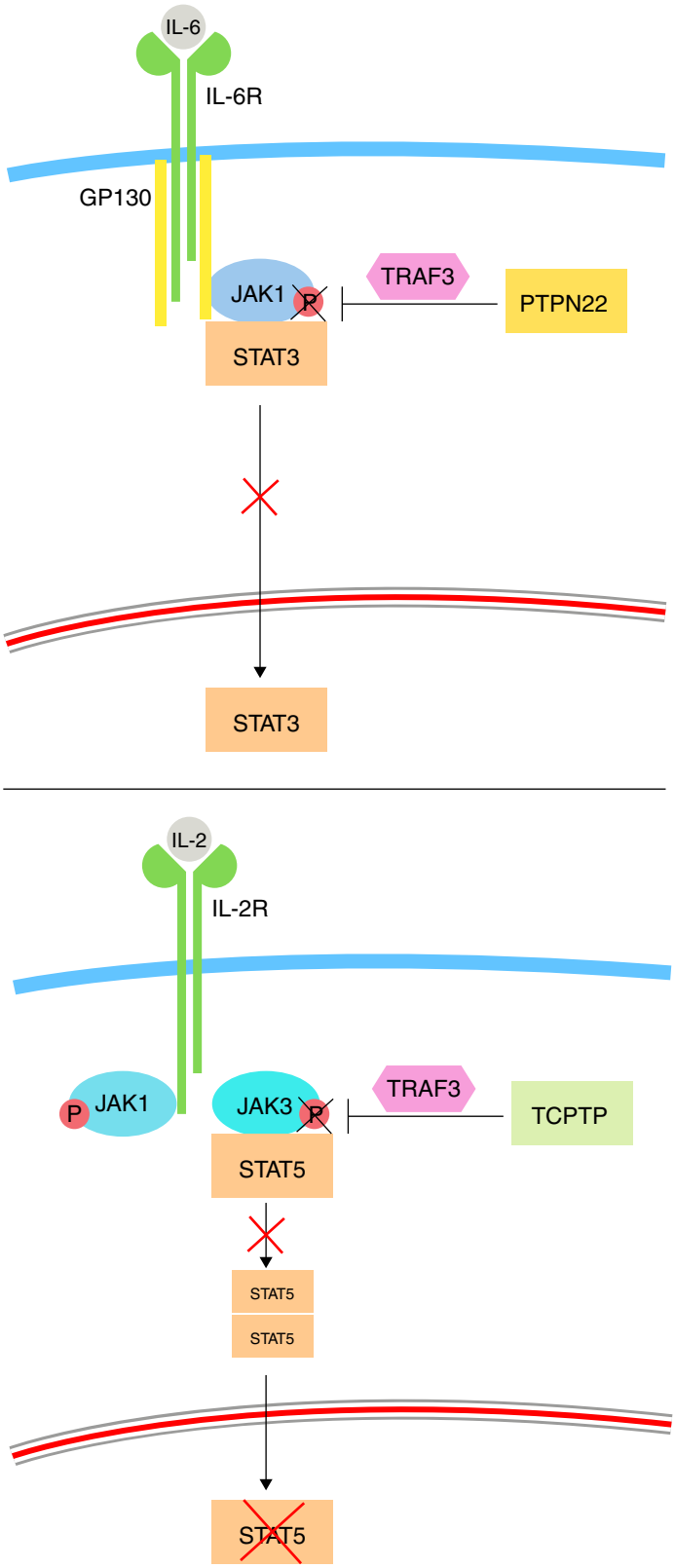

FIGURE 1 **TRAF3 regulation of IL-6/IL-2 signaling**. (Top) Stimulation of the B cell IL-6R results in phosphorylation of STAT3 by JAK1. This leads to STAT3 translocation to the nucleus to regulate transcription of IL-6 response genes. TRAF3 is recruited to JAK1 and the IL-6R following IL-6 stimulation, and TRAF3 subsequently recruits the phosphatase PTPN22 to the complex. PTPN22 then dephosphorylates JAK1 to prevent further phosphorylation of STAT3, effectively inhibiting signaling downstream of JAK1. (Bottom) Stimulation of the T cell IL-2R induces phosphorylation and activation of both JAK1 and JAK3, which in turn phosphorylate STAT5. This results in STAT5 dimerization, allowing the STAT5 complex to translocate to the nucleus. To inhibit the IL-2R signaling pathway, TRAF3 recruits the phosphatase TCPTP to the IL-2R complex to dephosphorylate JAK3, which inhibits the phosphorylation of STAT5 and downstream signaling events

phosphorylation.[31] It was thus reasonable to predict that B cell TRAF3 could limit IL-6R signaling via a similar mechanism. It was also shown previously that exogenously expressed TRAF3 and the phosphatase protein tyrosine phosphatase number 22 (PTPN22) associate in the epithelial cell line HEK293.[32] It was determined that IL-6 signaling to B cells induces TRAF3 to associate with the IL-6R and JAK1, to which TRAF3 recruits PTPN22 to inhibit activation of JAK1 and STAT3; the TRAF3-PTPN22 association requires the TRAF-C domain.[26] PTPN22-deficient mice also display an increased plasma cell compartment, consistent with this mechanism.[26] These findings demonstrated for the first time that PTPN22 is recruited to the IL-6R and revealed a new role and mechanism for TRAF3 in limiting the size of the plasma cell population via phosphatase recruitment.

2.3 | Implications for human disease

It is now well-appreciated that loss-of-function mutations and/or a reduced copy number of the *TRAF3* gene are relatively common in various human B cell malignancies, particularly lymphoma and MM (reviewed in Refs. 33 and 34). B cell-produced autocrine IL-6 has also long been recognized as a pathogenic factor in B cell cancers,[35] particularly MM,[36] consistent with its role as a plasma cell differentiation factor. Thus, the important physiologic role of TRAF3 in restraining B cell IL-6R signaling has significant implications for B cell tumorigenesis.

IL-6R signals to immune cells are also implicated in the pathogenesis of various chronic and acute inflammatory conditions.[29,37] Of particular interest, a human *PTPN22* polymorphism associated with an increased propensity to develop autoimmune disease fails to bind TRAF3.[32] This suggests that TRAF3's inhibitory role in IL-6R signaling is also highly relevant to prevention of chronic inflammation.

3 | TRAF3-MEDIATED INHIBITION OF IL-2R SIGNALING IN T LYMPHOCYTES

3.1 | Impact of TRAF3 on T regulatory cell differentiation

Interestingly, *Traf3* deletion in T cells does not recapitulate the in vivo phenotype of the B-*traf3*$^{-/-}$ mouse. There is no notable increase or decrease in mature CD4$^+$ or CD8$^+$ T cells, nor in their homeostatic survival, in T-*traf3*$^{-/-}$ mice. There is no increase in overall T cell numbers in the T-*traf3*$^{-/-}$ mouse, except for a ~ 2-fold increase in the T regulatory (T_{reg}) population.[8,10] Also, in sharp contrast to the enhanced antibody production of B-*traf3*$^{-/-}$ mice, T-*traf3*$^{-/-}$ mice display markedly deficient in vivo effector functions, including supporting a T-dependent humoral response, and ability to combat infection with an intracellular pathogen.[8]

As noted above, one of the most interesting observations of the T-*traf3*$^{-/-}$ mouse was that it unexpectedly displays a 2–3-fold increase in CD4$^+$Foxp3$^+$ T_{reg} while not altering the number of conventional T cells. The amplified T_{reg} numbers are a consequence of increased thymic T_{reg} development, not improved survival or proliferation, nor from an elevated number of inducible T_{reg} cells. During T_{reg} development, the absence of TRAF3 does not alter the number of T_{reg} precursors, but mature T_{reg} numbers increase following exposure of precursors to IL-2, reaching numbers 2-3-fold greater than that of the respective littermate control populations. Thus, the IL-2-driven maturation of T_{reg} precursors to mature T_{reg} is enhanced in the absence of TRAF3, suggesting that TRAF3 may normally restrain IL-2R signaling to T cells.[31]

3.2 | The mechanistic role of TRAF3 in IL-2R signaling via phosphatase recruitment

Stimulation of the IL-2R activates both JAK1 and JAK3, which in turn phosphorylate and activate STAT5, inducing downstream activation (Fig. 1).[38] In TRAF3-deficient conventional T cells (T_{con}), engagement of the IL-2R leads to enhanced increases in phosphorylation of JAK1, JAK3, and STAT5 compared to littermate control T cells.[31] Increased activation of STAT5 increases the association of STAT5 with the promoters for genes encoding IL-2Rα (CD25) and the cytokine-inducible Src homology 2 domain-containing protein CIS, two known gene targets of STAT5. The increased activation of IL-2R signaling in the absence of TRAF3 suggests that TRAF3 plays an inhibitory role in this pathway.

Stimulation of the IL-2R results in an induced association between TRAF3 and JAK3, indicating that TRAF3 is recruited to the IL-2R.[31] Previous research identified an association between both JAK1 and JAK3 with the TCPTP, which inhibits IL-2R signaling by dephosphorylating JAK1 and JAK3.[39] In the absence of T cell TRAF3, the association between JAK3 and TCPTP does not occur, whereas the association between IL-2R with either JAK1 or JAK3 is unchanged. The association between TRAF3 and TCPTP requires the RING and Zn-finger domains of TRAF3.[31] This is of particular interest, because TRAF3–PTPN22 association utilizes the TRAF-C domain,[26] which is the domain with which TRAFs most frequently associate with other proteins. TCPTP also regulates the phosphorylation of STAT1 induced by IFN-γ stimulation in CD4$^+$ T_{con} cells.[39] In the absence of TRAF3, IFN stimulation also increases the phosphorylation of STAT1,[40] so it will be of interest to determine whether TRAF3 also recruits TCPTP to IFN receptors. Together, these data identified a new inhibitory role for TRAF3 in IL-2R signaling, which is mediated by recruiting TCPTP to the IL-2R complex, where TCPTP dephosphorylates JAK1 and JAK3 to inhibit the downstream IL-2R signaling pathway. Furthermore, the inhibition of TCPTP by TRAF3 results in the proper regulation of T_{reg} development.

4 | TRAF3 REGULATION OF TYPE 1 IFN RECEPTOR (IFNαR) SIGNALING

4.1 | Impact of TRAF3 on IFNαR-mediated PD-1 expression by T lymphocytes

The inhibitory receptor termed programmed cell death protein 1 (PD-1) has garnered considerable recent attention in the T cell activation field. PD-1 is upregulated in activated T cells and serves

as a "checkpoint" to normally restrain T cell activation. Targeting and inhibiting PD-1 has led to dramatic advances in cancer immunotherapy,[41] but how PD-1 is upregulated in T cells is not well understood. Our lab recently identified a role for TRAF3 in regulating PD-1 through the IFNαR signaling pathway. In the absence of TRAF3, naive CD4$^+$ and CD8$^+$ T cells display a 4-fold increase in membrane expression of PD-1.[40] IFNαR signaling is a key pathway known to upregulate PD-1 expression.[42] Upon ligation of IFNαR in the canonical pathway (Fig. 2), JAK1 induced-phosphorylated STAT1 and STAT2 dimerize to form a complex that is released from IFNαR. The cytoplasmic STAT1/2 complex recruits the transcription factor IFN-response factor (IRF) 9 to form the trimolecular complex termed IFN stimulated gene factor (ISGF)3. The ISGF3 complex then translocates to the nucleus where it binds to the DNA-binding sequences called IFN-stimulated-response elements (ISRE), which initiate IFN-induced response genes, including *PCDC1*, the gene encoding PD-1.[42] We hypothesized that the increase in PD-1 expression levels seen in TRAF3-deficient T cells is due to the loss of TRAF3 as an inhibitor of IFNαR signaling.

4.2 | The mechanism for TRAF3 inhibition of IFNαR signaling

IFNαR is a heterodimeric transmembrane receptor composed of two subunits, IFNαR1 and IFNαR2. Binding of type 1 IFN to IFNαR induces multiple signaling pathways, classified as canonical and noncanonical.[43] The canonical pathway is JAK/STAT dependent, whereas the noncanonical pathways signal through either PI3K or MAPK. Both canonical and noncanonical pathways originate from JAK1 activation. As described above, TRAF3 can associate with JAK1,[26] so there is reasonable evidence to predict that TRAF3 regulates both canonical and noncanonical pathways downstream of IFNαR-induced JAK1 signaling. We recently tested this prediction in mouse primary T cells and human T cell lines.

Similar to the inhibitory roles of TRAF3 in B cell IL-6R and T cell IL-2R signaling, the loss of TRAF3 in primary T cells increases canonical IFN-induced STAT1 phosphorylation at both Y701 and S727 sites.[40] Interestingly, a unique feature of TRAF3's regulation of IFNαR signaling is that increases in phosphorylation of STAT1 correlate with increases in total STAT1 protein levels, a feature not observed in TRAF3-mediated regulation of IL-6R or IL-2R signaling. These results were confirmed in a second model system, using CRISPR/Cas9 technology to delete TRAF3 from an immortalized human T cell line (HuT28.11) previously employed to study TRAF3 in T cells.[26] The known association between TRAF3 and phosphatases in both B and T lymphocytes[26,31,44] suggests a potential regulatory role for TRAF3 via phosphatase recruitment to the IFNαR complex after type 1 IFN stimulation. Because TRAF3 regulates PTPN22 in both B[26] and T[44] cells, we first examined if TRAF3 inhibits IFNαR signaling via PTPN22. Stimulation of PTPN22-deficient HuT28.11 cells with type 1 IFN recapitulates the phenotype seen in TRAF3-deficient T cells.[40] STAT1 is a known target for PTPN22, and association between PTPN22 and JAK1 results in JAK1 dephosphorylation, inhibiting downstream signaling.[45] Thus in the absence of TRAF3, we predict that PTPN22 is not recruited efficiently to JAK1, allowing constitutive phosphorylation of JAK1 upon IFNαR activation (Fig. 2).

Alterations in IFNαR signaling in TRAF3-deficient T cells are not restricted to the canonical pathway. T cell proliferation and the activation of extracellular signal-regulated kinase (Erk), a member of the noncanonical MAPK pathway in the IFNαR-signaling pathway, also increase with IFN stimulation in the absence of TRAF3. Interestingly, IFN-induced proliferation and Erk activation in PTPN22-deficient T cells are not detectably different from the response of the HuT28.11 parent cell line, in contrast to the phenotype of the TRAF3-deficient Hut28.11 cells.[40] These data suggest that the regulatory role of TRAF3 in IFN-stimulated MAPK signaling is not completely dependent upon the recruitment of PTPN22, implicating the potential involvement of other phosphatases that inhibit IFNαR signaling.

For example, the IFNαR-associated kinase Tyk2 is deactivated by tyrosine-protein phosphatase nonreceptor type 1B (PTP1B) to inhibit Vav and downstream MAPK signaling.[46] Tyk2-associated PTP1B also dephosphorylates Erk upon IFN stimulation.[47] While the association between TRAF3 and PTP1B is as yet unexplored, it is worth noting that the protein sequence for PTP1B harbors a potential TRAF-binding site (TTQE) at amino acids 164–167.[48] If TRAF3 regulates PTP1B recruitment to the IFNαR complex after stimulation, loss of TRAF3 would result in increased phosphorylation of Tyk2 and Erk. Together, these data present PTP1B as a possible target for TRAF3 regulation of the noncanonical MAPK pathway, a possibility that is the subject of ongoing investigation.

A second IFN-stimulated noncanonical pathway features activation of Akt (Fig. 2). Similar to the canonical pathway, loss of TRAF3 and PTPN22 both increase Akt activation, although TRAF3 deficiency again results in a more marked increase in the phosphorylation of Akt.[40] Because loss of PTPN22 does not induce the same degree of Akt phosphorylation as seen in TRAF3-deficient T cells, this reinforces the concept, discussed above, that TRAF3 employs both PTPN22 and at least one additional phosphatase to inhibit IFNAR signaling. As described in an earlier section, an alternative phosphatase known to be regulated by TRAF3 is TCPTP (also known as PTPN2), which associates with TRAF3 to inhibit both JAK1 and JAK3 in T cell IL-2R signaling.[31] In addition to regulating JAK proteins, TCPTP inhibits Akt activation in the insulin receptor signaling pathway.[49] Thus, TRAF3-mediated regulation of a number of phosphatases has the potential to regulate various pathways downstream of IFNαR in T cells. Further studies are required to determine the T cell phenotypic effect of TRAF3's regulation of IFNαR.

5 | TRAF3 AS AN ENHANCER OF TCR SIGNALING, VIA RESTRAINING THE FUNCTION OF INHIBITORY SIGNALING PROTEINS

5.1 | TRAF3 regulation of TCR signaling

In addition to increased T_{reg} number, the T-*traf3*$^{-/-}$ mouse displays a profound defect in in vivo responses to infection with an intracellular

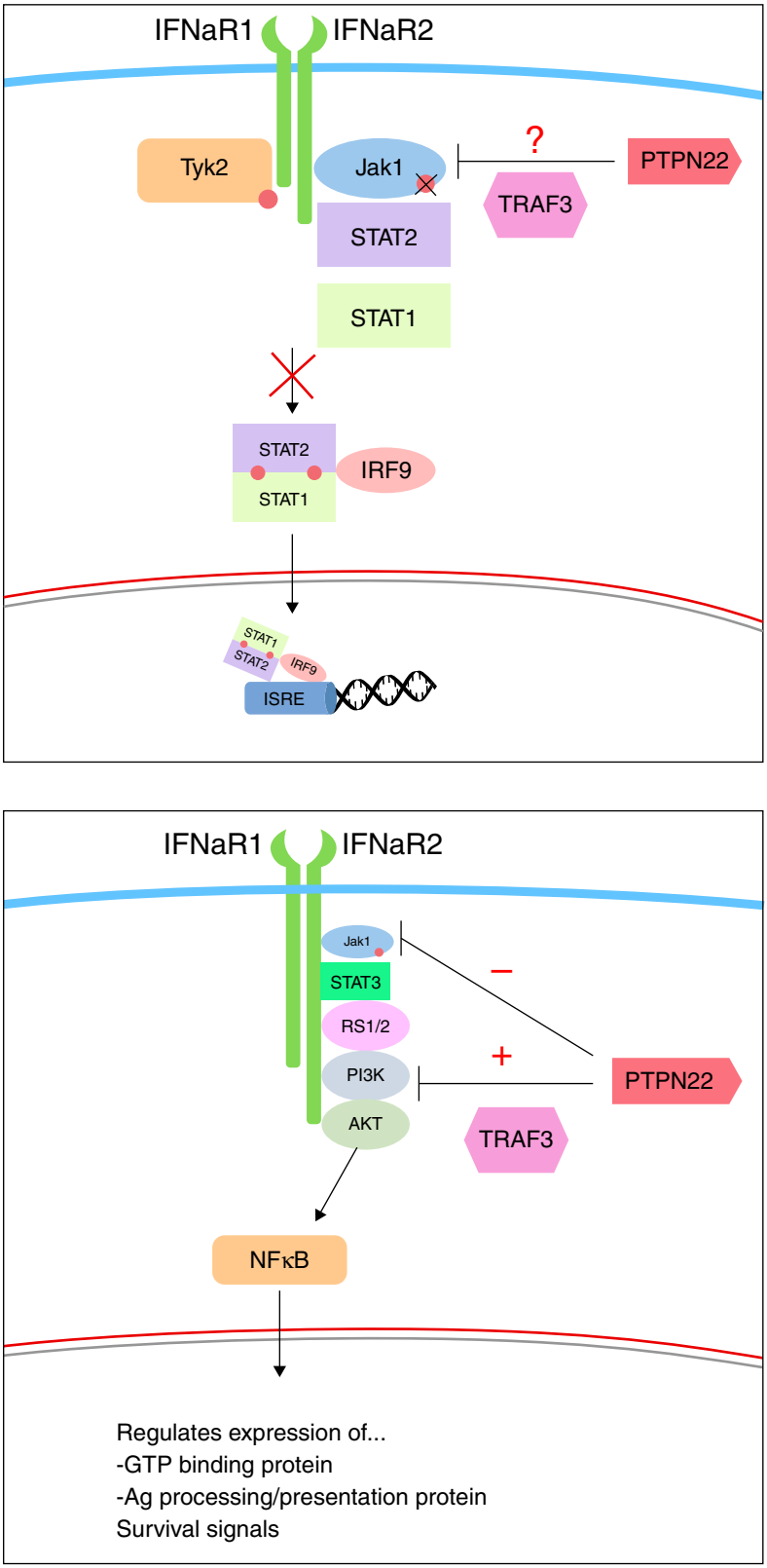

FIGURE 2 **The regulatory roles of TRAF3 in canonical and noncanonical IFNαR signaling.** The IFNαR1/2pathway is initially activated through phosphorylation of JAK1, which phosphorylates STAT molecules. In the canonical pathway (top), this involves STAT1 and STAT2, whose dimerization causes their dissociation from IFNαR. The cytoplasmic STAT1:STAT2 complex recruits IRF9 to form the ISGF3 complex. ISGF3 translocates to the nucleus, where it interacts with the ISRE DNA-binding sequence, inducing IFN response genes. JAK1 activation by IFNAR also leads to the recruitment of PI3K, further stabilized by STAT3 (noncanonical pathway [bottom]). PI3K recruits and activates AKT, engaging the NFκB pathway to regulate transcription of a variety of genes. TRAF3 recruitment to IFNαR inhibits both pathways, by recruiting PTPN22 to the receptor complex, to dephosphorylate key IFNαR signaling proteins, as shown

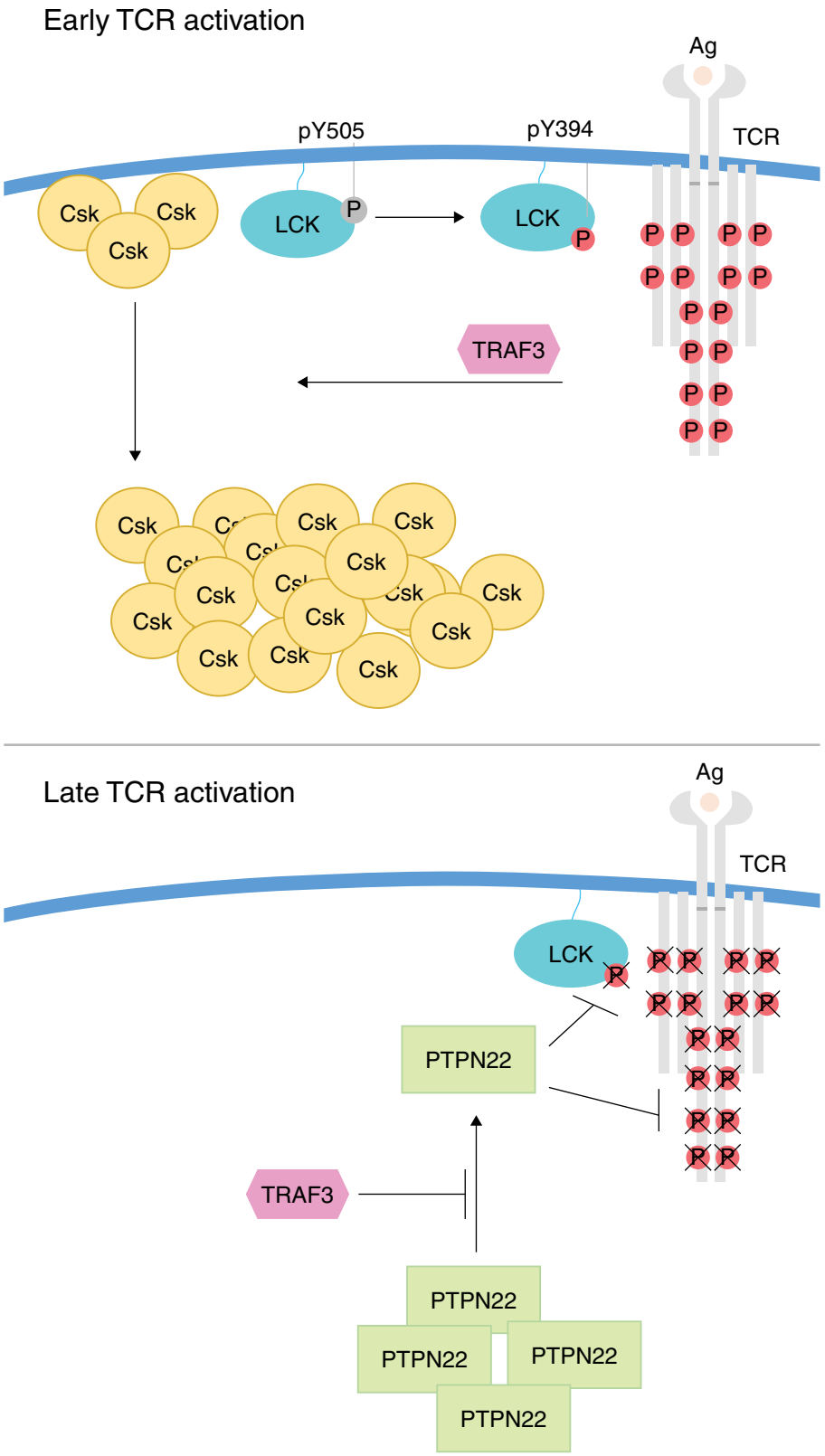

FIGURE 3 **TRAF3 enhancement of TCR/CD28 signaling by regulating the localization of the inhibitors Csk and PTPN22.** TRAF3 enhances TCR signaling via at least two distinct mechanisms. Early in induction of TCR signaling, TRAF3 regulates Csk levels in the plasma membrane by associating with Csk and translocating it to the cytoplasm (Top). This decrease in Csk membrane levels allows for the activation of Lck through decreased phosphorylation of Lck on the inhibitory site Y505. This in turn promotes localization of activated Lck to the TCR complex, enhancing TCR activation. (Bottom) A second mechanism by which TRAF3 promotes TCR/CD28 signaling is by controlling the release of cytosolic PTPN22. TRAF3 controls the release of PTPN22 from the cytoplasm to the membrane, inhibiting PTPN22 from dephosphorylating the TCR signaling proteins Lck and the CD3 subunits of the TCR complex

pathogen, or to immunization with a model T-cell-dependent antigen (Ag).[8] As there are a number of TNFRSF receptors that costimulate the T cell response to Ag (e.g., CD30, CD137, CD120), it was initially thought that this phenotype results from compromised function of these receptors. However, isolated TRAF3-deficient resting T cells from these mice (depleted of T_{reg}) unexpectedly show markedly defective in vitro responses to TCR + CD28 stimulation, with reduced proliferative responses and greatly reduced cytokine production.[10] These surprising results led to the identification of TRAF3 as a TCR/CD28-associating protein. Stimulation of T cells with agonistic anti-CD3 and anti-CD28 antibodies recruits TRAF3 to the TCR/CD28 complex, whereas stimulation of either receptor alone does not result in TRAF3 association. The specific mechanism(s) by which TRAF3 is recruited to the TCR–CD28 complex are not yet defined. Possibilities include direct association with CD28, as well as indirect associations with CD28-interacting proteins, such as linker of activated T cells (LAT), Grb2, and/or Gads. Analysis of TCR-signaling events revealed markedly reduced activation of TCR-signaling proteins, including zeta-chain-associated protein kinase 70 (Zap70), LAT, and Erk in TRAF3-deficient T cells.[8] This defect in TCR signaling in turn results in a profound reduction in differentiation of invariant natural killer T cells in these mice.[50]

These initial findings identified a new role for TRAF3 in T cell activation and function. Further investigation into the molecular mechanisms for TRAF3-mediated enhancement of TCR signaling revealed that TRAF3 regulates the subcellular localization of several key inhibitors of TCR signaling, to enhance TCR signals[44] (Fig. 3). In the absence of TRAF3, T cells have increased membrane levels of C-terminal Src kinase (Csk), which inhibits activation of lymphocyte-specific protein tyrosine kinase (Lck), a key mediator of early TCR signaling. TRAF3 associates with Csk and promotes its dissociation from the plasma membrane after TCR + CD28 signaling. We also identified a regulatory role for TRAF3 in restraining TCR complex interaction with the phosphatase PTPN22. Upon TCR activation, TRAF3 regulates the release of PTPN22 from the cytoplasm to the membrane, where PTPN22 inhibits TCR signaling by dephosphorylating the CD3 subunits of the TCR complex, Lck, and other TCR-signaling proteins. Thus in addition to the previously described inhibitory roles for the associations between TRAF3 and phosphatases, the association between TRAF3 and PTPN22 in TCR signaling plays an *enhancing* role, by inhibiting the movement of PTPN22 to the plasma membrane. Together, the regulation of PTPN22 and Csk by TRAF3 results in proper T cell activation in conventional T cells including response to infection, immunization, and cytokine production. The role of TRAF3 in TCR signaling shows the dynamic roles TRAF3 can play in a variety of signaling pathways in lymphocytes.

5.1.1 | Concluding remarks

The roles of TRAF3 in B and T lymphocyte signaling are clearly diverse and multifaceted, and these regulatory roles can be either enhancing or inhibitory, depending upon receptors, binding partners, and cell types.

The early lethality of whole-mouse deletion of *Traf3* indicates that germline deletions of *TRAF3* will be very rare in humans, and this lethality indicates that TRAF3 performs multiple important functions for multiple cell types. Seven years ago, a single patient with a single-allele putative "dominant negative" *TRAF3* mutation was described. The authors examined skin fibroblasts of this patient, which validated results of prior experiments performed in TRAF3-deficient embryonic fibroblasts, that indicated decreased type I IFN production in response to TLR stimuli in these cells. The patient cells recapitulated this phenotype.[51] There was no characterization of patient B or T lymphocyte functions. The patient was initially identified via an inadequate ability to clear a *Herpes simplex* virus infection; we predict that this was likely due to T cell dysfunction as well as that of myeloid cells, but this is untested. We also predict that this patient could be at increased risk for B cell malignancies with age. Overall, the many roles played by TRAF3 in multiple cell types indicate that systemic targeting of TRAF3 would be dangerous; any such targeting would need to be local in nature.

Findings highlighted here illustrate how TRAF3 recruitment of kinases and phosphatases can have multiple impacts on inhibitory receptor signaling pathways and downstream lymphocyte biology. We believe that the number and diversity of TRAF3 roles in lymphocyte signaling pathways have just begun to be appreciated and understood.

AUTHORSHIP

Conceptualization, writing—original draft, writing—review and editing, A.M.W. and G.A.B; supervision, G.A.B.; funding acquisition, G.A.B. All authors reviewed the manuscript.

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DISCLOSURES

The authors declare no competing financial interests.

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