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mTOR signaling in the differentiation and function of regulatory and effector T cells

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Abstract

The mechanistic target of rapamycin (mTOR) signaling pathway integrates environmental signals and cellular metabolism to regulate T cell development, activation and differentiation. Recent studies reveal the importance of exquisite control of mTOR activity for proper T cell function, and detailed molecular mechanisms that regulate mTOR signaling in different T cell subsets. Here, we review the latest advances in our understanding of the mTOR pathway and its regulation in the differentiation and function of regulatory T cells and effector T cells.

Introduction

“Let me warn you, Icarus, to take the middle way...”

Ancient wisdom from East and West has taught us the peril of extremes. Perhaps the recent studies on the mechanistic target of rapamycin (mTOR) signaling and T cell biology have reaffirmed the verity of these teachings. mTOR signaling consists of two complexes, mTORC1 and mTORC2, which share the catalytic subunit mTOR but are distinguished by the scaffold proteins RAPTOR and RICTOR, respectively. Current model posits that PI3K-AKT pathway activates both mTORC1 and mTORC2. As a sensor for a plethora of environmental cues, mTOR controls cell growth and proliferation [1]. In adaptive immune cells, mTOR dictates multiple T cell lineage fates and functions [2]. While both mTORC1 and mTORC2 suppress differentiation of regulatory T cells (Tregs) induced *in vitro* (iTregs), mTORC1 is required for functional competency of thymic-derived Tregs (tTregs) [3]. In effector CD4⁺ T cells, mTOR promotes Th1, Th2 and Th17 differentiation. Suppression of mTORC1 also enhances memory CD8⁺ T cell differentiation [4]. Research in the past three years has revealed the importance of a finely controlled mTOR activity for proper T cell function and immune homeostasis, much as the Oracle at Delphi has taught – nothing in excess. Importantly, these studies have also uncovered the detailed molecular mechanisms underlying the delicate control of mTOR signaling in T cells, and underscored the vast scope of upstream signals that mTOR senses. Here, we review the latest advances in our

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understanding of how a fine-tuned mTOR signaling controls the differentiation and function of Tregs and effector T cells.

A balanced mTOR activity maintains Treg stability and function

Our previous study found that deletion of RAPTOR, but not RICTOR, specifically in Tregs led to severe systemic autoimmunity, partly due to defective lipid biosynthesis. TCR and IL-2 drive mTORC1 activation, which promotes the suppressive activity of Tregs by enhancing proliferation and expression of Treg effector molecules including CTLA-4 and ICOS. Furthermore, mTORC2 activity is elevated in the absence of RAPTOR, and deletion of RICTOR partially ameliorates the autoimmune diseases in mice with Treg-specific deletion of RAPTOR [5]. Thus, we concluded that mTORC1, but not mTORC2, is critically required for tTreg functional competency. Consistent with our findings, recent study of human Tregs showed that weak TCR stimulation of conventional T cells (Tconvs) induces iTreg differentiation, and the high mTORC1 activity of these iTregs correlates with increased suppressive activity. Furthermore, inhibition of glycolysis diminishes the suppressive activity of human iTregs, which is associated with decreased mTORC1 activity [6].

Does over-activation of mTOR signaling affect Tregs? Park *et al.* addressed this question by examining mice with Treg-specific deletion of TSC1, an upstream negative regulator of mTORC1 [7]. Treg-specific TSC1 deficiency does not affect overall T cell differentiation and homeostasis at steady state. However, TSC1-deficient Tregs exhibit reduced *in vivo* suppressive activity in a T cell-mediated colitis model. In an inflammatory environment, TSC1-deficient Tregs lose FOXP3 expression and convert to effector-like T cells producing proinflammatory cytokines, IL-17 and IL-1 β . This loss of Treg stability is due to increased mTORC1 activity, because knockdown of S6K1, a major downstream target of mTORC1, rectifies the increased IL-17 and IL-1 β production in TSC1-deficient Tregs. Thus, over-activation of mTORC1 promotes Treg instability and conversion to effector T cells, leading to the loss of suppressive function in inflammatory conditions. This is reminiscent of TSC1 deficiency in Tconvs, which abrogates naïve T cell quiescence, increases apoptosis and impairs anti-bacterial immunity [8-10]. Interestingly, TSC1 deficiency in thymocytes selectively increases tTreg differentiation, but not peripheral tTregs. Reduced mTORC2 activity, but not increased mTORC1 activity, is responsible for increased tTreg differentiation in the absence of TSC1, suggesting distinct regulatory mechanisms between thymic and peripheral tTregs differentiation [11].

For mechanisms controlling mTORC2 activity in Tregs, the answer came from studies on the function of PTEN, a crucial negative regulator of PI3K pathway. To investigate how dysregulation of PI3K impacts Tregs, we and others deleted PTEN specifically in Tregs [12,13]. Surprisingly, PTEN deficiency in Tregs leads to highly increased mTORC2 activation, but minimal mTORC1 activation, suggesting that PI3K pathway preferentially promotes mTORC2 activation in Tregs [12]. Furthermore, mice with Treg-specific deletion of PTEN develop age-related autoimmune and lymphoproliferative disease, characterized by increased level of serum autoantibodies and glomerulonephritis [12]. At cellular level, PTEN deficiency in Tregs leads to increased follicular helper T (Tfh) cells, germinal center (GC) B

cells and IFN- γ producing Th1 cells, but not Th2 or Th17 cells. Mechanistically, PTEN-deficient Tregs have increased instability and lose Foxp3 expression. This instability is associated with increased CpG methylation status of the conserved noncoding sequence 2 (CNS2) of FOXP3 locus, which controls the heritable maintenance of Foxp3 expression [13-15]. Importantly, Treg-specific deletion of RICTOR completely restores immune homeostasis in mice with PTEN-deficient Tregs [12]. Therefore, excessive mTORC2 activity destabilizes Tregs and impairs Treg-mediated suppression of Tfh and Th1 cells through epigenetic modification of *Foxp3* locus. Collectively, these studies demonstrated that over-activation of either mTORC1 or mTORC2 is detrimental to Treg stability, with distinct defects in their ability to suppress specific effector T cells. Hence, maintenance of Treg stability and proper function requires an optimal level of mTOR activity — ‘*Goldilocks mTOR*’, with either too much or too little being deleterious (Figure 1).

mTOR determines effector T cell fates

mTOR in Tfh differentiation

Earlier studies have shown that mTOR signaling controls multiple effector T cell fates, including Th1, Th2 and Th17 cells [16-20]. Several recent studies addressed how mTOR controls differentiation of follicular helper T (Tfh) cells. Ray *et al.* found that Tfh cells have lower mTORC1 and mTORC2 activity, as well as reduced metabolic functions than Th1 cells in a LCMV infection model. Knockdown of mTOR or RAPTOR, but not RICTOR, increases Tfh frequency and reduces Th1 frequency, without significant effects on germinal center (GC) B cell numbers. Moreover, IL-2 drives mTORC1 activity to promote Th1 at the expense of Tfh differentiation, indicating a negative role of mTORC1 in Tfh differentiation [21]. However, other studies using genetic models reached different conclusions. Examination of a mouse line harboring an mTOR hypomorphic mutation revealed that reduced mTORC1 activity significantly dampens Tfh differentiation, suggesting a positive function of mTORC1 in Tfh differentiation [22]. Furthermore, T cell-specific deletion of mTOR, RAPTOR or RICTOR leads to severely reduced Tfh differentiation both at steady state and upon antigen immunization or viral infection [23,24]. Conversely, PTEN deficiency or PI3K over-activation in T cells results in higher Tfh differentiation [23,25]. As mTORC1 is crucial for T cell quiescence exit and hence initial T cell activation [19,26], we circumvented this complication using the OX40-cre system to induce deletion in CD4⁺ T cells after initial activation. OX40-cre-mediated deletion of *Rptor* or *Rictor* also leads to a substantial reduction of Tfh differentiation and function, as indicated by highly reduced GC B cells and immunoglobulin production [23]. Notably, mTORC1 and mTORC2 promote Tfh differentiation through overlapping and distinct mechanisms. Both complexes function downstream of ICOS, an essential costimulatory receptor for Tfh cells, to promote glucose metabolism. Direct modulation of glucose metabolism through overexpression of the glucose transporter, GLUT1, enhances Tfh differentiation. Additionally, mTORC2 controls Tfh differentiation by inhibition of Foxo transcription factors [23] and promoting expression of TCF1 [24], an essential transcription factor for Tfh differentiation [27,28]. It is not clear what lies behind the divergent observations between gene silencing studies and genetic models. Gene dosage effect could play a role, but the mTOR hypomorphic mutation modestly diminishes mTORC1 activity yet significantly reduces Tfh cells [22,29]. Another

possibility is the different timing of mTOR inhibition between retrovirus-mediated gene silencing and *in vivo* genetic deletion. Of note, silencing of mTOR exerts a strong inhibitory effect on Th1 differentiation in LCMV infection [21], suggesting that Tfh and Th1 cells could have different sensitivities to reduced mTOR activity. Further studies are warranted to fully elucidate the detailed mechanisms

mTOR in CD8⁺ T cell differentiation

Previous studies using rapamycin, the macrolide mainly inhibiting mTORC1, showed that mTORC1 is required for effector CD8⁺ T cell differentiation [30]. Consistent with this finding, genetic deletion of RHEB, an upstream activator of mTORC1, reduces effector CD8⁺ T cell generation, while deletion of TSC2 (another upstream inhibitor of mTORC1) increases effector CD8⁺ T cells upon vaccinia infection [31]. However, TSC1 deficiency reduces effector CD8⁺ T cells upon infection of *Listeria monocytogenes* due to increased apoptosis [8,32]. It is unclear why the phenotypes of *Tsc1*^{-/-} CD8⁺ T cells differs from those of *Tsc2*^{-/-} CD8⁺ T cells, although different infection models might play a role. In terms of mTORC2, RICTOR-deficient CD8⁺ T cells had normal effector cell generation and function, indicating that mTORC2 is dispensable for effector CD8⁺ T cell differentiation [31].

Memory CD8⁺ T cell formation is enhanced by rapamycin treatment [33,34] or silencing RAPTOR expression [33], suggesting a negative role of mTORC1 in memory CD8⁺ T cell differentiation. This conclusion was further established in mouse genetic models. RHEB-deficient CD8⁺ T cells generate more memory cells despite reduced effector cell differentiation [31]. Conversely, deletion of TSC1 in antigen-experienced CD8⁺ T cells leads to normal effector cell differentiation but highly reduced differentiation of memory-precursors and memory cell formation, associated with excessive mTORC1 activity and dysregulated cell metabolism [35]. Interestingly, RICTOR deficiency enhances generation and recall response of memory CD8⁺ T cells, associated with enhanced metabolic fitness, including increased spare respiratory capacity and fatty acid oxidation [31], both critical for memory CD8⁺ T cell formation [36]. Moreover, this elevated memory CD8⁺ T cell response is driven by nuclear accumulation of Foxo1 [37]. Thus, both mTORC1 and mTORC2 negatively regulate memory CD8⁺ T cell differentiation. However, it is not clear how mTORC1 activity affects the function of memory CD8⁺ T cells. While rapamycin treatment enhances recall response during secondary LCMV infection [33], RHEB-deficient memory CD8⁺ T cells fail to respond to secondary immunization [31]. Again, there could be nuances of differential mTOR activities, i.e., a modest reduction of mTORC1 promotes memory differentiation and function, but a more substantial reduction could have opposite effects. Further investigations are needed to address these questions.

How is mTOR activity regulated in T cells?

Regulation of mTOR activity in Tregs

As mentioned in the introduction, mTOR senses various environmental cues. We have reported that IL-2, S1PR1, and ICOS are important upstream signals that activate mTOR in Tregs, Tconvs, and Tfh cells, respectively [5,23,38,39] (Figure 2). Semaphorins are repulsive

axonguidance factors that modulate neural patterning and development. But semaphorin 4A (SEMA4A) is also expressed on certain immune cells and its receptor, neuropilin-1 (NRP1), was identified as a marker for tTregs but not iTregs [40,41]. Treg-specific deletion of NRP1 does not affect immune homeostasis at steady state, but NRP1-deficient Tregs are unable to suppress anti-tumor immune response or T cell-mediated colitis [42,43]. Mechanistically, ligation of NRP1 by SEMA4A recruits PTEN to immunologic synapse to restrict both mTORC1 and mTORC2 activation, and thus promotes Treg stability and suppressive activity [42]. These findings are consistent with the fact that excessive mTOR activity impairs Treg function. Recently, Gerriets *et al.* showed that TLR1 and TLR2 signaling promotes mTORC1 activity, glycolysis and proliferation of Tregs, but at the same time reduces their suppressive activity. Interestingly, FOXP3 expression reduces mTORC1 activity and glycolysis, and promotes mitochondrial oxidative metabolism. Thus, the counteraction between inflammatory signals emanated from TLR1/TLR2 and FOXP3 balances mTORC1 signaling and glucose metabolism to control the proliferation and suppressive function of Tregs [44]. Tregs are crucial for maintaining intestinal immune homeostasis [3]. The vitamin A metabolite all-trans retinoic acid (RA) endows guthoming phenotypes in T cells by upregulating the integrin $\alpha 4\beta 7$ and chemokine receptor CCR9. Treatment with rapamycin or RAPTOR deficiency, but not RICTOR deficiency, prevents RA-induced CCR9 expression in Tregs, suggesting that RA promotes gut tropism through mTORC1 [45].

In addition to extracellular signals, intracellular signaling events also impinge upon mTOR in Tregs. Autophagy targets intracellular substrates for lysosomal degradation and recycling in response to stress and it is known to be suppressed by mTORC1 signaling. Surprisingly, Treg-specific deficiency of *Atg7*, a critical gene in autophagy, results in increased mTORC1 activity, which activates glycolytic metabolism through the metabolic regulator MYC transcription factor. Increased glycolysis destabilizes Tregs and impairs their suppressive activity, leading to systemic autoimmune disorders. The instability of ATG7-deficient Tregs can be restored by treatment with rapamycin or MYC inhibitors [46]. Deficiency of ATG16L1, another autophagy component, in Tregs increases apoptosis and glycolytic metabolism and leads to spontaneous inflammatory disease, particularly in gastrointestinal organs [47]. Thus, autophagy suppresses mTORC1 and glycolysis to promote Treg stability and survival.

Protein phosphatase 2A (PP2A) is a highly conserved serine-threonine phosphatase consisting of three distinct subunits, the scaffold A subunit (PP2AA), the regulatory B subunit (PP2AB) and the catalytic C subunit (PP2AC). PP2A interacts with RAPTOR and inhibits mTORC1 activity. Deletion of the PP2AA, which prevents maturation of the PP2AC and impairs its catalytic activity, in Tregs leads to increased mTORC1 activation and glycolysis, loss of Treg suppressive activity and multi-organ autoimmunity. Treatment with rapamycin ameliorates the autoimmune disease in mice with Treg-specific deletion of PP2AA. Thus, like autophagy, PP2A maintains Treg suppressive activity through restraining mTORC1 [48].

MicroRNAs (miRNAs) regulate mTOR signaling in Tregs

Ablation of miRNA biogenesis impairs Treg development [49-51]. Through an overexpression screening, Warth *et al.* found that miR-99a and miR-150 promote iTreg differentiation. Interestingly, RA-induced miR-99a cooperates with constitutively expressed miR-150 to target the 3' UTR of the *Mtor* mRNA and suppress mTOR activity [52]. In a separate study, miR-15b/16 was identified to promote iTreg generation by targeting *Rictor* and *Mtor* mRNA [53]. These findings are consistent with a negative role of mTOR signaling in iTreg induction and *de novo* FOXP3 expression [2]. Therefore, multiple miRNAs suppress mTOR signaling components to promote iTreg differentiation.

Regulation of mTOR activity in effector CD4⁺ T cells

One of the classic upstream signals that mTOR senses is amino acids, including leucine, arginine, lysine and glutamine [1]. T cells require amino acid transporters to uptake amino acids. ASCT2 is a glutamine transporter and its expression on T cells is enhanced after TCR ligation. ASCT2-deficient CD4⁺ T cells have highly reduced uptake of glutamine and leucine. ASCT2 deficiency impairs mTORC1 activation, and consequently leads to reduced Th1 and Th17 differentiation and function. Furthermore, TCR activation-mediated ASCT2 induction, glutamine uptake and mTORC1 activation are partly dependent on the CARMA1-BCL10-MALT1 (CBM) complex, a signaling nexus linking TCR and CD28 signals to downstream events [54]. Another group reported that CARMA1 and MALT1, but not BCL10, mediate TCR induced mTORC1 activation [55]. The reason behind the discrepancy is not clear, and further study is needed to elucidate the role of BCL10 in TCR-induced mTOR activation. Thus, amino acid transporter ASCT2 facilitates glutamine and leucine uptake, which activates mTORC1 and promotes effector T cell differentiation.

IL-9-producing CD4⁺ T cells are recently designated as Th9 cells [56]. Th9 cells play important role in allergic inflammatory diseases [57] and host defense against gastrointestinal worm infection [58], but little is known regarding the signaling requirement for Th9 differentiation. Recently, Wang *et al.* identified the NAD⁺-dependent deacetylase Sirtuin-1 (SIRT1) as a negative regulator of Th9 differentiation. T cell-specific deletion of SIRT1 promotes IL-9 production in CD4⁺ T cells, exacerbates allergic airway inflammation, and delays tumor formation in a xenograft melanoma model. Importantly, SIRT1-deficient T cells exhibit elevated mTORC1 activity and glycolysis. Deletion of mTOR kinase or inhibition of glucose metabolism largely restores SIRT1 deficiency induced Th9 differentiation [59]. Therefore, SIRT1 blocks Th9 differentiation by suppressing mTORC1 activity and mTORC1-dependent glucose metabolism.

mTORC1 is known to promote Th17 differentiation [16,17,20], but it is less clear how mTORC1 activity is regulated during Th17 differentiation. Koga *et al.* showed that Th17 differentiation requires the calcium/calmodulin-dependent protein kinase IV (CaMK4). CaMK4 promotes IL-17 expression partly by interacting with AKT and enhancing AKT/mTORC1 activity. Silencing CaMK4 in T cells from SLE patients or healthy individuals reduces Th17 differentiation, highlighting its potential as therapeutic target [60]. Thus, Th17 differentiation requires CaMK4-dependent AKT/mTORC1 activation.

Regulation of mTOR in effector CD8⁺ T cells

Similar as CD4⁺ T cells, amino acid uptake is important for mTORC1 activation in CD8⁺ T cells. Sinclair *et al.* showed that T cell activation strongly induces expression of System L amino-acid transporter, which is composed of heavy chain, CD98 (*Slc3a2*) and light chain, LAT1 (*Slac7a5*). It mediates leucine uptake and sustains mTORC1 activity in effector CD8⁺ T cells. Loss of LAT1 in CD8⁺ T cells prevents metabolic reprogramming and clonal expansion [61]. Together with the data on glutamine transporter ASCT2, these results demonstrate that TCR signaling activates mTORC1 partly through induction of amino acid transporters.

Previous study showed that phosphoinositide-dependent kinase 1 (PDK1)-dependent mTORC1 controls glucose metabolism in effector CD8⁺ T cells [62], but the upstream inputs are not clear. SEMA4A and another of its receptors (other than NRP1), PLEXIN B2, are expressed on CD8⁺ T cells. While ligation of NRP1 by SEMA4A dampens mTOR activity in Tregs, ligation of PLEXIN B2 by SEMA4A activates mTORC1 in CD8⁺ T cells. SEMA4A-deficient CD8⁺ T cells have reduced effector T cell response against *Listeria monocytogenes* infection [63]. Thus, SEMA4A-PLEXIN B2 axis enhances mTORC1 activity to drive effector CD8⁺ T cell differentiation against bacterial infection.

CD8⁺ T cells are instrumental in mediating anti-tumor immune response, yet their function is often compromised within tumor microenvironment by a variety of mechanisms, including engagement of immune checkpoint receptors. A recent study identified elevated extracellular potassium ion ($[K^+]_e$) concentration in tumor microenvironment as a new immune suppression mechanism [64]. Increased $[K^+]_e$ inhibits T cell function by suppressing AKT-mTORC1 signaling. This suppressive effect is dependent on PP2A, as knockdown of PP2A reverses potassium-mediated AKT-mTOR suppression. Furthermore, this suppression requires increased intracellular potassium ($[K^+]_i$) in T cells. Expression of the voltage-gated potassium channel Kv1.3, which mediates K^+ efflux and reduces $[K^+]_i$, on T cells promotes mTORC1 activation, effector cytokine production, and anti-tumor function [64]. Thus, potassium ion regulates mTORC1 activity and effector CD8⁺ T cell function through PP2A.

Asymmetric inheritance of mTOR determines CD8⁺ T cell fate

What are the mechanisms by which mTOR signaling promotes effector CD8⁺ T cell differentiation but suppresses memory CD8⁺ T cell formation? One theory on effector vs memory differentiation posits that a single naïve T cell gives rise to descendants with different fates by asymmetric cell division [65]. Three recent studies revealed that PI3K-mTORC1-MYC-mediated metabolic pathways are asymmetrically divided and they dictate differential cell fates in the daughter cells [66-68]. Daughter T cells proximal to antigen-presenting cells (APC) inherit higher level of amino acid transporters and mTORC1 activity. Furthermore, elevated mTORC1 sustains higher level of MYC in proximal T cells. Interestingly, treatment with rapamycin, or over-activation of mTORC1 through deletion of TSC1, abolishes the MYC asymmetry. MYC and mTORC1 together maintain higher level of proliferation, glycolysis and glutamine metabolism [66,67]. Importantly, *in vivo* functional analysis demonstrated that MYC^{hi} T cells undergo greater proliferation during primary

response, whereas MYC^{lo} T cells have greater expansion during secondary recall response [67]. Therefore, asymmetric distribution of mTORC1-MYC signaling drives differential metabolic programs in daughter cells, which in turn determine their divergent fates. Metabolic manipulation hence has the potential to influence effector vs memory T cell response.

Conclusion

Despite extensive advances in our understanding of mTOR signaling and T cell biology, we still have much to explore, especially in terms of mechanisms controlling mTOR activity in T cell differentiation. We have yet to understand how mTORC1-MYC asymmetry is established in CD8⁺ T cell fate decisions and whether and how mTOR regulates memory CD4⁺ T cell formation. Importantly, the realization that T cells require exquisitely fine-tuned mTOR activity for proper differentiation and function cautions against therapeutic interventions that drastically alter mTOR activity. On this note, we still do not understand exactly how much reduction of mTOR activity enhances memory differentiation without adverse effect on memory cell function or other lineages. Further investigation should provide more insight into strategies to harness mTOR signaling or immunometabolism to improve human health.

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Highlight

- A fine-tuned mTOR activity is required for proper function of T cells
- Both mTORC1 and mTORC2 contribute to Tfh cell differentiation
- Multiple upstream signaling inputs impinge upon mTOR in T cell responses
- Asymmetric distribution of mTORC1 and Myc determines CD8+ T cell fates

Figure 1.

Optimal mTOR activity (*Goldilocks mTOR*) is required for proper T cell differentiation and function. (Upper panel) Over-activation of mTORC1 destabilizes Tregs and impairs their suppressive function. This abolishes naïve T cell quiescence, disrupts Myc asymmetric distribution, promotes Th9 differentiation, and reduces memory CD8⁺ T cell differentiation. Loss of mTORC1 activity reduces Treg suppressive function, impairs effector CD4⁺ and CD8⁺ T cell differentiation, abolishes Myc asymmetry, and diminishes memory T cell function. However, a modest reduction of mTORC1 activity enhances memory CD8⁺ T cell differentiation and function. It is currently unclear the extent to which the reduction of mTORC1 is beneficial. (Lower panel) Over-activation of mTORC2 promotes Treg instability and impairs Treg-mediated suppression of Th1 and Tfh differentiation. While loss of mTORC2 does not affect Treg function, it increases thymic tTreg generation and reduces Th1 and Tfh differentiation. In CD8⁺ T cells, mTORC2 deficiency does not affect effector T cell generation and function, but enhances memory T cell formation. Finally, loss of both mTORC1 and or mTORC2 enhances iTreg differentiation.

Current Opinion in Immunology

Figure 2.

Multiple upstream inputs impinge upon mTOR signaling in T cells under various contexts. There are positive signals, including S1PR1, TLR1, TLR2, TCR, IL-2, ICOS, SEMA4A/ PLEXIN B2, CaMK4, RA, and amino acid transporters ASCT2 and System L amino acid transporter (a heterodimer composed of LAT1 and CD98). ASCT2 and System L amino acid transporter mediate uptake of glutamine and leucine, respectively, which potentially activate mTORC1. The expression of these amino acid transporters is induced by TCR activation. Negative signals include potassium channel Kv1.3-mediated transport of potassium ion (K^+) (it engages PP2A), NRP1, SIRT1, autophagy, microRNA (miR)-99a, miR150, miR15b/16, and Foxp3. Please see main texts for details. Abbreviations: RA, retinoic acid; PP2A, protein phosphatase 2A; NRP1, neuropilin-1; S1PR1, sphingosine-1-phosphate receptor 1; PTEN, phosphatase and tensin homolog; TLR, toll-like receptor; Gln, glutamine; Leu, leucine; SEMA4A, semaphorin; CaMK4, calcium/calmodulin-dependent protein kinase IV; SIRT1, sirtuin1.