

Invited Review

TNF Receptor-Associated Factor (TRAF) Signaling Network in CD4⁺ T-Lymphocytes

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CD4⁺ T helper cells (T_H cells), such as T_H1, T_H2, T_H17, T_{FH}, and T_{reg} cells, play critical roles in host defense against infection and in the pathogenesis of immune-mediated diseases. Antigen-presenting cells, such as dendritic cells, deliver three kinds of signals essential for the activation, differentiation, and survival of naïve CD4⁺ T cells: the first signal is transmitted through T-cell receptors (TCRs) providing the specificity of the immune response and initiating the earliest signals leading to T-cell activation, the second signal through costimulatory receptors promoting the survival and clonal expansion of the antigen-primed T cells, and the third signal through cytokine receptors directing the differentiation of naïve CD4⁺ T cells into the various T_H subsets. Tumor necrosis factor receptor (TNFR)-associated factors (TRAFs), which are composed of six TRAF proteins (TRAF1-TRAF6) with a conserved C-terminal TRAF domain, are intracellular signaling adaptors that mediate the link between receptor-proximal activation events and intracellular signaling proteins. There is growing evidence that TRAFs recruited to TCRs, costimulatory TNFRs, and cytokine receptors play crucial roles in key signaling events in CD4⁺ T cells and control the lineage commitment,

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functionality, and life-and-death decisions of different T_H subsets. In this review, we summarize the TRAFs' physiological functions in T-cell immunity and the molecular mechanisms by which TRAFs regulate the three signals required for the activation, differentiation, and survival of $CD4^+$ T cells and other T-cell subsets.

Keywords: $CD4^+$ T cells; signalosome; T-cell biology; TNFR; TRAF

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Introduction

$CD4^+$ T helper cells (T_H cells) are vital for host defense against infection and are prominently involved in the pathogenesis of immune-mediated diseases, such as allergies, inflammatory bowel disease, and rheumatoid arthritis. Naïve $CD4^+$ T cells, which mature in the thymus, patrol secondary lymphoid organs, such as the lymph nodes, spleen, and Peyer's patches in the small intestine. By using α/β T-cell receptors (TCRs), naïve $CD4^+$ T cells scan for specific agonist peptide-major histocompatibility complex (MHC) class II ligands displayed on antigen-presenting

cells (APCs). Upon recognizing their cognate antigen, naïve $CD4^+$ T cells differentiate into effector T_H subsets that control the functions of B cells, macrophages, and $CD8^+$ cytotoxic T cells through cell-to-cell contact or by secreting cytokines.

The primary $CD4^+$ effector cell subsets are T_H type 1 cells (T_H1 cells), T_H type 2 cells (T_H2 cells), interleukin 17 (IL-17)-producing T_H cells (T_H17 cells), follicular helper T cells (T_{FH} cells), and peripherally derived regulatory T cells (p T_{reg} cells) (Fig. 1). For naïve $CD4^+$ T cells to differentiate into effector T_H cells, three signals are required: signal 1 from TCRs, signal 2 from costimulatory receptors, and sig-

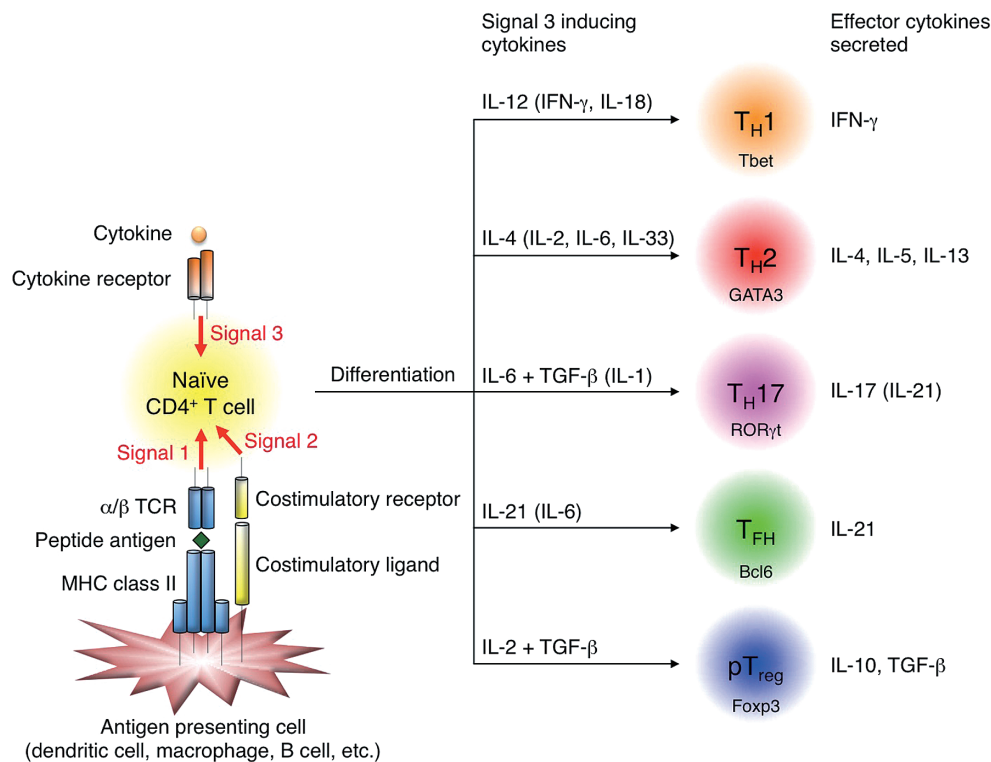


Fig. 1. Three signals required for T helper cell differentiation.

Three distinct signals are required for naïve $CD4^+$ T cells to differentiate into the various functional classes of effector T helper (T_H) cells, including T_H1 , T_H2 , T_H17 , T_{FH} , and p T_{reg} cells. T_H1 cells activate macrophages by secreting IFN- γ , which leads to the killing of intracellular pathogens. T_H1 cells are also responsible for delayed-type hypersensitivity. T_H2 cells contribute to humoral immunity by assisting B cells, and are involved in host defense against extracellular parasites. T_H2 cells potentiate allergic responses and asthma. T_H17 cells promote protective immunity against extracellular bacteria and fungi by secreting IL-17. T_H17 cells are also responsible for autoimmunity and for immune-mediated inflammatory diseases. T_{FH} cells assist B cells and are essential for germinal-center formation, class switching, affinity maturation, and the development of high-affinity antibodies. Lastly, p T_{reg} cells mediate immunosuppression and anti-inflammatory responses by regulatory mechanisms that depend on cell-to-cell contact or the immunoregulatory cytokines IL-10 and TGF- β .

nal 3 from cytokine receptors (Murphy et al. 2007). These three signals activate a variety of intracellular signaling cascades that are essential for inducing lineage-specific transcription factors: Tbet for T_H1 cells, GATA3 for T_H2 cells, ROR γ t for T_H17 cells, Bcl6 for T_{FH} cells, and Foxp3 for pT_{reg} cells (Murphy and Reiner 2002; Weaver et al. 2007; Sakaguchi et al. 2008; Korn et al. 2009; Littman and Rudensky 2010; Zhu et al. 2010; Crotty 2011). Signal 1 provides the specificity of the immune response and initiates the earliest signals leading to T-cell activation. Signal 2, which functions with signal 1 to activate T cells, primarily promotes the survival and clonal expansion of antigen-primed T cells. Signal 3 directs the differentiation of naïve CD4⁺ T cells into the various T_H subsets (Croft 2003; Krogsgaard and Davis 2005; Watts 2005; Yoshimura et al. 2007; Smith-Garvin et al. 2009; Yokosuka and Saito 2009; Nakayama and Yamashita 2010; Oh and Ghosh 2013; Yamane and Paul 2013) (Fig. 1).

Tumor necrosis factor receptor (TNFR)-associated factors (TRAFs) are intracellular signaling proteins that were initially identified as molecules interacting with various members of the TNFR superfamily and that mediate the link between receptor-proximal activation events and intracellular signaling proteins. Six TRAF family proteins, which share a conserved C-terminal TRAF-C domain, have been identified (Inoue et al. 2000; Chung et al. 2002; Ha et al. 2009) (Fig. 2). There is growing evidence that TRAFs recruited to TCRs, costimulatory TNFRs, and cytokine receptors control key signaling events in CD4⁺ T cells and are required for the lineage commitment, functionality, and life-and-death decisions of T_H subsets. The costimulatory TNFRs are composed of 4-1BB, CD27, CD30, DR3, GITR, HVEM, OX40, and TNFR2. The cytokine receptors that recruit TRAFs are receptors for IL-1, IL-2, IL-6, IL-18, IL-33, and TGF- β (Figs. 3 and 4). TRAFs also participate

in the signal transduction by receptors, such as the Toll-like receptors (TLRs), the nucleotide binding-oligomerization domain (NOD)-like receptors (NLRs), and the retinoic acid-inducible gene (RIG)-I-like receptors (RLRs) (Takeda et al. 2003; Ha et al. 2009; Kawai and Akira 2010; Hacker et al. 2011). This review summarizes findings on the importance of TRAF-family proteins in regulating the three critical signals for the activation, differentiation, and survival of conventional CD4⁺ T cells and other T-cell subsets.

1. TRAF structure and function

TRAFs function both as E3 ubiquitin ligases and as adaptor proteins to mediate signal transduction. The TRAF proteins, with the exception of TRAF1 (see Fig. 2), have an N-terminal really interesting new gene (RING)/zinc-finger domain that functions as an ubiquitin E3 ligase to promote the assembly of polyubiquitin chains on signaling proteins. This polyubiquitination is critical for activation of downstream signaling cascades, such as those mediated by the canonical and noncanonical nuclear factor κ B (NF- κ B) and by the mitogen-activated protein kinases (MAPKs), which include the extracellular signal-regulated protein kinase (ERK), c-Jun NH₂-terminal kinase (JNK), and p38 (Chung et al. 2002; Ha et al. 2009; Karin and Gallagher 2009; Hayden and Ghosh 2014).

TRAF proteins also contain a C-terminal coiled-coil (TRAF-N)/TRAF-C domain that contributes to their homo- and hetero-oligomerization and functions in sequence-specific interactions with receptor cytoplasmic tails or intracellular signaling proteins. The coiled-coil domains of the TRAF2 trimer and the TRAF1:(TRAF2)₂ heterotrimer interact with the cellular inhibitor of apoptosis 1 (cIAP1) and cIAP2 of the ubiquitin E3 ligase (Zheng et al. 2010). The TRAF-C domain of TRAF2 recognizes two consensus amino acid motifs: a major motif (P/S/A/T)X(Q/E)E (where

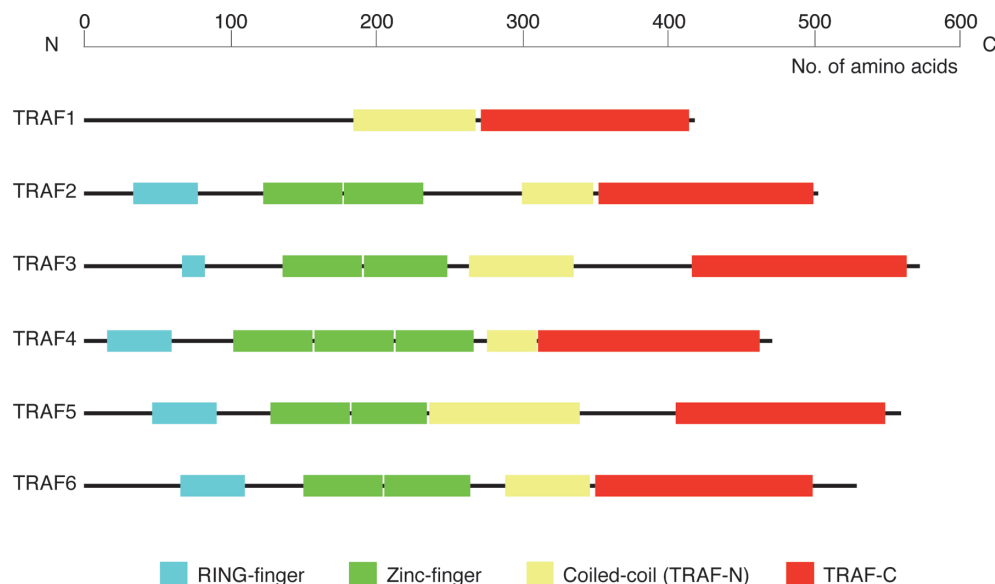


Fig. 2. TRAF-protein domain organization.

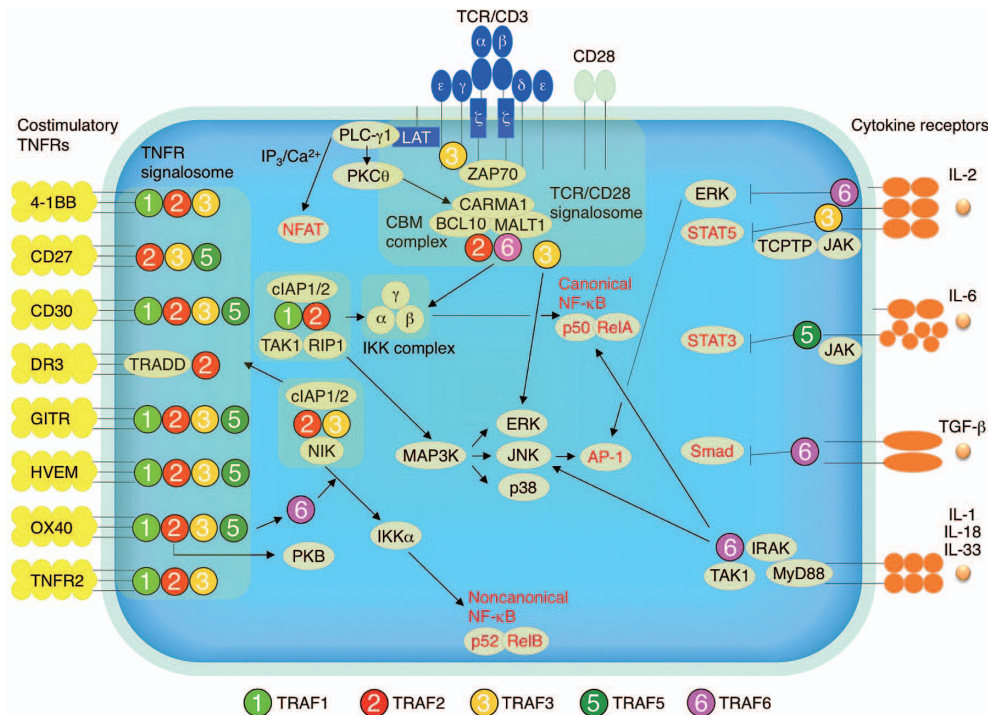


Fig. 3. TRAF proteins regulate signal 1, signal 2, and signal 3.

TRAF2, TRAF3, and TRAF6 control the TCR/CD28 signalosome, which contributes to signal 1 and activates the canonical NF- κ B and MAPK pathways. TRAF1, TRAF2, TRAF3, and TRAF5 control the TNFR signalosome, which contributes to signal 2 and activates the canonical NF- κ B, noncanonical NF- κ B, and MAPK pathways. Some TRAFs control signal 3 via the cytokine receptors for IL-1, IL-2, IL-6, IL-18, IL-33, and TGF- β . TNFRs recruit TRAFs and organize the TNFR signalosome, which helps form the cIAP1/2-TRAF1/2-RIP1-TAK1 complex and activates IKK β , leading to canonical NF- κ B activation. NIK is constitutively degraded in the cIAP1/2-TRAF2/3 complex. The recruitment of TRAF2/3 to TNFRs protects NIK from degradation, which in turn activates IKK α and leads to noncanonical NF- κ B activation.

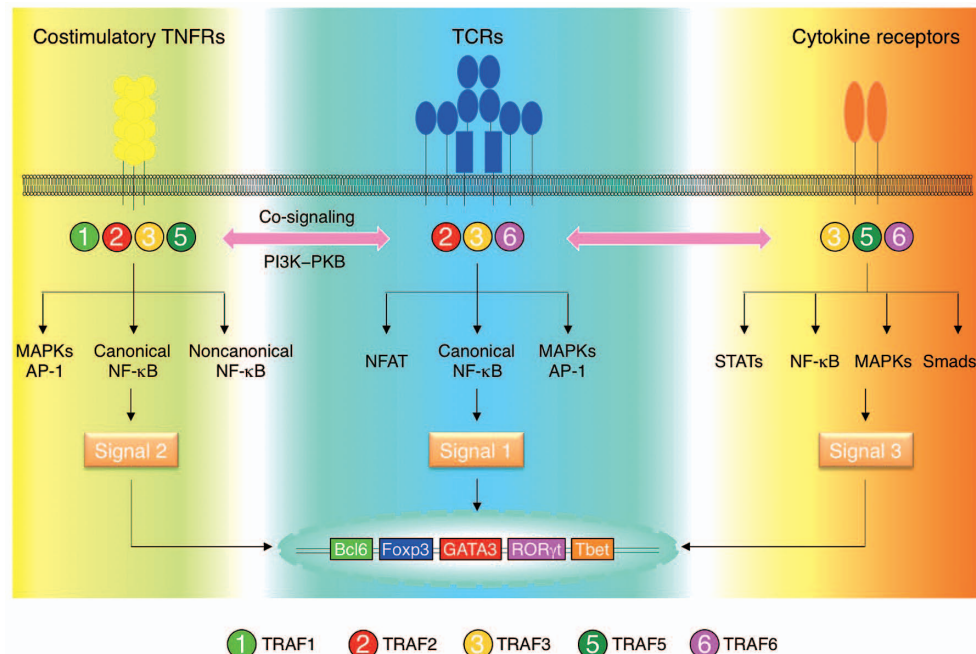


Fig. 4. TRAF signaling network controls signal 1, signal 2, and signal 3 in CD4⁺ T cells.

TRAFs control three signals that are required for naïve CD4⁺ T cells to differentiate into effector T_H cell subsets. These three signals activate a variety of intracellular signaling cascades, such as the canonical NF- κ B, noncanonical NF- κ B, MAPK, NFAT, Smad, and STAT pathways, which are required for inducing lineage-specific transcription factors: Tbet for T_{H1} cells, GATA3 for T_{H2} cells, ROR γ t for T_{H17} cells, Bcl6 for T_{FH} cells, and Foxp3 for pT_{reg} cells.

‘X’ indicates any amino acid) and a minor motif PXQXXD (Ye et al. 1999). Based on their binding specificity for TNFR-family molecules, the TRAF-C domains of TRAF1, TRAF3, and TRAF5 are thought to recognize the major and minor TRAF2-binding motifs (Park et al. 1999; Ye et al. 1999). The TRAF-C domain of TRAF6 recognizes the consensus motif PXEXX(Ar/Ac) (‘Ar’ indicates an aromatic residue such as F, Y, or W; ‘Ac’ indicates an acidic residue such as D or E) (Ye et al. 2002). The TRAF4-binding motif is unknown.

2. Roles of TRAFs in T-cell biology

Studies using mice deficient in one of the individual *Traf* gene (*Traf1* to *Traf6*) have helped elucidate the roles of individual TRAF proteins (Xu et al. 1996; Yeh et al. 1997; Lomaga et al. 1999; Naito et al. 1999; Nakano et al. 1999; Shiels et al. 2000; Tsitsikov et al. 2001; Regnier et al. 2002). Each TRAF molecule differentially regulates intracellular signaling that critically influences the development, homeostasis, and functionality of T cells (Table 1). We firstly summarize phenotypes of T cells derived from respective *Traf*^{-/-} mice.

2.1. TRAF1

TRAF1 is required for maximal CD8⁺ T-cell responses to influenza virus (Sabbagh et al. 2006). *Traf1*^{-/-} memory CD8⁺ T cells cannot efficiently control the lymphocytic choriomeningitis virus (LCMV) in chronic stages of infection (Wang et al. 2012). TRAF1 exhibits a prosurvival effect in CD8⁺ T cells via 4-1BB-mediated upregulation of the prosurvival Bcl-x_L and 4-1BB-ERK-dependent down-regulation of the proapoptotic Bim (Sabbagh et al. 2008). These results demonstrate that the 4-1BB-TRAF1 signaling is critical for the effector function and long-term survival of antigen-primed CD8⁺ T cells.

2.2. TRAF2

Traf2^{-/-} mice have an inflammatory phenotype with elevated serum TNFα, and age and die prematurely. The survival of *Traf2*^{-/-} mice is markedly improved by eliminating TNFα signaling (Yeh et al. 1997; Nguyen et al. 1999). However, *Traf2*^{-/-}*Tnfa*^{-/-} mice still develop fetal autoimmune inflammation via constitutive activation of noncanonical NF-κB, whose activation is tightly controlled by NF-κB inducing kinase (NIK). The inflammatory phenotype of *Traf2*^{-/-}*Tnfa*^{-/-} mice is CD4⁺ T-cell intrinsic, and the constitutive activation of noncanonical NF-κB in *Traf2*^{-/-}*Tnfa*^{-/-} CD4⁺ T cells drives the expansion of pathogenic T_H1 and T_H17 cells (Lin et al. 2011). The colonic lamina propria of *Traf2*^{-/-}*Tnfr1*^{-/-} mice contains higher numbers of T_H17 cells (Piao et al. 2011). The early death of *Traf2*^{-/-}*Tnfa*^{-/-} mice can be rescued by deletion of one NIK allele (Lin et al. 2011), indicating that the TRAF2-mediated negative control of NIK/noncanonical NF-κB signaling is critical for both embryogenesis and preventing harmful inflammation driven by pathogenic CD4⁺ T cells.

In *ΔT-Traf2*^{-/-} mice, whereas the number of splenic CD4⁺ T cells are unchanged, the number of CD8⁺ T cells is reduced by ~50%, i.e., ~50% reduction in naïve (CD44^{low}CD62L^{high}) CD8⁺ T cells, ~40% reduction in effector memory (T_{EM}, CD44^{high}CD62L^{low}) CD8⁺ T cells, and ~70% reduction in central memory (T_{CM}, CD44^{high}CD62L^{high}) CD8⁺ T cells. *ΔT-Traf2*^{-/-} CD8⁺ T cells are defective in the lymphopenia-induced proliferation mediated by homeostatic cytokines IL-15, showing a critical role for TRAF2 in the maintenance of peripheral T_{CM} CD8⁺ T cells by modulating sensitivity to IL-15 (Villanueva et al. 2015).

2.3. TRAF3

The antigen-specific T-cell response is impaired in *Traf3*^{-/-} chimeric mice generated by reconstituting *Traf3*^{-/-} liver stem cells in irradiated wild-type mice (Xu et al. 1996). The population of Foxp3⁺ T_{reg} cells is increased two- to three-fold in T-cell specific *Traf3*^{-/-} mice (*ΔT-Traf3*^{-/-} mice) (Xie et al. 2011; Yi et al. 2014a), and *ΔT-Traf3*^{-/-} mice show defective immunity to *Listeria monocytogenes* (Xie et al. 2011).

The number of T_{EM} CD4⁺ T cells is increased two-fold in *ΔT-Traf3*^{-/-} mice, with a corresponding decrease in the number of naïve CD4⁺ T cells. In contrast, the number of T_{CM} CD8⁺ T cells is reduced by ~90% due to impaired responsiveness to IL-15, although the numbers of T_{EM} CD8⁺ T cells and naïve CD8⁺ T cells are unchanged (Yi et al. 2014b; Villanueva et al. 2015).

The Foxp3⁺ T_{reg} cells from T_{reg}-specific *Traf3*^{-/-} (*ΔT_{reg}-Traf3*^{-/-}) mice underexpress ICOS, a costimulatory receptor that mediates T_{reg}-specific functions and IL-10 induction. This reduction in ICOS causes altered CD4⁺ T cell homeostasis and exaggerated humoral immune responses. The frequency of T_H1 type of T_{EM} CD4⁺ T cells in the peripheral lymphoid organs, such as lamina propria of the small and large intestines, is increased in *ΔT_{reg}-Traf3*^{-/-} mice. Upon immunization with red blood cells from sheep (SRBCs), *ΔT_{reg}-Traf3*^{-/-} mice exhibit a defect in production of follicular T_{reg} (T_{FR}) cells, which are localized in the B cell follicles and specialized for the control of germinal center (GC) reactions. The T_{FH} cells from *ΔT_{reg}-Traf3*^{-/-} mice immunized with SRBCs express higher levels of cytokines, such as IL-4, IL-10, IL-17, and IFN-γ, and of transcription factors, such as Bcl6, GATA3, and RORγt. These dysfunctional T_{FH} cells promote the formation of GCs and production of high-affinity antibodies, indicating that TRAF3 is required for maintaining the ICOS expression in T_{reg} cells and that the ICOS deficiency compromises the induction of T_{FR} cells and the control of GC reactions (Chang et al. 2014).

These studies demonstrate that TRAF3 plays diverse roles in the homeostasis and activation of peripheral CD4⁺ and CD8⁺ T-cell subsets.

2.4. TRAF4

Lymph node T cells from *Traf4*^{-/-} mice proliferate and

Table 1. Phenotypes of TRAF KO mice and T cells.

TRAFs	Phenotypes of germline KO mice	References	T cell types	Phenotypes of TRAF KO T cells	References	
TRAF1	No apparent defects	Tsitnikov et al. 2001	CD3 (germline <i>Traff1</i> ^{-/-})	↑ Anti-CD3-mediated proliferation; ↑ TNFR2-mediated signaling	Tsitnikov et al. 2001	
	Normal T-dependent humoral response		CD4 (germline <i>Traff1</i> ^{-/-})	↑ Antigen-dependent proliferation; ↑ T _H 2	Bryce et al. 2006	
			CD8 (germline <i>Traff1</i> ^{-/-})	↓ Cell survival; ↓ T-cell immunity to influenza virus	Sabbagh et al. 2006	
			CD8 (germline <i>Traff1</i> ^{-/-})	↓ 4-1BB-mediated ERK (↓ Bcl-x _i ; ↑ Bim)	Sabbagh et al. 2008	
			CD8 (germline <i>Traff1</i> ^{-/-})	↓ 4-1BB-mediated canonical NF-κB; ↑ Noncanonical NF-κB	McPherson et al. 2012	
TRAF2	Progressively runted and die within weeks after birth	Yeh et al. 1997	CD8 (germline <i>Traff1</i> ^{-/-})	↑ T-cell immunity to lymphocytic choriomeningitis virus (LCMV)	Wang et al. 2012	
			Thymocytes (germline <i>Traff2</i> ^{-/-})	↑ TNFα-mediated apoptosis	Yeh et al. 1997	
			CD3 (germline <i>Traff2</i> ^{-/-})	↓ 4-1BB-mediated IL-2	Saouli et al. 1998	
			CD3 (<i>Lck-Cre/Traff2</i> ^{lox/lox})	↑ Noncanonical NF-κB	Gardam et al. 2008	
	Elimination of TNF signaling partially rescues the lethal phenotype of <i>Traff2</i> ^{-/-} mice		CD4 (germline <i>Traff2</i> ^{-/-} <i>Tnfr1</i> ^{-/-})	Dysregulated T cell homeostasis; ↑ T _H 1/T _H 17	Lin et al. 2011	
TRAF3	Progressively runted and die within weeks after birth	Nguyen et al. 1999	CD4 (germline <i>Traff2</i> ^{-/-} <i>Tnfr1</i> ^{-/-})	↓ Anti-CD3/CD28-mediated canonical NF-κB; ↑ Noncanonical NF-κB	Lin et al. 2011	
	↑ T-dependent humoral response (germline <i>Traff2</i> ^{-/-} <i>Tnfr1</i> ^{-/-} ; <i>Traff2</i> ^{-/-} <i>Tnfr2</i> ^{-/-})		CD4 (germline <i>Traff2</i> ^{-/-} <i>Tnfr1</i> ^{-/-})	↑ T _H 17 in the colon	Piao et al. 2011	
			CD8 (<i>Lck-Cre/Traff2</i> ^{lox/lox})	↓ Lymphopenia-induced proliferation; ↓ CD44 ^{hi} CD122 ⁺ (T _{reg}) T cells; ↓ IL-15-mediated proliferation	Villanueva et al. 2015	
			Thymocytes (<i>Lck-Cre/Traff2</i> ^{lox/lox})	↑ PKB	Villanueva et al. 2015	
			CD3 (germline <i>Traff3</i> ^{-/-})	↑ Antigen-specific T cell response; ↓ T-dependent humoral response	Xu et al. 1996	
TRAF4	Progressively runted and die within weeks after birth	Xu et al. 1996	CD3 (<i>Lck-Cre/Traff3</i> ^{lox/lox})	↑ Noncanonical NF-κB	Gardam et al. 2008	
			CD3 (<i>Od4-Cre/Traff3</i> ^{lox/lox})	↓ Anti-CD3/CD28-mediated proliferation, cytokines, ZAP70, LAT, PLC-γ1, ERK	Xie et al. 2011	
			CD3 (<i>Od4-Cre/Traff3</i> ^{lox/lox})	↓ T-cell immunity to <i>Listeria monocytogenes</i> ; ↓ T-dependent humoral response	Xie et al. 2011	
			CD4 (<i>Foxp3</i> ^{3rd-Cre/Traff3^{lox/lox})}	↓ ICOS, IL-10; ↓ T _{reg} function; ↓ follicular T _{reg} cells; ↓ Anti-CD3/CD28-mediated ERK, AP-1	Chang et al. 2014	
			CD4 (<i>Od4-Cre/Traff3</i> ^{lox/lox})	↑ IL-2-mediated JAK1, JAK3, STAT5; ↑ Foxp3 ⁺ T _{reg} cells	Yi et al. 2014a	
TRAF5	1/3 of mice die before birth and 2/3 of mice show defects	Shiels et al. 2000 Regnier et al. 2002	CD3 (<i>Od4-Cre/Traff3</i> ^{lox/lox})	↑ T _H 1 CD4 ⁺ T cells; ↓ T _H 1 CD8 ⁺ T cells; ↓ IL-15-mediated STAT5, ERK	Yi et al. 2014b	
	Normal T-dependent humoral response		CD8 (<i>Lck-Cre/Traff3</i> ^{lox/lox})	↓ CD44 ^{hi} CD122 ⁺ (T _{reg}) T cells	Villanueva et al. 2015	
			CD3 (germline <i>Traff4</i> ^{-/-})	Normal anti-CD3/CD28-mediated proliferation and cytokines	Cheriflis-Vicini et al. 2008	
TRAF6	No apparent defects	Nakano et al. 1999	Thymocytes (germline <i>Traff5</i> ^{-/-})	↓ CD27-mediated proliferation	Nakano et al. 1999	
	A mild defect in T-dependent humoral response		CD4 (germline <i>Traff5</i> ^{-/-})	↑ T _H 2	So et al. 2004	
			CD4 (germline <i>Traff5</i> ^{-/-})	↓ GITR-mediated proliferation, IL-2, canonical NF-κB, p38, ERK	Esparza et al. 2006	
			CD8 (germline <i>Traff5</i> ^{-/-})	↓ Cell survival; ↓ T-cell immunity to <i>Listeria monocytogenes</i>	Kraus et al. 2008	
			CD4 (germline <i>Traff5</i> ^{-/-})	↑ IL-6-mediated STAT3; ↑ T _H 17	Nagashima et al. 2014	
TRAF6	Perinatal and postnatal lethality	Lomaga et al. 1999 Naïto et al. 1999	Thymocytes (germline <i>Traff6</i> ^{-/-})	↓ IL-1α-mediated proliferation	Naïto et al. 1999	
			CD4 (germline <i>Traff6</i> ^{-/-})	↑ T _H 2; ↓ IL-18-mediated IFN-γ in T _H 1 cells	Chiffolleau et al. 2003	
			CD4 (<i>Od4-Cre/Traff6</i> ^{lox/lox})	Dysregulated T cell homeostasis; ↑ T _H 2; ↑ Autoantibodies; ↑ PI3K-PKB	King et al. 2006	
			CD8 (<i>Od4-Cre/Traff6</i> ^{lox/lox})	↓ Cell survival; ↓ Fatty acid metabolism	Pearce et al. 2009	
			CD4 (<i>Od4-Cre/Traff6</i> ^{lox/lox})	↑ TGF-β-mediated Smad2/3; ↑ T _H 17	Cejas et al. 2010	
			Thymocytes (germline <i>Traff6</i> ^{-/-})	↑ IL-2-mediated JAK, ERK, AP-1	Motegi et al. 2011	
			Thymocytes (germline <i>Traff6</i> ^{-/-})	↓ Foxp3 ⁺ T _{reg} cells	Shimo et al. 2011	
			CD4 (<i>Od4-Cre/Traff6</i> ^{lox/lox})	↑ T _H 9; ↓ noncanonical NF-κB	Xiao et al. 2012	
			CD4 (<i>Foxp3</i> ^{3rd-Cre/Traff6^{lox/lox})}	↓ Foxp3 expression; ↑ T _H 2	Muto et al. 2013	
			CD8 (<i>Od4-Cre/Traff6</i> ^{lox/lox})	↓ Lymphopenia-induced proliferation; ↓ IL-18-mediated proliferation	Walsh et al. 2014	
			CD3, CD4 and CD8 T cells	↑, Enhanced; ↓, Reduced		

produce cytokines normally in response to anti-CD3/CD28, IL-2, or phytohemagglutinin (PHA), suggesting that TRAF4 does not play a major role in T-cell responses (Cherfils-Vicini et al. 2008).

2.5. TRAF5

Traf5^{-/-} mice are more susceptible to developing an asthma-like T_H2 phenotype when systemically immunized with ovalbumin (OVA) in alum adjuvant and then subjected to airway challenge with aerosolized OVA (So et al. 2004). The ability of *Traf5*^{-/-} naïve CD4⁺ T cells to differentiate into T_H17 cells is enhanced by IL-6. Accordingly, the T_H17-cell-associated experimental autoimmune encephalomyelitis (EAE) is exaggerated in *Traf5*^{-/-} mice (Nagashima et al. 2014). These results demonstrate that TRAF5 limits the differentiation of inflammatory CD4⁺ T cells such as T_H2 and T_H17 cells.

Antigen-specific CD8⁺ T-cell expansion and memory formation are defective in *Traf5*^{-/-} mice infected with *Listeria monocytogenes*. The effect of TRAF5 on the antibacterial immunity is T-cell intrinsic, and TRAF5 has been suggested to promote the prosurvival signals mediated by CD27 (Kraus et al. 2008).

2.6. TRAF6

Traf6^{-/-} chimeric mice, which are generated by reconstituting *Traf6*^{-/-} fetal liver cells in irradiated wild-type mice, develop a lethal inflammatory disease mediated by activated T_H2 cells (Chiffolleau et al. 2003). Accordingly, Δ T-*Traf6*^{-/-} mice develop systemic inflammatory disease, with high counts of activated T_{EM} CD4⁺ T cells that produce large amounts of T_H2 cytokines. Humoral immunity is also enhanced in Δ T-*Traf6*^{-/-} mice, which have elevated serum levels of immunoglobulin M (IgM), IgG, IgE, and anti-DNA autoantibodies. In Δ T-*Traf6*^{-/-} CD4⁺ T cells, the phosphorylation of protein kinase B (PKB or Akt) and of p85, the regulatory subunit of phosphoinositide 3-kinase (PI3K), is enhanced, suggesting that TRAF6 regulates peripheral tolerance through inhibiting the hyperactivation of the PI3K-PKB pathway (King et al. 2006).

OX40 signaling via TRAF6 promotes the differentiation of IL-9-producing T_H9 cells, which enhance allergic airway inflammation. OX40 cosignaling upregulates TRAF6, which does not directly bind OX40, and this event augments NIK expression leading to noncanonical NF- κ B activation. TRAF6 deficiency abrogates the NIK/noncanonical NF- κ B-dependent T_H9 differentiation, indicating that TRAF6 downstream of OX40 supports the differentiation of T_H9 cells through activation of the noncanonical NF- κ B pathway (Xiao et al. 2012).

Treg-specific *Traf6*^{-/-} (Δ Treg-*Traf6*^{-/-}) mice develop allergic skin diseases, arthritis, and lymphadenopathy. Under lymphopenic conditions, the Foxp3⁺ T_{reg} cells from Δ Treg-*Traf6*^{-/-} mice rapidly lose Foxp3 and become exFoxp3 cells. These exFoxp3 cells can become T_H1, T_H2, and T_H17 effector cells and produce proinflammatory cyto-

kines, indicating that TRAF6 is required to stabilize the Foxp3 expression (Muto et al. 2013).

Δ T-*Traf6*^{-/-} CD8⁺ T cells exhibit altered expression of genes that regulate fatty acid metabolism and display impaired memory T-cell development after infection with *Listeria monocytogenes*, indicating that TRAF6 has a critical role after infection by regulating a metabolic switch in CD8⁺ T cells that promotes survival and development into long-lived memory T cells (Pearce et al. 2009). Δ T-*Traf6*^{-/-} naïve CD8⁺ T cells are also defective in the lymphopenia-induced proliferation mediated by homeostatic cytokines IL-7 and IL-18, showing a critical role for TRAF6 in the homeostatic proliferation of CD8⁺ T cells (Walsh et al. 2014).

These studies demonstrate that TRAF6 signaling provides T cells with critical signals needed for the homeostasis and activation of peripheral CD4⁺ and CD8⁺ T-cell subsets.

3. TRAFs regulate signal 1, signal 2, and signal 3

Each TRAF-family molecule is differentially incorporated into the signaling complexes (signalosomes) of various TCRs, costimulatory TNFRs, cytokine receptors, and is involved in the spatiotemporal regulation of T-cell signaling (Figs. 3 and 4, and Table 1).

3.1. TRAFs control TCR signaling (signal 1)

The TCR signalosome, which is composed of co-receptors, kinases, phosphatases, and adaptors, transmits antigen-specific first signals into T cells. TRAF2, TRAF3, and TRAF6 regulate the signaling activity of the TCR signalosome that is critical for the activation of transcription factors, such as activation protein-1 (AP-1), nuclear factor of activated T cells (NFAT), and canonical NF- κ B (Figs. 3 and 4).

It might be important to note that T cells purified from *Traf1*^{-/-}, *Traf2*^{-/-}, *Traf6*^{-/-} or TRAF2 dominant-negative transgenic (TRAF2.DN-Tg) mice exhibit enhanced proliferation in response to anti-CD3 mAb or its cognate antigens *in vitro* (Saoulli et al. 1998; Tsitsikov et al. 2001; Chiffolleau et al. 2003; Bryce et al. 2006; King et al. 2006; Motegi et al. 2011). The enhanced response has been suggested to be due to altering thresholds for signals generated by TCR stimulation or cell cycle activity in T cells that have developed in the absence of particular TRAF molecules.

3.1.1. TRAF2 and TRAF6 in signal 1

TRAF2 and TRAF6 exert a significant impact on the TCR-induced canonical NF- κ B activation. The TCR signalosome induces the formation of the CARMA1-BCL10-MALT1 (CBM) complex; in turn, CBM complex recruits the IKK (I κ B kinase) complex, which consists of two catalytic subunits (IKK α and IKK β) and a regulatory subunit (IKK γ), to activate the canonical NF- κ B. Within the CBM complex, TRAF6 associates with MALT1, which contains three potential TRAF6-binding sites (Thome 2008), and

mediates the Lys63 (K63)-linked polyubiquitination of MALT1 and BCL10, thereby promoting the recruitment of the IKK complex via the ubiquitin-binding IKK γ subunit and the ubiquitination of IKK γ by TRAF6 (Sun et al. 2004; Oeckinghaus et al. 2007; Thome et al. 2010).

In *Traf2*^{-/-} CD4⁺ T cells, the canonical NF- κ B activation downstream of TCR engagement is severely impaired (Lin et al. 2011). In Jurkat T cells, silencing *TRAF2* and *TRAF6* expression through RNA interference inhibits the canonical NF- κ B activation and IL-2 production mediated by anti-CD3/CD28 (Sun et al. 2004). However, the canonical NF- κ B in mature Δ T-*Traf6*^{-/-} CD4⁺ T cells and *Traf6*^{-/-} CD4⁺ thymocytes shows no apparent deficiency (King et al. 2006; Motegi et al. 2011). The discrepancy between these results could be due to the expression levels of TRAF6 in T cells, i.e., Jurkat T cells constitutively express TRAF6, whereas mouse primary T cells express low amounts of TRAF6 in the steady states and rapidly upregulate TRAF6 in response to anti-CD3/CD28. These studies show that TRAF2 or TRAF6 incorporated into the TCR signalosome promotes the activation of canonical NF- κ B pathway.

3.1.2. TRAF3 in signal 1

In contrast to TRAF2 and TRAF6, TRAF3 shows an inhibitory function in the TCR signaling. CD4⁺ or CD8⁺ T cells from Δ T-*Traf3*^{-/-} mice display an impaired proliferative response and decreased IL-2, IL-4, IFN- γ , and TNF α production in response to anti-CD3 (or anti-CD3/CD28). T cells stimulated with anti-CD3/CD28 induce the phosphorylation of early TCR-signaling molecules, such as ZAP-70, LAT, PLC- γ 1, and ERK; this phosphorylation is defective in Δ T-*Traf3*^{-/-} T cells. However, the canonical NF- κ B activation is not affected by the TRAF3 deficiency. In wild-type T cells stimulated with anti-CD3/CD28, TRAF3 is co-immunoprecipitated with the CD3/CD28 complex but not with either CD3 or CD28 alone (Xie et al. 2011), indicating that TRAF3 promotes signal 1 and is part of the TCR/CD28 signalosome. A putative TRAF3-binding site in CD28's cytoplasmic tail, ²⁰³PYQPYA²⁰⁸, has been suggested to be critical for this binding (Xie et al. 2011).

3.2. TRAFs control costimulatory TNFR signaling (signal 2)

Costimulatory receptors are structurally categorized into at least two major families: the Ig superfamily, of which the best characterized molecule is CD28, and the TNFR superfamily, which includes 4-1BB, CD27, CD30, DR3, GITR, HVEM, OX40, and TNFR2. Unlike CD28, which binds PI3K p85, TNFR-family molecules do not directly bind protein kinases, but rather transduce signals through TRAFs. When bound by specific ligands, these costimulatory TNFRs recruit TRAF1, TRAF2, TRAF3, and TRAF5 (Figs. 3 and 4). The cytoplasmic tails of these TNFRs contain TRAF-binding motifs: in 4-1BB, ²³⁶QEED²³⁹ and ²⁴⁷EEEE²⁵⁰ (Arch and Thompson 1998; Jang et al. 1998); in CD27, ²⁴⁶PIQED²⁵⁰ (Akiba et al. 1998;

Yamamoto et al. 1998); in CD30, ⁵⁵⁸PHYPEQET⁵⁶⁵ and ⁵⁷⁶MLSVEEEGKED⁵⁸⁶ (Gedrich et al. 1996; Lee et al. 1996; Aizawa et al. 1997; Boucher et al. 1997; Duckett et al. 1997); in GITR, ²¹¹STED²¹⁴ and ²²¹PEEE²²⁴ (Kwon et al. 1999; Esparza and Arch 2005); in HVEM, ²⁶⁴VTTVAVEET²⁷² (Hsu et al. 1997); in OX40, ²⁶¹TPIQEEQAD²⁶⁹ (Arch and Thompson 1998; Kawamata et al. 1998); and in TNFR2, ⁴¹⁷KDEQVPFSKEECA⁴³⁰ and ⁴⁵⁰PLGVDPAGMK⁴⁵⁹ (Rothe et al. 1994; Boucher et al. 1997). DR3 recruits TRAF2 via the TNFR-associated death domain protein (TRADD) (Park et al. 2000). TRAFs recruited to these costimulatory TNFRs are critical for activating the canonical NF- κ B, noncanonical NF- κ B, and MAPK pathways (Figs. 3 and 4).

3.2.1. TRAF1 in signal 2

TRAF1 is recruited to 4-1BB (Arch and Thompson 1998; Jang et al. 1998; Saoulli et al. 1998), CD30 (Gedrich et al. 1996; Lee et al. 1996), GITR (Kwon et al. 1999), HVEM (Marsters et al. 1997), OX40 (Kawamata et al. 1998), and TNFR2 (Rothe et al. 1994).

4-1BB-mediated activation of ERK is severely impaired in *Traf1*^{-/-} CD8⁺ T cells (Sabbagh et al. 2008). This ERK activation promotes CD8⁺ T-cell survival through downregulation of the proapoptotic molecule Bim (Wang et al. 2007; Sabbagh et al. 2008). Upon 4-1BB triggering, TRAF1 associates with leukocyte-specific protein 1 (LSP1) in activated CD8⁺ T cells, and the TRAF1-LSP1 complex is essential for the 4-1BB-dependent ERK activation (Sabbagh et al. 2013). These results show that the 4-1BB-TRAF1-LSP1-ERK-Bim axis plays a major role in CD8⁺ T-cell survival and memory-cell generation.

3.2.2. TRAF2 in signal 2

TRAF2 is essential in TNFR-mediated cosignaling. TRAF2 is recruited to 4-1BB (Arch and Thompson 1998; Jang et al. 1998; Saoulli et al. 1998), CD27 (Akiba et al. 1998; Gravelstein et al. 1998; Yamamoto et al. 1998), CD30 (Gedrich et al. 1996; Lee et al. 1996; Harlin et al. 2002), DR3 (Chinnaiyan et al. 1996; Pobezinskaya et al. 2011), GITR (Kwon et al. 1999; Esparza and Arch 2005), HVEM (Hsu et al. 1997; Marsters et al. 1997; Cheung et al. 2009), OX40 (Arch and Thompson 1998; Kawamata et al. 1998), and TNFR2 (Rothe et al. 1994).

TNFR-family molecules assemble signaling complexes that contain TRAF2, cIAP1, cIAP2, RIP1, IKK $\alpha/\beta/\gamma$, and MAPK kinase kinases (MAP3Ks) such as MEK kinase 1 (MEKK1), TGF- β -activated kinase 1 (TAK1), and apoptosis signal-regulating kinase 1 (ASK1) and that promote the activation of canonical NF- κ B and MAPK pathways (Takeda et al. 2008; Ha et al. 2009; Karin and Gallagher 2009; Silke and Brink 2010; So and Croft 2012; Varfolomeev et al. 2012; Hayden and Ghosh 2014). For the canonical NF- κ B pathway, activated IKK β in the TNFR complex phosphorylates inhibitor of NF- κ B (I κ B), which leads to the Lys48 (K48)-linked ubiquitination and subse-

quent proteasomal degradation of I κ B. This loss of I κ B liberates the NF- κ B dimers, such as p50-RelA, and facilitates their entry into the nucleus (Hayden and Ghosh 2014). For the MAPK pathway, the TNFR complex activates the first enzyme of the MAP3K, to phosphorylate a second MAPK kinase (MAP2K), which in turn phosphorylates and activates a MAPK such as ERK, JNK, or p38 (Karin and Gallagher 2009).

4-1BB cosignaling is impaired in T cells from *Traf2*^{-/-} or TRAF2.DN-Tg mice (Saoulli et al. 1998). A signaling complex formed by 4-1BB and TRAF2 activates canonical NF- κ B in CD8⁺ T cells, and the 4-1BB signaling promotes CD8⁺ T-cell survival through the canonical NF- κ B-dependent upregulation of the prosurvival molecules Bcl-x_L and Bfl-1 (Lee et al. 2002; Nam et al. 2005). 4-1BB cosignaling via TRAF2 also plays a role for activating ASK1, which leads to JNK and p38 (Cannons et al. 1999, 2000).

GITR cosignaling is critical for activating canonical NF- κ B and MAPKs (Kanamaru et al. 2004; Esparza and Arch 2005; Snell et al. 2010). The GITR-induced canonical NF- κ B, which upregulates Bcl-x_L, is impaired in small interfering RNA-mediated TRAF2-knockdown CD8⁺ T cells (Snell et al. 2010).

OX40-mediated canonical NF- κ B and PKB activation is important for clonal expansion and survival of antigen-primed T cells (Croft et al. 2009). CD4⁺ T cells expressing TRAF2.DN-Tg show impaired memory T-cell expansion and survival following cognate antigen and OX40 stimulation (Prell et al. 2003). OX40 assembles a signalosome by recruiting TRAF2, RIP1, PKC θ , the CBM complex, the IKK α /β/γ complex, p85 PI3K, and PKB. When TRAF2 is knockdown via short hairpin RNA, T cells are impaired in recruiting PKC θ , the IKK complex, p85 PI3K, and PKB to OX40; accordingly, OX40-mediated canonical NF- κ B and PKB activation is also impaired (So et al. 2011a, b).

These results demonstrate that TRAF2 incorporated into the TNFR signalosome is critical for activating the canonical NF- κ B and that the TRAF2 signalosome provides antigen-primed T cells with a high levels of prosurvival signaling activity needed for T-cell longevity (So and Croft 2012, 2013).

3.2.3. TRAF3 in signal 2

TRAF3's recruitment to TNFRs is critical for activating the noncanonical NF- κ B pathway. TRAF3 is recruited to 4-1BB (Jang et al. 1998), CD27 (Yamamoto et al. 1998), CD30 (Gedrich et al. 1996; Boucher et al. 1997), GITR (Kwon et al. 1999), HVEM (Marsters et al. 1997), OX40 (Kawamata et al. 1998), and TNFR2 (Cabal-Hierro et al. 2014).

TRAF3 binds to NIK, and a complex containing TRAF2, TRAF3, cIAP1, and cIAP2 limits the activation of the noncanonical NF- κ B pathway through the constitutive proteasomal degradation of NIK. After TRAF2 and TRAF3 are recruited to the TNFRs, the molecular complex containing NIK is disrupted through TRAF3 degradation, and NIK

accumulates in the cell and subsequently phosphorylates IKK α . IKK α phosphorylates p100, which triggers the processing of p100 into p52 and the translocation of NF- κ B dimers (such as p52-RelB) into the nucleus. Thus, in unstimulated T cells, TRAF2 or TRAF3 negatively regulates NIK's activity (Liao et al. 2004; Varfolomeev et al. 2007, 2012; Vallabhapurapu et al. 2008; Zarnegar et al. 2008). Accordingly, the noncanonical NF- κ B pathway is constitutively activated in T cells from Δ T-*Traf2*^{-/-} or Δ T-*Traf3*^{-/-} mice (Gardam et al. 2008; Xie et al. 2011).

NIK expressed by CD4⁺ T cells plays an essential role in graft-versus-host disease, and NIK signaling via OX40 is required for the lethal autoimmunity (Murray et al. 2011). NIK is also important for generation and maintenance of CD4⁺ and CD8⁺ memory T cells in response to LCMV infection (Rowe et al. 2013). 4-1BB signaling activates the noncanonical NF- κ B pathway through the cIAP1-dependent degradation of TRAF3 in CD8⁺ T cells (McPherson et al. 2012). These results indicate that T-cell intrinsic NIK/noncanonical NF- κ B signaling promotes the effector/memory T-cell survival and that the TNFR-TRAF3 axis plays a major role for initiating the noncanonical NF- κ B pathway.

3.2.4. TRAF5 in signal 2

TRAF5 is recruited to CD27 (Akiba et al. 1998), CD30 (Aizawa et al. 1997), GITR (Esparza et al. 2006), HVEM (Hsu et al. 1997; Marsters et al. 1997), and OX40 (Kawamata et al. 1998). *Traf5*^{-/-} CD4⁺ T cells have impaired GITR cosignaling and defective GITR-mediated canonical NF- κ B, p38, and ERK activation (Esparza et al. 2006). The GITR-mediated canonical NF- κ B is diminished in small interfering RNA-mediated TRAF5-knockdown CD8⁺ T cells (Snell et al. 2010). Although the proliferation and survival mediated by CD27 cosignaling are impaired in *Traf5*^{-/-} T cells, the CD27-mediated activation of canonical NF- κ B and MAPKs is not altered, suggesting functional redundancy between TRAF2 and TRAF5 in CD27 cosignaling (Nakano et al. 1999; Kraus et al. 2008). These results show that TRAF5 works as a positive regulator in CD27 and GITR cosignaling.

3.3. TRAFs control cytokine receptor signaling (signal 3)

TRAFs are recruited directly or indirectly by the cytokine receptors for IL-1 (Cao et al. 1996), IL-2 (Motegi et al. 2011; Yi et al. 2014a), IL-6 (Nagashima et al. 2014), IL-8 (Manna and Ramesh 2005), IL-17 (Gaffen 2011), IL-18 (Kojima et al. 1998), IL-25 (Maezawa et al. 2006), IL-33 (Funakoshi-Tago et al. 2008), GM-CSF (Meads et al. 2010), type I IFNs (Yang et al. 2008), IFN λ s (Xie et al. 2012), and TGF- β (Sorrentino et al. 2008). Receptors for IL-1, IL-2, IL-6, IL-18, IL-33, and TGF- β are expressed on T cells; thus, these cytokines are major sources of signal 3. TRAFs recruited to these receptors contribute to signal 3 through positive or negative regulation of key transcription factors, such as AP-1, NF- κ B, signal transducers and activators of transcription (STAT), and Smad (Figs. 3 and 4).

3.3.1. IL-2 in signal 3

IL-2, a potent T-cell growth factor that is produced by activated T cells and is necessary for T-cell proliferation, development, and function, promotes the differentiation of naïve CD4⁺ T cells into the T_H2 and pT_{reg} lineages (Cote-Sierra et al. 2004; Sakaguchi et al. 2008; Littman and Rudensky 2010) (Fig. 1). The IL-2-receptor (IL-2R) consists of three proteins: the α , β , and γ chains. Resting T cells express the β and γ heterodimer IL-2R $\beta\gamma$, which binds IL-2 with moderate affinity; thus resting T cells respond to higher concentrations of IL-2. Upon T-cell activation, the IL-2R α chain appears and creates the receptor IL-2R $\alpha\beta\gamma$; this receptor's high IL-2-binding affinity allows activated T cells to respond to lower concentrations of IL-2 than can naïve T cells. The IL-2R's β and γ chains, which are responsible for signal transduction, activate the Janus kinase (JAK)-STAT pathway (Sugamura et al. 1996; Yamane and Paul 2012; Liao et al. 2013).

TRAF3 negatively regulates IL-2R signaling. The IL-2-mediated phosphorylation of JAK1, JAK3, and STAT5 is enhanced in ΔT -*Traf3*^{-/-} CD4⁺ T cells (Yi et al. 2014a). T cell protein tyrosine phosphatase (TCPTP) associates with JAK1/3 to inhibit IL-2R signaling by dephosphorylating JAK proteins (Simoncic et al. 2002). TRAF3 mediates TCPTP's recruitment to IL-2R, and the TRAF3 RING/zinc-finger domain, but not the TRAF-C domain, is critical for the TRAF3-TCPTP interaction (Yi et al. 2014a). Thus, TRAF3 inhibits JAK-STAT signaling by recruiting TCPTP to the IL-2R complex.

TRAF6 also negatively regulates IL-2R signaling, although its inhibitory mechanism differs from that of TRAF3. The IL-2-mediated activation of JAK1, ERK, and AP-1 is enhanced in *Traf6*^{-/-} CD4⁺ thymocytes. IL-2R β contains the TRAF6-binding consensus motif ²⁹⁸PLEVLE³⁰³ within the Box2 JAK-binding site. The interaction of TRAF6 and IL-2R β inhibits JAK1 binding to IL-2R β ; thus, TRAF6 limits the JAK1 activation mediated by IL-2 (Motegi et al. 2011).

These results demonstrate that TRAF3 and TRAF6 function as negative regulators for IL-2R signaling in T cells and suggest that these TRAFs control the differentiation and maintenance of many types of T cells by modulating the IL-2 signaling network.

3.3.2. IL-6 in signal 3

IL-6, which is produced by dendritic cells in response to infection or the pro-inflammatory cytokines IL-1 and TNF α , contributes to the differentiation of naïve CD4⁺ T cells into the T_H17 lineages (Korn et al. 2009; Kimura and Kishimoto 2010; Littman and Rudensky 2010) (Fig. 1). The IL-6-receptor consists of the IL-6-binding chain IL-6R and the 130-kD signal-transducing chain gp130. The binding of IL-6 in a complex with IL-6R and gp130 recruits and activates STAT3 (Taga and Kishimoto 1997).

TRAF5 negatively regulates IL-6-receptor signaling (Nagashima et al. 2014). The STAT3 phosphorylation

mediated by IL-6, but not by IL-10 or IL-21, is enhanced in *Traf5*^{-/-} CD4⁺ T cells. TRAF5, which is highly expressed in naïve CD4⁺ T cells, constitutively associates with gp130 and inhibits the IL-6-mediated STAT3 recruitment and activation. The gp130 cytoplasmic tail, which includes the amino acid sequence ⁷⁷⁴VFSRSESTQPLLDSEERPEDLQLVD⁷⁹⁸, contains an SXXE motif (⁷⁷⁶SRSE⁷⁷⁹) and two diacidic amino acids (⁷⁸⁸EE⁷⁸⁹ and ⁷⁹²ED⁷⁹³) that have been identified as TRAF2-binding consensus motifs in TNFRs (Arch and Thompson 1998; Park et al. 1999; Ye et al. 1999). STAT3 binds to the four distal phosphorylated-tyrosine motifs of gp130, ⁷⁶⁵YRHQ⁷⁶⁸, ⁸¹²YFKQ⁸¹⁵, ⁹⁰⁴YLPQ⁹⁰⁷, and ⁹¹⁴YMPQ⁹¹⁷ (Stahl et al. 1995). The TRAF5-binding region is located between those first two motifs, which suggests that TRAF5 inhibits the recruitment of STAT3 to these two motifs through steric hindrance in gp130 or through modulation of an optimal configuration of gp130 that might be required for activating JAK or STAT3.

Expression of *Traf5* and *Il6st* (which encodes gp130) mRNA under steady-state conditions is significantly higher in T cells than in B cells, natural killer T cells, natural killer cells, and macrophages. Anti-CD3/CD28 stimulation significantly downregulates the *Traf5* expression in wild-type CD4⁺ T cells, suggesting that the constitutive TRAF5-gp130 binding is critical for limiting the initial instructive IL-6 signals required for T_H17 development (Nagashima et al. 2014).

These results demonstrate that TRAF5 works as an anti-inflammatory factor to limit immune responses mediated by effector CD4⁺ T cells that had been primed with IL-6 and suggest that TRAF5 also regulates signaling pathways in other gp130 family of cytokines, such as cardiotrophin 1 (CT-1), ciliary neurotrophic factor (CNTF), IL-11, IL-27, leukemia inhibitory factor (LIF), and oncostatin M (OSM). Further studies are needed to determine if the TRAF5-gp130 interaction universally controls signaling via the receptors for gp130 family of cytokines.

3.3.3. TGF- β in signal 3

TGF- β is required for the differentiation of naïve CD4⁺ T cells into the T_H17 and pT_{reg} lineages (Sakaguchi et al. 2008; Korn et al. 2009; Kimura and Kishimoto 2010; Littman and Rudensky 2010) (Fig. 1). Three homologous TGF- β isoforms, TGF- β 1, TGF- β 2, and TGF- β 3, act through a complex of type I (TGF β RI) and type II (TGF β RII) serine/threonine kinase receptors that induce the phosphorylation of intracellular Smad proteins. TGF- β 1 is predominantly expressed in the immune system (Heldin et al. 1997; Li et al. 2006).

In ΔT -*Traf6*^{-/-} CD4⁺ T cells, T_H17 development is enhanced due to elevated Smad2/3 phosphorylation mediated by TGF- β (Cejas et al. 2010). TGF- β -dependent Smad activation blocks the IL-2 transcription in T cells, to support T_H17 differentiation (Laurence et al. 2007). Thus, TRAF6 negatively regulates TGF- β R-Smad signaling in CD4⁺ T cells. Although TRAF6 constitutively associates with

TGF β RI and promotes the TAK1-p38/JNK pathway in non-T cells (Sorrentino et al. 2008; Yamashita et al. 2008), the enhanced T_H17 development seen in $\Delta T\text{-}Traf6^{-/-}$ CD4⁺ T cells does not result from a defect in TGF- β -mediated TAK1-p38/JNK signaling (Cejas et al. 2010).

3.3.4. The IL-1-family cytokines in signal 3

The IL-1 family of ligands, IL-1 (IL-1 α and IL-1 β), IL-18, and IL-33, are important in the differentiation, function, and maintenance of particular T_H subsets, including T_H1, T_H2, and T_H17 cells (Nakanishi et al. 2001; Boraschi and Tagliabue 2013; Garlanda et al. 2013; Basu et al. 2015; Endo et al. 2015) (Fig. 1). IL-1 is synthesized by monocytes, macrophages, and neutrophils after activation with microbial products such as lipopolysaccharide (LPS). IL-18 is produced by macrophages and dendritic cells in response to microbial products. IL-33 is expressed by fibroblasts, endothelial cells, and epithelial cells. The activating IL-1-receptor is a complex of two chains, the ligand-binding chain IL-1R1 and the accessory chain IL-1R3. Similarly, the receptor for IL-18 consists of the ligand binding IL-1R5 and the accessory IL-1R7, and the receptor for IL-33 consists of the ligand binding IL-1R4 and the accessory IL-1R3. IL-1, IL-18, and IL-33 activate Toll-IL-1-receptor (TIR) domain-containing receptors, i.e., IL-1R1, IL-1R3, IL-1R4, IL-1R5, and IL-1R7, to recruit the adaptor myeloid differentiation factor 88 (MyD88) and the IL-1-receptor associated kinase (IRAK). IRAK contains three potential TRAF6-binding sites (Ye et al. 2002). The subsequent recruitment of TRAF6 activates the NF- κ B and MAPK pathways (Inoue et al. 2000; Chung et al. 2002; Boraschi and Tagliabue 2013; Garlanda et al. 2013).

IL-1 α -mediated cell proliferation is impaired in

Traf6^{-/-} thymocytes (Naito et al. 1999), indicating a requirement for TRAF6 in IL-1-receptor signaling. Similarly, IL-18-mediated IFN- γ production is reduced in *Traf6*^{-/-} T_H1 cells (Chiffolleau et al. 2003), and IL-18-driven homeostatic-like proliferation is severely impaired in $\Delta T\text{-}Traf6^{-/-}$ naïve CD8⁺ T cells (Walsh et al. 2014), indicating a requirement for TRAF6 in IL-18-receptor signaling. These results clearly show that TRAF6 is essential for signaling via the receptors for IL-1 family of ligands and suggest that the IL-1R-TRAF6 axis promotes the differentiation or maintenance of T_H1, T_H2, and T_H17 cells.

Conclusion

TRAF-family molecules regulate signals that are mediated by TCRs, costimulatory TNFRs, and cytokine receptors expressed on CD4⁺ T cells and are required for the activation, differentiation, and survival of various T_H-cell subsets. Each receptor forms a functional signalosome that contains a specific set of TRAFs and provides a platform for phosphorylation, ubiquitination, and other posttranslational modifications via protein-protein interactions. Although TRAF1, TRAF2, TRAF3, TRAF5, and TRAF6 are all critical in signaling activities in T cells, it is not clear how the various receptors recruit their particular TRAFs after ligand stimulation. It is tempting to speculate that the signals mediated through these receptor types coordinate and integrate multiple intersecting signaling pathways through TRAFs (Figs. 3, 4 and 5). The proposed synergy among the three receptors might be mediated through: (1) protein-protein interactions between different TRAF signalosomes formed by these receptors, (2) TRAF protein degradation mediated by signaling via one of the receptors, which can disrupt already existing TRAF signalosomes or

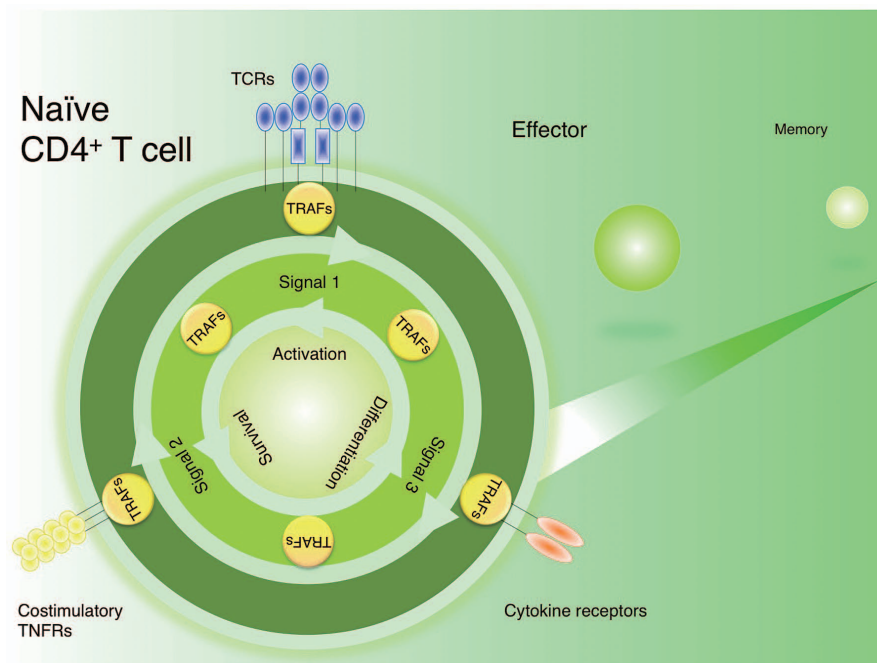


Fig. 5. TRAF signaling network controls activation, differentiation, and survival of CD4⁺ T cells.

inhibit the formation of new TRAF signalosomes in other receptors, (3) new TRAF protein synthesis mediated by signaling via one of the receptors, which can promote the formation of new TRAF signalosomes in other receptors. Further research is required to understand the mechanisms by which TRAFs cooperatively regulate intersecting signaling activities in CD4⁺ T cells.

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Conflict of Interest

The authors declare no conflict of interest.

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