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T cell Receptor Signal Transduction in T lymphocytes

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Abstract

The T cell receptor (TCR) recognizes self or foreign antigens presented by major histocompatibility complex (MHC) molecules. Engagement of the TCR triggers the formation of multi-molecular signalosomes that lead to the generation of second messengers and subsequent activation of multiple distal signaling cascades, such as the Ca^{+2}-calcineurin-NFAT, RasGRP1-Ras-Erk1/2, PKCθ-IKK-NFκB, and TSC1/2-mTOR pathways. These signaling cascades control many aspects of T cell biology. Mechanisms have been evolved to fine-tune TCR signaling to maintain T cell homeostasis and self-tolerance, and to properly mount effective responses to microbial infection. Defects or deregulation of TCR signaling has been implicated in the pathogenesis of multiple human diseases.

Keywords

T cell receptor; T cell activation; T cell development; T cell tolerance; T cell anergy; Mammalian target of rapamycin; Diacylglycerol kinase

Introduction

T cells play a major role in mounting an effective adaptive immune response. Evidence demonstrates that signals from T cell receptor (TCR) are critical for T cell development, homeostasis, activation, and tolerance. T cell development in the thymus requires the expression of a functional TCR, following proper VDJ recombination [1,2]. Moreover, signal strength from the TCR on $CD4^+CD8^+$ double-positive (DP) thymocytes dictate the fate of these immature T cells during thymic selection [3]. DP thymocytes that receive weak TCR signals are positively selected and undergo maturation to either CD4 or CD8 single-positive (SP) mature T cells [4], whereas those that receive strong TCR stimuli proceed for apoptosis by negative selection [5]. Negative selection deletes highly self-reactive T cells to establish central T cell tolerance. Thymic selection ensures generation of mature T cells that are mostly self–tolerant, are capable of mounting effective immune responses against foreign antigens, and can maintain homeostasis [6].

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In the peripheral lymphoid organs and tissues, naïve T cells are activated upon TCR engagement with foreign peptides presented by dendritic cells and other antigen-presenting cells in response to pathogenic infection [7]. Intracellular signal cascades generated from the TCR leads to plethoric effects, such as increased cell size, transcription, translation, and subsequent production of various cytokines, chemokines, and effector molecules [8]. TCR signaling in mature T cells is also tightly regulated via multiple mechanisms, and dysregulaton of TCR signaling can cause T cell hyperactivation that can lead to autoimmune diseases [9].

Proximal TCR Signal Transduction

TCR complex

Intense investigations during the mid-1980s, using biochemical and immunochemical methods, unveiled the structural components of TCR complex [9]. These efforts, followed by powerful genetic manipulation studies, led to the current knowledge regarding the composition of the TCR complex. The TCR complex is composed of TCRα and β, and the CD3 proteins [10,11]. TCRα and β are generated by somatic VDJ recombination, and TCRαβ dimer is responsible for recognition of peptide-MHC complexes [12,13]. The invariant CD3 proteins, consisting of δ, ε, γ and ζ chains, associate with TCR via hydrophobic interaction [14]. Unlike TCRα and β chains, CD3 proteins are not directly involved in antigen recognition, but their cytosolic domains contain the immunoreceptor tyrosine-based activation motifs (ITAMs) that are responsible for transmitting the TCR signal [15,16]. ITAMs consist of two tyrosine residues flanked by leucine/isoleucine and spaced by bulky aromatic aminoacid. Engagement of TCR triggers ITAM phosphorylation at the tyrosine residues by Src-family protein tyrosine kinases (PTKs), leading to the formation of a proximal signaling complex and activation of downstream signaling events [17].

Proximal protein tyrosine kinases and substrates

Studies using PTK inhibitors revealed the roles of PTKs in TCR signaling. Cytosolic PTKs, such as the Src-family PTKs, Lck and Fyn, and the Syk-family kinase ζ-associated protein of 70 kDa (ZAP-70), are activated by TCR ligation (Figure 1) [18–20]. Lck activation triggers the phosphorylation of ITAMs of CD3 proteins [21]. Evidence garnered from genetically modified mouse models has demonstrated that Lck is required for T cell development [22]. Dephosphorylation of the inhibitory Y505 residue of Lck by CD45 tyrosine phosphatase facilitates its auto-phosphorylation at Y394, which is a key event for Lck activation [23,24]. In contrast, phosphorylation of Y505 by C-terminal Src kinase (Csk) inhibits Lck activation, which is important for maintaining T cell homeostasis in the absence of TCR engagement [25]. Several lines of evidence have shown that Fyn is also involved in phosphorylation of ITAMs [26]. However, Fyn is not essential for T cell development. Phosphorylated ITAMs form docking sites for several PTKs and other proteins. The phosphorylation of ITAMs at ζ chain recruits ZAP-70 via its SH2 domains, which specifically bind to phospho-tyrosine residues on ITAMs [27,28]. This binding triggers phosphorylation of ZAP-70 by Lck, leading to its activation. Direct association of ZAP-70 with the activated TCR/CD3 complex results in phosphorylation/activation of other proteins and recruitment of adaptors, leading to the formation of a multi-nucleated signaling complex [29].

Formation of signalosomes nucleated by adaptor molecules and activation of PLCγ

Several studies have identified the primary role of ZAP-70 phosphorylation in the regulation of phospho lipase C γ1 (PLCγ1) activation, cytosolic Ca^{+2} influx, and activation of distal signaling pathways, such as NFAT, AP-1 and NF-κB [30]. However, the manner in which

ZAP-70 mediate these events was unknown until the characterization of the components of the proximal signaling complex. Rigorous investigations have identified adaptor proteins, protein kinases, and key regulatory molecules that assemble the proximal signalosome for TCR signal transduction. ZAP-70 phosphorylates two key adaptor proteins: Linker for the activation of T cells (LAT), a transmembrane adaptor protein, and the cytosolically localized SH2, containing leukocyte phosphoprotein of 76 kDa (SLP-76) [31,32]. These two adaptors together form a proximal signaling complex and orchestrate the recruitment of various effector proteins [33,34]. Phosphorylated LATs recruit SH2 domain-bearing proteins, including PLCγ1, the p85 regulatory subunit of PI3K, Grb2, and Gads, to form a sub cellular assembly [35,36]. SLP-76 joins the complex by binding to Gads and PLCγ1 via a proline-rich region. Diminished activation of Ras was manifested in LAT- and SLP-76-deficient mouse models and Jurkat T cell line models due to the impairment in the formation of the proximal signaling complex [37,38]. Grb2 is constitutively bound with Son of Sevenless (Sos), which is a dual-specific GTP exchange factor (GEF) for both Ras and Rho GTPases. Grb-Sos complex binds to phosphorylated tyrosines on LAT, leading to activation of Ras. However, LAT mutants, defective of PLCγ1 binding, fail to induce complete activation of Ras, suggesting that Grb2 is not sufficient for Ras activation, at least in T cells [9,39].

Concurrently, SLP-76 also undergoes ZAP-70 mediated phosphorylation, leading to the recruitment of Vav1, a GEF, IL-2-induced tyrosine kinase (Itk, a Tec family of PTK), and other adaptor proteins, such as non-catalytic tyrosine kinase (Nck) and ADAP [40]. Coordinated and precise loading of these effector molecules into the complex is not only important for maintaining the stability of the complex but also for its optimal activation. Ligation of TCR induces cellular polarization by regulating cytoskeleton and integrin affinity. Recruitment of Vav1 to SLP-76 via its SH2 domain activates Rho family of GTPases, such as Rac1, to promote actin reorganization [41]. ADAP also binds to SLP-76 at C-terminally located phosphorylated tyrosines to activate integrin signaling [42].

A key event in connecting proximal signaling to distal signaling branches of the TCR signaling pathway is the activation of PLCγ1 [43]. Mutational analyses of PLCγ1 have demonstrated that association of PLCγ1 with LAT, Gads, and SLP-76 is required for its optimal activation [39]. PLCγ1 activation also requires Itk, which is activated by both SLP-76 and Lck [44,45]. Association of Itk with SLP-76 maintains its close proximity to its substrate PLCγ1, whereas Lck directly phosphorylates Itk at Y511 and promotes its activation [40,46]. Activated Itk phosphorylates PLCγ1, resulting in its activation. Additionally, Rlk, another member of Tec family PTKs, is also known to participate in the activation of PLCγ1, as Itk and Rlk double-deficient mouse models exhibited complete loss of PLCγ1 activity [47,48].

Activated PLCγ1 hydrolyzes membrane-bound phosphatidylinositol 4, 5-bisphosphate (PIP_2) into two essential second messengers—inositol-3-phosphate (IP_3) and diacylglycerol (DAG) in equal stoichiometry [49]. IP_3 and DAG initiate a variety of distal signaling pathways. IP_3 triggers the activation of a Ca^{+2}-dependent calcineurin-NFAT signaling pathway. The membrane-bound DAG can activate at least couple of major signaling pathways that include RasGRP1, PKCθ, and PDK1-mediated pathways [49,50].

Distal Signaling Pathway

The Ras-Erk1/2-AP1 pathway

Phosphorylation of extracellular signal-regulated kinase 1/2 (Erk1/2) has been a designated biochemical marker to assess the quality of TCR signaling for decades. However, the exact pathway that regulates activation of Erk1/2 was unclear until the identification of Ras

[51,52]. Ras is a small G protein whose activated state is dependent on the presence of GTP. The GTP-bound form is active, while the GDP-bound form is inactive. Ras-GTP activates Raf-1, which, itself, is a serine/threonine kinase. Raf-1 phosphorylates and activates mitogen-activated protein kinase kinase (Mek1/2), which subsequently phosphorylates and activates Erk1/2. Erks trigger the phosphorylation and activation of transcription factor, Elk [53]. Elk induces the expression of c-fos transcription factor, leading to the formation of AP-1, a dimeric complex of Jun/Fos that plays a critical role in immune response.

Two guanine nucleotide exchange factors, RasGRP1 and Sos, are known to be responsible for Ras activation in T cells. RasGRP1 binds to DAG, which induces its membrane translocation and activation [54]. Deficiency of RasGRP1 causes defective activation of the Ras- Erk1/2 pathway, mTOR, and PI3K/Akt [55,56]. RasGRP1 is crucial for conventional αβ T cell and *i*NKT but not γδ T cell development [55,57,58]. RasGRP1 also mediates the activation of conventional αβ T cells and is critical for γδ T cell activation and expression IL-17 [58]. Different from RasGRP1, Sos is recruited to LAT via Grb, and its activation is independent of DAG. Recent evidence indicates that So spromotes Ras activation and is dependent on RasGRP1; it may also play a selective role for Ras activation downstream of the pre- TCR and is important for β-selection [51,59–61]. Although RasGRP1 is not essential for β-selection, it appears to be important for efficient β selection [58]. Moreover, abnormal expression of RasGRP1 and Ras were noted in systemic lupus erythematous (SLE) patients and in SLE mouse models, indicating that the RasGRP1 and Ras pathway may be implicated in the generation of SLE [62], furthermore suggesting the therapeutic potential in targeting this pathway.

IP_3-Ca^{+2}-NFAT pathway

Calcium is an essential second messenger and plays key roles in the activation of T cells. $IP_{3,}$ generated by PLCγ1, binds to its receptor on the endoplasmic reticulum, leading to a release of intracellular calcium stores. It has been noticed for years that calcium depletion in the ERs can trigger an influx of extracellular Ca^{+2} into T cells through a calcium release-activated calcium channel (CRAC) [63]. Only until recently, it was found that ERs constitutively express a transmembrane protein called stromal interaction molecule (STIM), which senses the intracellular Ca^{+2} store levels and facilitates the Ca^{+2} influx from Orai1 type CRAC channels [64].

Ca^{+2} influx triggers the activation of downstream events that subsequently activate transcriptional targets [50]. Ca^{+2} activates calcineurin, a protein phosphatase, leading to dephosphorylation and subsequent nuclear translocation of NFAT. Nuclear NFATs, together with AP-1 transcription factors (Jun/Fos) derived from DAG signaling, bind to cognate DNA response elements and induce the expression of genes related to T cell activation such as IL-2 and other effector molecules. However, in the absence of DAG-mediated signaling, selective activation of the Ca^{+2}-calcineurin-NFAT pathway induces T cell anergy by promoting the expression of anergy-relative molecules, such as several E3 ubiquitin ligases and DGKα [65–68]. In addition to NFAT, Ca^{+2} also activates calmodulin-dependent kinase (CaMK), which in turn activates transcription factor cyclic-AMP-responsive- element-binding protein (CREB) and myocyte enhancer factor 2 (MEF2) to mediate T cell activation [69]. Besides regulating T cell activation and anergy, Ca^{+2}-mediated signaling is also important for positive selection of thymocytes and for *i*NKT cell development [70–73]. Impaired Ca^{+2} signaling causes SCID in humans, due to the missense mutation in Orai1, which leads to aberrant calcineurin- NFAT activation [74,75].

The PKCθ-IKK-NFκB pathway

In addition to activating RasGRP1, DAG also triggers PKCθ signaling. PKCθ belongs to a class of novel-type PKCs that are primarily activated by DAG, through the C1 lipid-binding domain [76]. Although T cells express other forms/classes of PKCs, PKCθ plays major and non-redundant roles in T cell activation, particularly in the peripheral immune compartment [77,78]. Intense investigations have deciphered the underlying mechanisms by which IKKs are regulated. Activated PKCθ phosphorylates adaptor proteins, CARMA1, Bcl10, and MALT1, which subsequently triggers the formation of a tri-molecular complex called the CARMA1/Bcl10/MALT1 (CBM) complex [79–81]. Association of Bcl10 with MALT1 may recruit E3 ubiquitin ligase, called tumor necrosis factor receptor-associated factor 6 (TRAF6), to degrade IKKγ, or NF-κB essential modifier (NEMO), a regulatory protein of the IKK complex [82,83]. This relieves the inhibition on catalytic IKKs α and β, leading to phosphorylation of IκB. Phosphorylation of IκB by IκB kinases (IKK) induces ubiquitination and degradation of IκB. As result, NF-κB is released and translocated into the nucleus to regulate gene expression [84,85]. Genetic evidence has clearly demonstrated the critical roles of the PKCθ-IKK-NFκB pathway for T cell activation, survival, homeostasis, and effector function along with *i*NKT and regulatory T cell (Treg) development [86,87]. Deregulation of this pathway can cause several consequences, such as severe combined immunodeficiency (SCID), autoimmunity, lymphoma, and defective T cell activation and survival [88–90].

TSC1/2-mTOR signaling

Mammalian target of rapamycin (mTOR) has evolved as a master regulator of cell growth, proliferation, and metabolism by integrating extracellular signals/stimuli, such as nutrients, energy, stress, and growth factors/cytokines. mTOR, a serine/threonine kinase, exists in two functionally distinct complexes— namely, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2)— to phosphorylate and activate a unique choice of substrates [91]. mTORC1 complexes with raptor, an adaptor protein, and other accessory proteins to phosphorylate substrates p70 ribosomal S6 kinase1 (S6K1), 4 elongation factor-binding protein 1 (4E-BP1), and Unc-51 like kinase (ULK1) in a rapamycin-sensitive and dependent manner (Figure 2) [92–94]. However, mTORC2 is insensitive to acute rapamycin treatment, and it associates with rictor to phosphorylate PKCα and Akt on serine 473 residue [95,96]. Phospho-S6K1 phosphorylates S6, a key component of ribosome, whose phosphorylation is critical for the reconstitution of ribosome and for protein synthesis. Hyper-phosphorylation of 4E-BP1 releases the inhibition on eIF4E, a key eukaryotic translational initiation factor, leading to initiation of cap-dependent translation [97]. In contrast, the mTORC2 substrates PKCα and Akt, upon phosphorylation, directly act as signaling proteins to modulate various cellular processes, which include cytoskeletal reorganization and cell survival [98]. mTORC1 is activated by a Ras-like, small GTPase, called Rheb. The TSC1/2 heterodimeric complex is a key negative regulator of mTORC1 [99]. TSC2 is a GTPase-activating protein, by which TSC2 inhibits the activation of Rheb [100]. Phosphorylation of TSC2 by Akt and Erk induces inactivation of TSC, releasing the inhibition on Rheb, and leading to the activation of mTOR [101,102]. TSC1 binds to and stabilizes TSC2.

TCR engagement induces the activation of both mTORC1 and mTORC2 through the PI3K-Akt and DAG-RasGRP1-Ras-Mek1/2- Erk1/2 pathways [56,103]. DGKα and ζ inhibit mTORC1 and mTORC2 activation via negative control of the DAG-RasGRP1-Ras- Erk1/2 pathway [56]. Studies from cell line models have shown that Akt or Erk1/2 can phosphorylate TSC2 and promote the activation of mTORC1 [101]. The underlying mechanisms by which mTORC2 is regulated are not known.

Using rapamycin, shRNA, and genetically engineered mouse models, multiple studies have demonstrated that mTOR regulates the key processes in adaptive immunity. Inhibition of mTORC1 with rapamycin induces T cell anergy [104]. In contrast, elevated mTORC1 signaling, due to TSC1 deficiency, prevents T cell anergy [105].

Signals from both TCR and specific cytokine milieus play a crucial role in the generation of appropriate $CD4^+$ helper effector cells (Th) [106]. mTORC1 and mTORC2 differentially regulate the generation of effector T cell types [107]. Naïve CD4 T cells derived from rictor-deficient mice are impaired in differentiation into IL-4-producing Th2 and IFNγ-producing Th1 effector cells, whereas T cells from Rheb or raptor-deficient mice are defective in Th17 differentiation [108,109]. mTORC2 induces the phosphorylation of PKCθ at S660/676 and Akt at S473 to promote Th2 and Th1 differentiation, respectively [109]. mTORC1 promotes Th17 differentiation by down-regulating Socs5 to promote STAT3 activity [108]. In addition to controlling Th differentiation, mTORC1 positively controls primary but inhibits memory CD8 T cell-mediated, anti-viral immune response and regulates the T cell trafficking by modulating L-selectin (CD62L) and CCR7 [103,110,111]. Given the importance of mTOR, its activity also needs to be tightly controlled. Indeed, several recent studies have demonstrated that dysregulation of mTOR signaling, due to deficiency of TSC1, results in increased T cell death, resistance to T cell anergy, loss of T cell quiescence, and the abnormal survival and function of macrophages and mast cells [105,112–119].

TCR Signaling in Tregs

Tregs mediated immune suppression is crucial for immune tolerance, dysregulation of which results autoimmunity [120–122]. Natural Tregs (nTreg) are a subset of T cells whose lineage differentiation is primarily governed by Forkhead box transcription factor FOXP3 in the thymus [123]. In addition, FOXP3 can be induced in peripheral conventional T cells to generate inducible Tregs (iTreg) especially in mucosal tissues. Tregs constitutively express the high affinity IL-2 receptor α-chain (CD25), GITR, and CTLA-4 [124–126]. They also produce suppressive cytokines such as TGF-β and IL-10 [127].

Emerging investigations have shed light on some of key aspects of TCR signaling in Treg development and function. Like conventional T cells, both avidity and duration of TCR signals play important roles in determining Treg differentiation. The signals received from TCR, IL-2R, TGF-βR and retinoic acid play pivotal roles in induction of AP-1, NFAT, and NF-κB that modulate OXP3 transcription and subsequently Treg differentiation [128]. The proximal TCR signaling components are absolutely required for Treg development and function. Mice deficient of Lck, ZAP-70, and LAT are devoid of both conventional T cells and Tregs, suggesting that these two subsets of T cells at least share some common promoximal components for TCR signal transduction. ZAP-70 mutation in its ITAM binidng SH2 domain leads to decreased nTreg, resulting in autoimmunity [129,130]. Impaired PLCγ1 activation also impacts the Treg development and function. A mutation in PLCγ1 binding site of LAT (Y136F) causes severe impairment in Treg development due to attenuation of downstream signals such as Ca^{+2} influx [131].

Multiple distal signaling cascades play important roles in Treg development and function. Ca^{+2} influx and its downstream signaling play essential roles for generating and maintaining the sustainable calcineurin-NFAT signaling pathway that is important for Treg development and function [132]. Combined deficiency of endoplasmic reticulum Ca^{+2} sensors STIM1 and STIM2 markedly reduces Treg cellularity and impairs immune suppression [133]. However, the ablation of Orai Ca^{+2} channels appears not affecting Treg development and function suggesting that these channels may not be crucial for Ca^{+2} entry in Treg [134]. The NF-κB signaling pathway is another major contributor of nTreg development. Specific

ablation of PKCθ, CARMA1, Bcl 10 or TAK in mice inhibits the generation of nTregs in the thymus [135–138]. In contrast to its role for nTreg development, PKCθ inhibits Treg function as blockade of PKCθ activity enhances Treg-mediated suppression [139]. Furthermore, PKCθ signaling negatively regulates iTreg function via Akt-Foxo1/3A axis [140]. Tregs display decreased Erk1/2 activation [141]. Although the RasGRP1-Ras-Erk1/2 pathway plays indispensable roles for postive selection of conventional T cells in the thymus, deficiency of RasGRP1 does not cause a drastic decrease of Tregs or γδ T cells [58,142]. The independence of Treg development on RasGRP1 is consistent with the notion that Tregs are selected with relatively strong TCR signaling during positive selection and RasGRP1 is preferentially required for positive selection of developing T cells with low affinity TCRs to self-peptide/MHC complexes [143]. Compared with conventional T cells, Tregs have decreased PI3K and mTOR activities [56,144]. Although mTOR signaling plays pivotal roles during T cell activation, the PI3K-Akt pathway and mTOR signaling appear to inhibit Treg differentiation and function [145]. Mouse model with inactive form of PI3K-p110 catalytic subunit exhibited increased thymic Treg cellularity [146]. Inhibition of mTOR by rapamycin or genitic deficiency of mTOR induces Treg differentiation *in vitro* [147–151]. Overall, both common signaling pathways and distinct regulations of TCR signaling exist between conventional T cells and Tregs. However, the limited availability of Tregs prevents in depth analyses of TCR in this subset of T cells.

Negative Control of TCR Signaling

TCR signaling play key roles in T cell development and function, deregulation of TCR signal has been proven detrimental to the host. Various negative regulatory molecules including phosphatases, ubiquitin ligases, and lipid kinases play key roles in T cells by fine-tuning TCR signaling.

Phosphatases

Formation of proximal signaling complex is initiated through phosphorylation and activation of Lck. The SH2-domain, containing protein tyrosine phosphatase (SHP-1), inactivates Lck by dephosphorylating its active site [152]. SHP-1 dampens TCR signaling events, both at early and late time points by mediating Lck dephosphorylation. TCR ligation of antagonists or weak antigens induce Lck-mediated phosphorylation as well as activation of SHP-1. As a result, SHP-1 dephosphorylates Lck, leading to its rapid inactivation. At later time points, TCR stimulation induces the expression of adhesion molecules called carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1). Phosphorylated immunoreceptor tyrosine-based inhibitory motifs (ITIM) of CEACAM1 recruit SHP-1 and dephosphorylate Lck to terminate the TCR signaling pathway [153,154]. Under optimal conditions, Erk1/2 indirectly regulates the SHP-1 activity. Erk1/2 phosphorylates Lck at S59 to prevent the binding of SHP-1, leading to sustained TCR signaling [155].

Suppressor of T cell receptor signaling (Sts), another novel class of proteins, is hypothesized to possess protein phosphatase activity. Sts-1 targets Syk, whereas Sts-2 dephosphorylates ZAP-70 [156,157]. Genetic ablation of these two isoforms resulted in T cell hyperproliferation and autoimmunity. The underlying mechanisms are not known but may be uncovered in future studies. In addition to dephosphorylating Lck, phosphatases act on many other downstream signaling events, such as dephosphorylating PIP_3 by Pten and MAPKs by dual specificity phosphatases, to negatively control TCR signaling [158,159].

Ubiquitination and degradation: E3 ubiquitin ligases

Interest has been growing in the ability to understand the roles of protein degradation mechanisms that regulate TCR signaling. Protein degradation occurs mainly by ubiquitin-

mediated proteosomal and/ or lysosomal mediated processes. Ubiquitination or conjugation of ubiquitin (Ub) to protein involves a cascade of enzymatic reactions [160]. First, Ub activating enzyme, E1 initiates the formation of activated Ub from an inactivated state. Second, activated Ubis further transferred to a Ub-conjugating enzyme called E2. Finally, E3 ubiquitin ligases couple the transfer of the activated Ub from E2 to the target proteins. Thus, E3 ligases facilitate the actual attachment of Ub to substrates by defining the substrate specificity [161]. Although the list of E3 ligases is increasing, the manners which specify the binding to their substrates are still not well defined [162].

Several studies have demonstrated that certain E3 ligases function as regulators of T cell tolerance and that their mutation or deletion can lead to the generation and activation of autoreactive T cells, subsequently resulting in the onset of autoimmunity [163]. E3 ligases primarily regulate the T cell activation by regulating TCR signaling to induce both central and peripheral T cell tolerance [164,165]. Casitas B cell lymphoma (Cbl-b) is a well-studied E3 ligase belonging to the RING finger family, which marks numerous target proteins by ubiquitination to initiate protein degradation. Cbl-b, along with other members of the Cbl family such as the protein c-Cbl, promotes negative regulation in the TCR signaling pathway [166]. Cbl proteins ubiquitinate TCRζ and several proteins in a proximal signaling complex and target them for degradation to attenuate the TCR signaling [167]. The intracellular domain of the TCR/CD3ζ chain is rich in multiple lysine residues, acting as a substrate for ubiquitination. Cbl-b triggers the conjugation of Ub to the TCRζ chain in activated T cells to sequester or degrade surface TCRs, thus resulting in the attenuation of TCR signaling. ZAP-70 facilitates Cbl-mediated TCRζ ubiquitination by acting as an adaptor [168]. Downstream of TCR, Src- and Syk-family PTKs undergo Cbl-mediated ubiquitination, followed by degradation, to dampen the TCR signaling. Furthermore, Cbl-b interacts with Vav1, specifically in naïve T cells, and this interaction is important for maintaining negative regulation of TCR signaling in the absence of antigen engagement [169]. In the absence of a DAG-AP1 signaling pathway, Ca^{+2} flux activates NFAT, which positively regulates the expression of Cbl-b via Egr transcription factors [170].

TRAF6 is a member of the RING finger family E3 ligase whose functions were well documented in APCs but poorly understood in T cells [171]. Similar to Cbl-b, the genetic ablation of TRAF6 had also been shown to induce T cell hyperproliferation [172]. The gene related to anergy in lymphocytes (GRAIL) is a transmembrane protein, the expression of which regulates T cell activation. Anergic T cells express high levels of GRAIL, accompanied by a characteristic decrease in IL-2 production [173]. Itch, a HECT family ubiquitin ligase, is known to regulate T cell tolerance, particularly T cell anergy, by modulating TCR signaling [174]. Itch, by virtue of its ubiquitin ligase activity, targets PLC-γ1 and PKCθ [175,176]. Further, Itch also marks Jun, causing diminished activation of AP-1 [177]. This is at least one of the mechanisms that accounts for autoimmune and proinflammatory-prone phenotypes in Itch-deficient mouse models. Roquin was identified as a novel RING finger E3 ligase and has been shown to play a role in T cell tolerance [178]; however, underlying mechanisms are yet to be elucidated. Because Cbl-b, GRAIL and Itch possess overlapping functions, it has been postulated that these E3 ligases translocate to immune synaptic regions where they target TCR signaling proteins [179]. This results in compromised immunological synaptic function and subsequent diminished TCR signaling.

Diacyglycerol kinases

Given the crucial roles of DAG in TCR signaling, it is important to understand the mechanisms through which DAG is regulated. DGKs are lipid kinases that phosphorylate DAG to produce phosphatidic acid (PA), thereby regulating the subcellular DAG levels [180,181]. As a result, TCR induced Ras-Mek-Erk signaling is attenuated when DGK

activity is increased [182,183]. Ten isoforms of DGKs are expressed in mammals, consisting of the kinase domain and at least two cysteine-rich C1 domains that share the DAG/phorbol ester-binding region of PKCs [118,181]. The genetic ablation of DGKα or ζ, DGK isoforms, expressed at high levels in T cells, resulted in increased activation of the Ras-Mek-Erk-AP1 pathway, the PKCθ-NFκB pathway, and mTOR signaling, which in turn led to T cell hyperactivation, loss of T cell anergy, and enhanced primary but impaired anti-viral responses by CD8 T cells [49,67,68,184–186]. Moreover, deficiency of both DGKα and ζ resulted in a severe T cell developmental blockade at the DP stage, indicating that these two isoforms perform redundant roles in T cells. Interestingly, treatment of DGKα and ζ double-deficient thymi with phosphatidic acid partially restored T cell development, suggesting that DGKα and ζ may function as a signal switch by terminating DAG mediated signaling and at the same time initiating PA-mediated signaling [187,188]. A particularly important question to be addressed is the identity of the downstream effector molecules that bind to and mediate PA signaling.

Dysregulation of TCR Signaling in Diseases

Given the importance of the TCR signal in T cell development, activation, and tolerance, it is not surprising that dysregulation in TCR signaling causes or contributes to various diseases. Defects in TCR signaling may lead to failure in generating optimal immune responses, which can lead to immune deficiency. For example, mutations on genes that encode for CD3δ, ε, and ζ chain results in SCID, which is characterized by the absence or defective function of T cells [189]. Defective expression of Lck is associated with suboptimal activity that in turn leads to immunodeficiency in both humans and mice [190,191]. Deficiency or mutation of ZAP-70 leads to a rare form of SCID in humans, with specific absence of peripheral $CD8^+$ cells and functionally impaired $CD4^+$ cells [192,193]. However, a mouse deficient in ZAP-70 is devoid of both $CD4^+$ and $CD8^+$, as its development was blocked at the $CD4^+$ $CD8^+$ DP stage.

Altered TCR signaling can cause abnormal thymic selection or uncontrolled T cell activation, which can cause autoimmune diseases. Reduced expression of CD3ζ is associated with SLE and rheumatoid arthritis (RA) [194–196]. Altered signal transduction from ZAP-70 causes aberrant changes in tyrosine phosphorylation and Ca^{+2} mobilization and, subsequently, thymic selection [197]. Mutations observed in ZAP-70 were transitions and deletions leading to transcriptional loss, destabilized protein, and loss of kinase function. Spontaneous mutations in the SH3 domain of ZAP-70 in mice produce autoimmune arthritis, which resembles human RA [129].

A deficiency of CD45 expression results in SCID, similar to its phenotype in humans [198,199]. Patients carrying certain CD45 polymorphisms were predisposed to multiple sclerosis (MS). A similar protein tyrosine phosphatase PTPN22 (LYP/PEP) acts on several of its substrates, including Lck, ZAP-70, and Syk, to inactivate a TCR signaling pathway by direct dephosphorylation [200]. A genetic variant of human PTPN2 is involved in a range of autoimmune diseases, such as type1 diabetes mellitus, SLE, RA, Graves' disease, and Hashimoto thyroiditis [201–205].

Conclusions

Initiation of T cell immune responses begins with the generation of signals from the TCR, which is further conveyed to various downstream effectors to shape T cell development, activation, homeostasis, and tolerance. Advances in biochemical, molecular biological, and genetic tools/reagents have significantly contributed to the appreciation of today's TCR signaling. While we have focused on discussing proximal TCR signaling events,

downstream signaling cascades, and negative control of TCR signaling, but the transduction and regulation of TCR signal is much more complex. Each of the signaling events may be controlled by counter-mechanisms to ensure proper TCR signaling and T cell homeostasis and activation. Cross talks among distal TCR signaling pathways and feedback mechanisms add to the complexity of regulation. Current knowledge of TCR signaling is brought to the stage mainly based on naïve conventional αβ T cells and T cell line models. TCR signaling in other rare but important populations of T cells, such as memory T cells, Tregs, and NKT cells, remains poorly understood. Identification of distinct regulation of TCR signaling among the different T cell subsets allows selective modulation of T cell responses. Although traditional immunoblotting and genetic approaches have greatly advanced our understanding of TCR signaling, new imaging technologies should illustrate the dynamic signal transduction and its regulation in different subcellular compartments. Comprehensive and in-depth knowledge of TCR signaling may improve the understanding of the pathogenesis and aid in the design of effective therapies for immunological diseases including autoimmunity, immunodeficiencies, and cancers.

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Abbreviations

PIP_3	Phosphatidylinositol-tris-phosphate
MHC	Major Histocompatibility Complex
Lck	Lymphocyte-specific protein tyrosine kinase
Syk	Spleen tyrosine kinase
SH2	Src Homology 2
SH3	Src Homology 3
PI3K	Phosphatidylinositol 3-kinases
Grb2	Growth factor receptor-bound protein 2
Gads	Grb2-like adaptor protein
NFAT	Nuclear Factor of Activated T-cells
AP-1	Activator Protein 1
NF-κB	Nuclear Factor-Kappa B
ADAP	Adhesion and Degranulation-promoting Adapter Protein
Rlk	Resting lymphocyte kinase
RasGRP1	RAS Guanyl-Releasing Protein 1
PKCθ	Protein Kinase C θ
PDK1	3-phosphoinositide dependent protein kinase-1
Elk	Ets-related protein
mTOR	Mammalian Target of Rapamycin
***i*NKT**	invariant Natural Killer T cell

CCR7	Chemokine receptor 7
Bcl10	B-cell lymphoma/leukemia 10
MALT1	Mucosa-Associated Lymphoid Tissue lymphoma translocation protein 1
IκB	Inhibitor of Kappa B
Treg	regulatory T cells
SLE	Systemic Lupus Erythematous
RA	Rheumatoid Arthritis
SCID	Severe Combined Immunodeficiency

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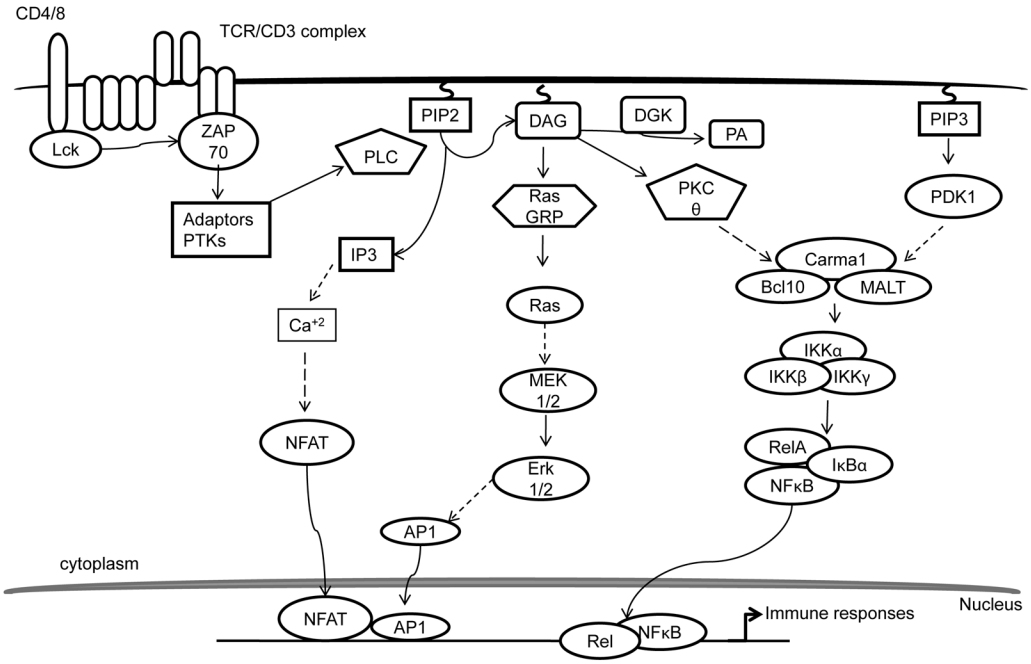

Figure 1.
Schematic illustration of TCR signaling.

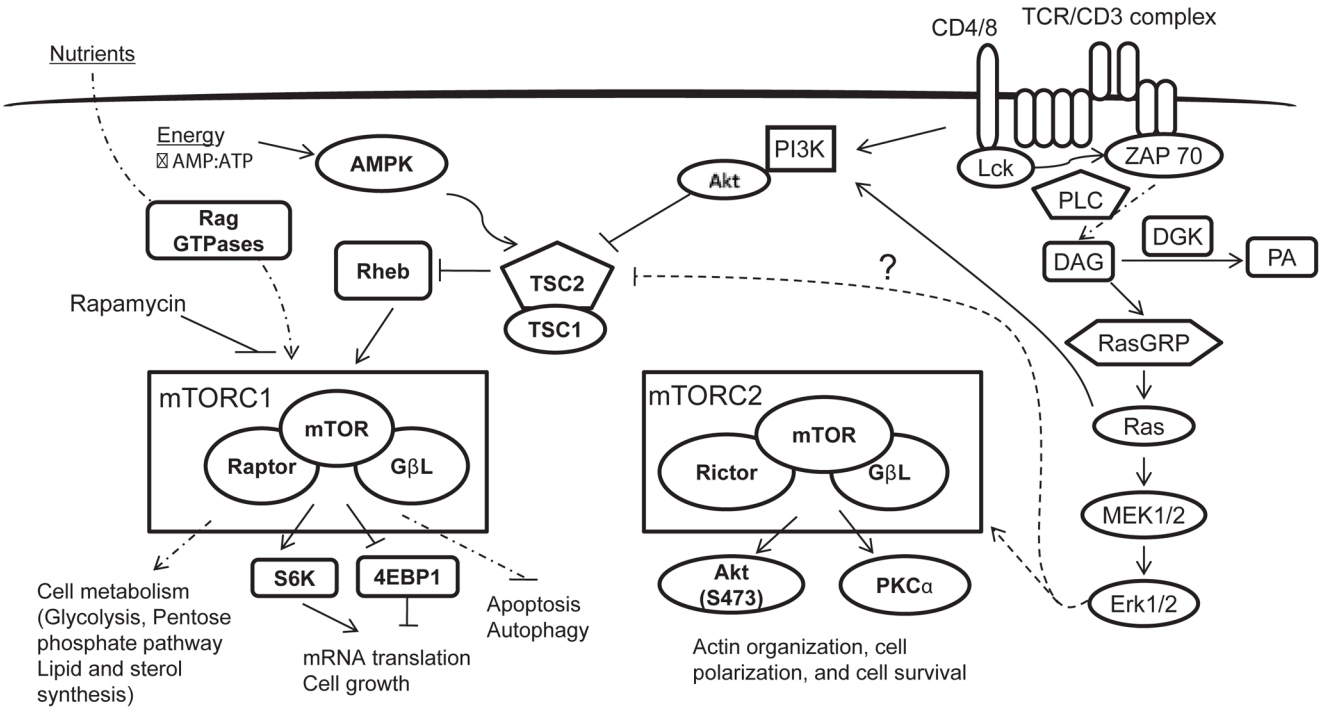

Figure 2.
mTOR signaling and its regulation in T cells.