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# Regulation of T cells by mTOR: The known knowns and the known unknowns

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## Abstract

Mammalian/mechanistic target of rapamycin (mTOR) is emerging as an important integrator of environmental cues critical for the regulation of T cell activation, differentiation and function. Recent studies leveraging pharmacologic inhibition or T cell specific genetic deletion of signaling components in the mTOR pathway have provided important insights into the mechanisms involved, and have been informative in defining targets downstream of mTOR that promote immune regulation. However, these studies have also presented confusing and at times contradictory findings, highlighting the complexities involved in examining the mTOR pathway in distinct contexts. Here we review the current understanding of the roles of mTOR in T cell biology, highlighting emerging concepts and areas of investigation where the precise role of mTOR has yet to be fully discerned.

### Keywords

mTOR; T cells; rapamycin

## Introduction

Over the last decade there has been a marked increase in our understanding of T cell differentiation. For CD4+ T cells, the $T_H1$ and $T_H2$ paradigm has evolved into multiple effector and regulatory subsets, with greater appreciation for plasticity between these subsets. Likewise, there has been great progress in better defining the properties of CD8+ memory and effector T cells. Concomitant with this expanded view of T cell differentiation have been observations supporting an important role for the mammalian/mechanistic Target Of Rapamycin (mTOR) in promoting the development of these different T cell subsets. This brief review article does not seek to provide a comprehensive roadmap of the role of mTOR in regulating T cell differentiation and function; there are several recent comprehensive



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works on this topic [1–3]. Rather, we hope to convey our perspective regarding current available observations and perhaps more importantly highlight areas in which the precise role of mTOR is unclear.

## Rapamycin as an immunosuppressant

Rapamycin was the name given to a compound discovered on Rapa Nui (Easter Island) derived from *Streptomyces hygroscopicus* that was originally developed as a potential new antibiotic [4]. Elegant studies in yeast demonstrated that rapamycin mediates its effects by binding to an evolutionarily conserved serine/threonine kinase which was subsequently named TOR (Target of Rapamycin) [5]. Ultimately, rapamycin was found to be a poor antibiotic, but rather had potent immunosuppressive activity. Originally, it was proposed that the ability of rapamycin to inhibit immune responses was due to its anti-proliferative activity. For example, treatment of cells with rapamycin promotes G1 arrest and leads to the failure of cells to down modulate the CDK inhibitor p27 [6]. However, those who have performed proliferation experiments with rapamycin and T cells realize that the anti-proliferative effects of this agent are modest and predominantly affect the speed with which the T cells proceed through the cell cycle [7].

Some of the first clues regarding a potentially expanded role for mTOR in regulating T cell function came from studies on T cell anergy, a process by which $T_H1$ cells that encounter antigen (Signal1) in the absence of costimulation (Signal 2) are hyporesponsive upon rechallenge [8]. It was observed that rapamycin could promote anergy even in the presence of costimulation [9]. Initially it was thought that this was due to the ability of rapamycin to inhibit proliferation. However, studies employing another cyclophilin binding compound, sanglifehrin A, ultimately disassociated the ability of rapamycin to induce anergy from its anti-proliferative function [10]. Further studies directly implicated mTOR in regulating activation versus anergy [11–14]. These studies describing a role for rapamycin in promoting T cell anergy were followed by a series of studies demonstrating the ability of rapamycin to promote the generation of regulatory T cells (see also Zeng and Chi, this issue for a recent review[15]). While activating $T_H1$ cells in the presence of rapamycin promoted anergy, it was found that activating freshly isolated primary T cells in the presence of rapamycin led to both the selective expansion of T regulatory cells as well as their *de novo* generation [16–21]. Thus, the induction of anergy and regulatory T cells were two additional explanations (in addition to modest anti-proliferative function) for the ability of rapamycin to suppress immune responses.

## Genetic deletion of mTOR impacts T cell differentiation

To better define the role of mTOR in T cells, we crossed mTOR-floxed mice with mice expressing CD4-Cre recombinase [22]. In these mice, mTOR is deleted in all conventional T cells at the CD4+CD8+ double positive stage of thymic development. Notably, we did not detect a significant decrease in mTOR protein until the single positive stages of T cell development. As such, our group has refrained from drawing any conclusions concerning the role of mTOR in thymic T cell development, which is an active area of investigation [21, 23–27]. mTOR-deficient T cells proliferate slowly in response to activation, but TCR-

induced signaling appears to be intact in that IL-2 production by naïve T cells is similar to that of the wild-type T cells. On the other hand, these mice revealed a critical role for mTOR in regulating differentiation of peripheral T cells. Specifically, we observed that mTOR-deficient CD4+ T cells fail to differentiate into $T_H1$, $T_H2$, and $T_H17$ subsets under appropriate skewing conditions. Instead, under these activating conditions, the T cells develop into Foxp3+ regulatory cells [22]. These genetic studies suggested a novel paradigm whereby antigen recognition in the absence of mTOR activity leads to a default T-regulatory cell differentiation pathway. This result has led us to view mTOR activation, critical for the integration of costimulatory, cytokine, environmental and metabolic cues, as 'signal two' necessary for T cell differentiation (see two reviews for further detail[28, 29]).

## Dissecting Signals leading to mTOR activation in T cells

Much insight regarding the role of mTOR signaling in regulating T cell differentiation and function has been derived by genetically deleting components of the mTOR signaling complexes. In general, mTOR is activated by a variety of environmental cues such as growth factors, nutrients, energy and stress [30]. For T cells, TCR engagement in the presence of costimulation as well as cytokine stimulation (for example IL-1, IL-2, IL-4, IL-12 and IFN-γ) markedly enhances mTOR activation [31]. mTOR signals via two distinct complexes: mTORC1 and mTORC2, which contain both common and distinct components (Figure 1). In particular, the scaffolding proteins Raptor and Rictor define the downstream substrates for mTORC1 and mTORC2 activation respectively [30]. mTORC1 activity is typically assayed by the phosphorylation of the kinase S6K1 and the translation initiation factor 4E-BP1, while mTORC2 activity is assessed by the phosphorylation of the kinase Akt at serine 473 [30]. While phosphorylation of these proteins represent the best described substrates of mTORC1 and mTORC2 activity, there is great interest in determining the details and ultimate consequences of differential mTOR signaling and assessment of downstream targets in T cells as well as in general, as illustrated in the discussion of SGK1, below.

Canonical upstream signals leading to mTOR activation include PI3-kinase-induced generation of PIP3 which in turn activates PDK1 which then activates Akt through phosphorylatation at threonine 308. Activated Akt then phosphorylates TSC2 inhibiting the GAP activity of the TSC complex, thus allowing for the small GTPase Rheb to activate mTORC1 [30]. It is important to note that Akt activation is both upstream of mTORC1 (phosphorylation at threonine 308) and downstream of mTORC2 (phosphorylation at serine 473). Our group has shown that the genetic deletion of Rictor, and hence mTORC2 activity, does not inhibit Akt phosphorylation at threonine 308 and has no negative consequences in terms of mTORC1 activation [32]. However, while Figure 1 depicts the canonical mTORC1 activation pathways, the necessity of Akt activation in T cells has been called into question. Observations from the Cantrell group demonstrate that while PDK1 is necessary for mTORC1 signaling of *in vitro* activated cytotoxic lymphocytes (CTLs), such activation still occurs upon pharmacological inhibition of Akt [7]. These findings suggest that, for T cells, a kinase other than Akt may be responsible for the inactivation of TSC and subsequent activation of mTOR. Discovering this kinase, presumably one or more additional AGC (camp-dependent, cGMP-dependent, and protein kinase C) kinases similar to Akt, S6K1 or

SGK1 that can act in lieu of Akt, would reveal this precise mechanism and provide important insight in terms of better understanding the activation of mTOR in T cells and in general. Interestingly, one study suggests that MALT1 and Carma1, adaptor proteins that are known to complex with Bcl10 to activate NF-κB, may directly regulate mTORC1 without the necessity of Akt [33].

Recently, there has been a flurry of reports examining the role of TSC1 in CD8+ T cells [34–37]. TSC1 along with TSC2 form the Tuberous Sclerosis Complex which serves to inhibit mTORC1 by acting as a GAP for Rheb [38]. As might be expected, TSC1 deficient T cells demonstrate increased mTOR activity. However, such cells also display increased activation induced apoptosis [34, 36, 37, 39] and poor CD8+ T cell effector responses. Mechanistically, this was thought to be secondary to an overall decrease in expression of the anti-apoptotic protein Bcl-2 as well as abnormal mitochondrial potential and increased reactive oxygen species [34, 36, 37]. Complementary to these findings, one study also observed an increase in the pro-apoptotic molecule Bim [36]. Mechanistically, this was linked to a decrease in mTORC2 activity and a subsequent increase in the activity of the FOXO transcription factors that are negatively regulated by mTORC2. These findings highlight the complexity of the mTOR signaling pathway. Specifically, one must be circumspect as to whether a particular finding is due to enhanced mTORC1 or decreased mTORC2 (see Box1). Current work in the field using genetic knockouts or pharmacological inhibition of mTOR related proteins does not always consistently assess both mTORC1 and mTORC2 activation, thus limiting our understanding of mTOR signaling in T cell function.

## Understanding the role of downstream mTOR signaling in T cells

As discussed above, the two mTOR signaling complexes, mTORC1 and mTORC2, can be distinguished by their associated adaptor proteins (Raptor for mTORC1 and Rictor for mTORC2). Likewise, the small GTPase Rheb is selectively involved in activating mTORC1 signaling [30]. Our lab created mice in which mTORC1 activity was selectively inhibited in T cells by crossing mice with Rheb floxed alleles to transgenic mice expressing Cre recombinase under the control of CD4 [32]. Similar to T cell lacking mTOR, T cells deficient in Rheb fail to become $T_H1$ or $T_H17$ cells under respective skewing conditions. This lack of differentiation was associated with decreased T-bet and RORγt expression. However, at this time it is not clear if these observations are the direct cause of or a consequence of abortive effector generation. To this end, an important area of future research will be to define precise links between mTOR signaling and canonical immunologic signaling pathways. For example, preliminary studies from our lab suggest a role for mTOR in promoting T-bet phosphorylation (Powell, unpublished findings). Not surprising, mice with T cell specific deletion of Rheb are relatively resistant to the development of EAE. Curiously, when challenged with MOG peptide, instead of developing paralysis, the mice with T cell specific deletion of Rheb develop ataxia and cerebellar inflammation consistent with “nonclassical” EAE, a disease associated with $T_H2$ responses.

Notably, when activated, the Rheb-deficient T cells do not become regulatory T cells as was the case with the mTOR deficient T cells [22, 32]. At first this observation seems at odds with reports that the mTORC1 inhibitor rapamycin promotes the generation of regulatory T

cells. In fact, there is no paradox because under relatively modest concentrations of rapamycin (particularly in naïve T cells), mTORC1 and mTORC2 are both inhibited [32]. While the precise mechanism of this effect is not known, the ability of rapamycin to inhibit both mTORC1 and mTORC2 has been well described in various tumor cells [40]. Interestingly, a series of studies by the Fowler lab has shown that rapamycin can promote the generation of $T_H2$ cells in T cells from Balb/c mice as well as humans [41, 42]. Although mTORC1 and mTORC2 were not always simultaneously measured in these studies, we believe these observations are consistent with (under these culture conditions) rapamycin preferentially inhibiting mTORC1 and thus promoting $T_H2$ development at the expense of $T_H1$ inhibition.

The role of mTORC1 in T cells has further been studied by deleting the adaptor protein Raptor. One group employed mice with Raptor floxed alleles which expressed Cre recombinase under the control of the Lck promoter (Lck-Cre Raptor$^{fl/fl}$) to examine the role of mTORC1 on $T_H1$ and $T_H17$ differentiation [43]. Unlike the previous studies using Rheb-deficient T cells, this study found that Raptor-deficient CD4+ T cells are impaired in their ability to become $T_H17$ cells, but not $T_H1$ cells [43]. The reason for these differences, which are potentially quite interesting, is not clear and unfortunately the authors did not comment on the previous Rheb studies. A second study, from the Chi group, using mice with Raptor floxed alleles which express Cre recombinase under the control of CD4, demonstrated that Raptordeficient T cells did not generate $T_H1$ responses but also failed to generate $T_H2$ responses [44]. These results while differing with the previously published Lck-Cre Raptor$^{fl/fl}$ report were more consistent with the original work done using Rheb-deficient T cells with regard to the effect of mTORC1 on $T_H1$ differentiation. In this second study, a discussion concerning the differences between these results and the previously published Raptor study would have been illuminating, but was not addressed. Of note, by employing Raptor-deficient T cells we have observed that even after initially purifying CD4+ T cells, after several days in culture a population of CD4- γδ+ T cells emerge which express high levels of IFN-γ (Powell, unpublished observations). We speculate that this may account for the production of IFN-γ in the former Raptor-deficient paper. Additionally, it is of potential great interest that while the Rheb-deficient T cells demonstrate robust $T_H2$ differentiation, the Raptor-deficient T cells (in the second study) did not. That is, the differences between Rheb versus Raptor-deficient T cells might offer a unique opportunity to dissect specific downstream mTORC1 driven signaling pathways involved in regulating T cell differentiation and function. For example, it will be interesting to determine what specific metabolic programs are blocked in Raptor-deficient T cells when compared to Rheb-deficient T cells in order to determine specific differences in the metabolic requirements of $T_H1$ versus $T_H2$ cells. As such, we propose that studying T cell signaling and biology represents a robust means to dissect the unique roles of Rheb and Raptor in regulating mTORC1 signaling.

The role of mTORC1 in regulating the function of T regulatory cells appears not to be straight-forward. Multiple studies have demonstrated the ability of rapamycin to promote the generation and expansion of regulatory T cells [16, 17, 21]. Likewise, mTOR-deficient T cells preferentially develop into regulatory T cells even when engaging antigen under normally activating conditions [22]. From a metabolic perspective, at first glance, such

findings seem to fit with data demonstrating that effector CD4+ T cells are highly glycolytic, while regulatory T cells are less glycolytic and employ to a greater degree than effector cells, fatty acid metabolism [45]. mTOR activity is important in promoting glycolytic metabolism [46]. Likewise, these data are consistent with studies that transfection with a constitutively active Akt construct inhibits $T_{reg}$ generation [21]. However, based on studies examining both human and mouse regulatory T cells, Matarese and colleagues have proposed a dynamic role for mTOR activation in T regulatory cells. In their model, there is initial downregulation of leptin- induced mTOR activation and then an increase in mTOR activation which promotes T regulatory cell proliferation [47]. Perhaps consistent with these findings, Chi and colleagues have recently proposed that mTORC1 is essential for regulatory T cell function [48]. Based on studies employing Raptor-deficient T cells, they propose that mTORC1 is essential for metabolic programming necessary for regulatory T cell function. Ostensibly, these findings seem to contradict data demonstrating that mTOR-deficient T cells differentiate into (seemingly) functional regulatory T cells [22]. Reconciling these differences, however, is the fact that Raptor-deficient T cells have hyperactive mTORC2 activity that appears to inhibit regulatory T cell function. Indeed, regulatory T cell function is restored (though not completely) in T cells deficient in both Raptor and Rictor [48]. Our group has been examining mTOR function in different subsets of regulatory T cells derived from wild-type C57BL/6 mice. Based on these observations, we have proposed that mTOR activity may promote the function of short-lived, $CD62L^{lo}$ 'effector' regulatory T cells, while decreased mTOR activity promotes metabolic and survival programs in long-lived $CD62L^{hi}$ 'memory' regulatory T cells ([29] and unpublished observations). This idea corroborates with recent work establishing that peripherally generated $T_{reg}$ cells can differentiate into distinct subsets [49, 50]. As in the case of T helper cells, these distinct T regulatory subsets may also have distinct metabolic requirements. We hypothesize that the generation of "effector" T regulatory cells can occur in the presence of relatively high mTOR activity (for example, as is the case in stimulating cells with anti-CD3 and high dose TGF-β), while TCR engagement in the setting of decreased mTOR activity leads to the generation of "memory" $T_{reg}$ cells. As future studies better define the role of mTOR in $T_{reg}$ generation and function this will reveal new potential pharmacologic targets for the regulation of regulatory T cells.

The role of mTORC2 in promoting T helper cell differentiation has also been examined. By deleting Rictor in T cells, both our group and the Boothby group were able to selectively inhibit mTORC2 in T cells while leaving mTORC1 activity intact [32, 51]. These studies revealed that in the absence of mTORC2, T cells fail to develop into $T_H2$ cells. Likewise, both groups showed that $T_H17$ differentiation remained intact in the absence of mTORC2. However, Rictor-deficient T cells from our mice also demonstrated a robust ability to differentiate into $T_H1$ cells [32]. Our observations that Rheb-deficient T cells failed to differentiate into $T_H1$ or $T_H17$ cells while Rictor-deficient T cells failed to differentiate into $T_H2$ cells led us to propose a model whereby mTORC1 is critical for $T_H1$ and $T_H17$ differentiation while mTORC2 is critical for $T_H2$ differentiation. Alternatively, the Boothby group revealed diminished $T_H1$ differentiation in the absence of mTORC2 signaling [51]. While the precise reasons for these discrepancies is unclear, comparisons of T cells from these two mouse strains with T cell specific deletion of Rictor (which employ different

tissue specific Cre recombinases, we utilized a cre recombinase under the control of the CD4 promoter while Boothby's group utilized the distal Lck promoter) offer the opportunity to more selectively dissect the role of mTORC2 in T cells. For example, unlike T cells from our mice, the Rictor-deficient T cells from the Boothby group did not demonstrate a robust increase in Foxo/KLF2 mediated transcription, but rather a substantial defect in PKC signaling. In contrast to these studies, the Chi group demonstrated a modest role for mTORC2 in regulating $T_H2$ differentiation in their models [44]. Interestingly, blocking mTORC2 signaling through the genetic deletion of mSIN1 did not affect T helper cell differentiation [52]. These findings suggest that comparisons between mSIN1 dependent mTORC2 activity and Rictor dependent mTORC2 activity might provide important insight into the mTORC2 mediated pathways that selectively regulate T cell function, analogous to substrate differences between Rheb- and Raptor-deficient cells. Towards this goal, our group has revealed an important role for the mTORC2 substrate SGK1 in regulating $T_H1$ and $T_H2$ differentiation [53]. SGK1 is an AGC kinase that becomes activated upon phosphorylation by mTORC2 at its hydrophobic loop domain. Consistent with our observations that mTORC2 is critical for $T_H2$ differentiation, the selective deletion of SGK1 in T cells markedly impaired their ability to become $T_H2$ cells. In contrast, such cells produced IFN-γ upon stimulation. Indeed, mice with T cell specific deletion of SGK1 were resistant to House Dust Miteinduced reactive airway disease. Alternatively, these mice produced robust IFN-γ in response to viral and tumor challenge [53]. These data illustrate the selectivity that can be achieved by targeting pathways downstream of mTOR signaling. For example, we have determined that deleting mTOR mitigates the differentiation of effector T helper cells, whereas selectively inhibiting mTORC2 primarily mitigates $T_H2$ differentiation but leaves $T_H1$ differentiation intact, and selectively deleting SGK1 (downstream of mTORC2) mitigates $T_H2$ differentiation while enhancing $T_H1$ differentiation. In this regard, our data suggest that pharmacologic inhibitors of SGK1 might prove useful in treating atopic diseases such as asthma as well as potentially enhancing vaccine efficacy and tumor immunotherapy. Of note, two other groups demonstrated that SGK1 is critical for pathogenic $T_H17$ generation and the ability of sodium to enhance the pathogenesis of EAE [54, 55]. The genesis of these studies was from a robust bioinformatics approach, and the role of mTOR signaling in these two papers was not investigated.

## mTOR CD8+ effector and memory cells

Given its importance in integrating cues to guide T helper differentiation, it is not surprising that an important role for mTOR signaling has also been implicated in CD8+ T cells. Indeed, the role of mTOR in regulating CD8+ T cell function and differentiation emerged from a series of papers connecting mTOR, metabolism, and memory formation [56–59]. Treatment with rapamycin led to enhanced CD8+ T cell memory generation and recall responses. In as much as rapamycin is known as an immunosuppressive agent, these findings were quite provocative. The mechanism for these observations was revealed in part by elucidating the different metabolic requirement for CD8+ effector and memory T cells. Unlike effector CD8+ T cells, memory cells are far less glycolytic and rely more on lipid metabolism [60]. Inhibition of mTOR promotes these lipid metabolic programs [58]. Based on these properties, rapamycin has been employed strategically in experimental models to enhance

vaccine efficacy against viruses and tumors [61–64]. Initially, work suggested that regimens of rapamycin throughout the expansion and contraction phase of an immune response enhanced both the quantity and quality of CD8+ T cell memory [65]. However, more recent work suggests that long-term blockade of mTORC1 signaling through rapamycin treatment abrogates CD8+ T cell memory formation, and instead a short course during the expansion phase is recommended [66]. Thus, the exact modulation of mTOR signaling to enhance vaccine potential must be further explored. Interestingly, for not precisely clear reasons, it has been shown that low dose rapamycin treatment can selectively inhibit transplant rejection while simultaneously enhancing anti-viral responses [67, 68]. Furthermore, our group has found that the selective inhibition of mTORC2 by deleting Rictor can enhance memory T cell generation without inhibiting effector function (Powell unpublished findings). Thus, while inhibition of mTOR has been shown to dramatically enhance CD8+ T cell memory formation in part by influencing T cell metabolism, the precise effect of rapamycin treatment on the mTOR signaling pathway has not been fully explored. It is currently unknown if the results are due to specific loss of mTORC1 signaling or by inhibition of both mTORC1 and mTORC2 complexes. Further work will need to address these issues but nonetheless the idea that blocking mTOR signaling can enhance the efficacy of vaccines has exciting translational implications.

## Concluding remarks

In the last several years the role of mTOR in contributing to T cell activation, differentiation and function has been firmly established. Furthermore, selective roles for mTORC1 and mTORC2 signaling in T cells have been clearly demonstrated. Much of these findings have been facilitated by the incisive use of genetically modified mice. In this work, we have tried to highlight some of the areas of nonconcordance using these models, which we believe afford unique opportunities to dissect in greater detail these signaling pathways (Table 1). In particular, it is clear that the effects of deleting Rheb and Raptor on inhibiting mTORC1 activity are both overlapping and unique. Given the different immunologic consequences observed in Rheb-deficient and Raptor-deficient T cells, this presents a robust model in order to finely dissect mTORC1 function on regulating T cells. Alternatively, it has also become clear that when perturbing mTOR signaling the role of negative feedback loops must be considered (for example the deletion of Raptor leading to enhanced mTORC2 activity). Likewise, a further challenge to the field is to be able to distinguish “generic” effects of these signaling pathways (for example on proliferation) from selective mediators of T cell reprogramming and function. The powerful genetic models that have been developed provide important insight into the necessity of mTOR signaling in T cell differentiation and function. However, there is still much work to be done with regard to dissecting both the direct and indirect role of mTOR signaling (Box 2). To this end, defining the precise role of mTOR on both the “immunologic” signaling pathways (for example STAT signaling, PKC signaling, Tbet, GATA-3, RORγt expression) and metabolic pathways (for example glycolysis, oxidative phosphorylation, fatty acid oxidation) will be important future tasks to advance our understanding. Our lab takes the view that mTOR plays a critical role in *coordinating* immunologic and metabolic reprogramming to promote effective T cell differentiation in response to antigen. Thus, a better understanding of the

specific pathways downstream of mTOR will inform the development of novel pharmacologic agents to selectively regulate T cell function.

## References


1. Powell JD, et al. Regulation of immune responses by mTOR. Annu Rev Immunol. 2012; 30:39–68. [PubMed: 22136167]
2. Pollizzi KN, Powell JD. Integrating canonical and metabolic signalling programmes in the regulation of T cell responses. Nat Rev Immunol. 2014; 14:435–446. [PubMed: 24962260]
3. Waickman AT, Powell JD. Mammalian target of rapamycin integrates diverse inputs to guide the outcome of antigen recognition in T cells. J Immunol. 2012; 188:4721–4729. [PubMed: 22556133]
4. Vezina C, et al. Rapamycin (AY-22,989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle. J Antibiot (Tokyo). 1975; 28:721–726. [PubMed: 1102508]
5. Schmelzle T, Hall MN. TOR, a central controller of cell growth. Cell. 2000; 103:253–262. [PubMed: 11057898]
6. Sengupta S, et al. Regulation of the mTOR complex 1 pathway by nutrients, growth factors, and stress. Molecular Cell. 2010; 40:310–322. [PubMed: 20965424]
7. Finlay DK, et al. PDK1 regulation of mTOR and hypoxia-inducible factor 1 integrate metabolism and migration of CD8+ T cells. J Exp Med. 2012; 209:2441–2453. [PubMed: 23183047]
8. Schwartz RH. T cell anergy. Annu Rev Immunol. 2003; 21:305–334. [PubMed: 12471050]
9. Powell JD, et al. Inhibition of cell cycle progression by rapamycin induces T cell clonal anergy even in the presence of costimulation. J Immunol. 1999; 162:2775–2784. [PubMed: 10072524]
10. Allen A, et al. The novel cyclophilin binding compound, sanglifehrin A, disassociates G1 cell cycle arrest from tolerance induction. J Immunol. 2004; 172:4797–4803. [PubMed: 15067056]
11. Colombetti S, et al. Prolonged TCR/CD28 engagement drives IL-2-independent T cell clonal expansion through signaling mediated by the mammalian target of rapamycin. J Immunol. 2006; 176:2730–2738. [PubMed: 16493028]
12. Vanasek TL, et al. Antagonistic roles for CTLA-4 and the mammalian target of rapamycin in the regulation of clonal anergy: enhanced cell cycle progression promotes recall antigen responsiveness. J Immunol. 2001; 167:5636–5644. [PubMed: 11698435]
13. Powell JD, Zheng Y. Dissecting the mechanism of T-cell anergy with immunophilin ligands. Curr Opin Investig Drugs. 2006; 7:1002–1007.
14. Zheng Y, et al. A Role for Mammalian Target of Rapamycin in Regulating T Cell Activation versus Anergy. J Immunol. 2007; 178:2163–2170. [PubMed: 17277121]
15. Zeng H, Chi H. Metabolic control of regulatory T cell development and function. Trends Immunol. 2014
16. Battaglia M, et al. Rapamycin selectively expands CD4+CD25+FoxP3+ regulatory T cells. Blood. 2005; 105:4743–4748. [PubMed: 15746082]
17. Kang J, et al. De novo induction of antigen-specific CD4+CD25+Foxp3+ regulatory T cells in vivo following systemic antigen administration accompanied by blockade of mTOR. J Leukoc Biol. 2008; 83:1230–1239. [PubMed: 18270248]
18. Kopf H, et al. Rapamycin inhibits differentiation of Th17 cells and promotes generation of FoxP3+ T regulatory cells. Int Immunopharmacol. 2007; 7:1819–1824. [PubMed: 17996694]
19. Coenen JJ, et al. Rapamycin, not cyclosporine, permits thymic generation and peripheral preservation of CD4(+)CD25(+)FoxP3(+) T cells. Bone Marrow Transplant. 2007; 39:537–545. [PubMed: 17351648]
20. Zeiser R, et al. Differential impact of mammalian target of rapamycin inhibition on CD4+CD25+Foxp3+ regulatory T cells compared with conventional CD4+ T cells. Blood. 2008; 111:453–462. [PubMed: 17967941]
21. Haxhinasto S, et al. The AKT-mTOR axis regulates de novo differentiation of CD4+Foxp3+ cells. J Exp Med. 2008; 205:565–574. [PubMed: 18283119]

22. Delgoffe GM, et al. The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment. Immunity. 2009; 30:832–844. [PubMed: 19538929]
23. Tang F, et al. A critical role for Rictor in T lymphopoiesis. J Immunol. 2012; 189:1850–1857. [PubMed: 22815285]
24. Lee K, et al. Vital roles of mTOR complex 2 in Notch-driven thymocyte differentiation and leukemia. J Exp Med. 2012; 209:713–728. [PubMed: 22473959]
25. Chen H, et al. Disruption of TSC1/2 signaling complex reveals a checkpoint governing thymic CD4+ CD25+ Foxp3+ regulatory T-cell development in mice. FASEB J. 2013; 27:3979–3990. [PubMed: 23882125]
26. Wei J, et al. Cutting Edge: Discrete Functions of mTOR Signaling in Invariant NKT Cell Development and NKT17 Fate Decision. J Immunol. 2014; 193:4297–4301. [PubMed: 25261481]
27. Guo D, et al. NOTCH and phosphatidylinositide 3-kinase/phosphatase and tensin homolog deleted on chromosome ten/AKT/mammalian target of rapamycin (mTOR) signaling in T-cell development and T-cell acute lymphoblastic leukemia. Leuk Lymphoma. 2011; 52:1200–1210. [PubMed: 21463127]
28. Powell JD, Delgoffe GM. The mammalian target of rapamycin: linking T cell differentiation, function, and metabolism. Immunity. 2010; 33:301–311. [PubMed: 20870173]
29. Powell JD, et al. A Modified Model of T-Cell Differentiation Based on mTOR Activity and Metabolism. Cold Spring Harb Symp Quant Biol. 2013
30. Zoncu R, et al. mTOR: from growth signal integration to cancer, diabetes and ageing. Nat Rev Mol Cell Biol. 2011; 12:21–35. [PubMed: 21157483]
31. Powell JD, et al. Regulation of Immune Responses by mTOR. Annu Rev Immunol. 2011
32. Delgoffe GM, et al. The kinase mTOR regulates the differentiation of helper T cells through the selective activation of signaling by mTORC1 and mTORC2. Nat Immunol. 2011; 12:295–303. [PubMed: 21358638]
33. Hamilton KS, et al. T cell receptor-dependent activation of mTOR signaling in T cells is mediated by Carma1 and MALT1, but not Bcl10. Sci Signal. 2014; 7 ra55.
34. O'Brien TF, et al. Regulation of T-cell survival and mitochondrial homeostasis by TSC1. Eur J Immunol. 2011; 41:3361–3370. [PubMed: 21805467]
35. Krishna S, et al. Role of tumor suppressor TSC1 in regulating antigen-specific primary and memory CD8 T cell responses to bacterial infection. Infect Immun. 2014; 82:3045–3057. [PubMed: 24818661]
36. Zhang L, et al. TSC1/2 signaling complex is essential for peripheral naive CD8+ T cell survival and homeostasis in mice. PLoS One. 2012; 7:e30592. [PubMed: 22363451]
37. Yang K, et al. The tumor suppressor Tsc1 enforces quiescence of naive T cells to promote immune homeostasis and function. Nat Immunol. 2011; 12:888–897. [PubMed: 21765414]
38. Kwiatkowski DJ, Manning BD. Tuberous sclerosis: a GAP at the crossroads of multiple signaling pathways. Hum Mol Genet. 2005; 14(Spec No. 2):R251–R258. [PubMed: 16244323]
39. Wu Q, et al. The tuberous sclerosis complex-mammalian target of rapamycin pathway maintains the quiescence and survival of naive T cells. J Immunol. 2011; 187:1106–1112. [PubMed: 21709159]
40. Sarbassov DD, et al. Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. Mol Cell. 2006; 22:159–168. [PubMed: 16603397]
41. Mariotti J, et al. Ex vivo rapamycin generates apoptosis-resistant donor Th2 cells that persist in vivo and prevent hemopoietic stem cell graft rejection. J Immunol. 2008; 180:89–105. [PubMed: 18097008]
42. Jung U, et al. Ex vivo rapamycin generates Th1/Tc1 or Th2/Tc2 Effector T cells with enhanced in vivo function and differential sensitivity to post-transplant rapamycin therapy. Biol Blood Marrow Transplant. 2006; 12:905–918. [PubMed: 16920556]
43. Kurebayashi Y, et al. PI3K-Akt-mTORC1-S6K1/2 axis controls Th17 differentiation by regulating Gfi1 expression and nuclear translocation of RORgamma. Cell Rep. 2012; 1:360–373. [PubMed: 22832227]

44. Yang K, et al. T cell exit from quiescence and differentiation into Th2 cells depend on Raptor-mTORC1- mediated metabolic reprogramming. Immunity. 2013; 39:1043–1056. [PubMed: 24315998]
45. Michalek RD, et al. Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4+ T cell subsets. J Immunol. 2011; 186:3299–3303. [PubMed: 21317389]
46. Duvel K, et al. Activation of a metabolic gene regulatory network downstream of mTOR complex 1. Molecular Cell. 2010; 39:171–183. [PubMed: 20670887]
47. Procaccini C, et al. An oscillatory switch in mTOR kinase activity sets regulatory T cell responsiveness. Immunity. 2010; 33:929–941. [PubMed: 21145759]
48. Zeng H, et al. mTORC1 couples immune signals and metabolic programming to establish T(reg)-cell function. Nature. 2013; 499:485–490. [PubMed: 23812589]
49. Gratz IK, Campbell DJ. Organ-specific and memory treg cells: specificity, development, function, and maintenance. Frontiers in immunology. 2014; 5:333. [PubMed: 25076948]
50. Srivastava S, et al. Type I interferons directly inhibit regulatory T cells to allow optimal antiviral T cell responses during acute LCMV infection. J Exp Med. 2014; 211:961–974. [PubMed: 24711580]
51. Lee K, et al. Mammalian target of rapamycin protein complex 2 regulates differentiation of Th1 and Th2 cell subsets via distinct signaling pathways. Immunity. 2010; 32:743–753. [PubMed: 20620941]
52. Chang X, et al. Sin1 regulates Treg-cell development but is not required for T-cell growth and proliferation. Eur J Immunol. 2012; 42:1639–1647. [PubMed: 22678916]
53. Heikamp EB, et al. The AGC kinase SGK1 regulates TH1 and TH2 differentiation downstream of the mTORC2 complex. Nat Immunol. 2014; 15:457–464. [PubMed: 24705297]
54. Wu C, et al. Induction of pathogenic TH17 cells by inducible salt-sensing kinase SGK1. Nature. 2013; 496:513–517. [PubMed: 23467085]
55. Kleinewietfeld M, et al. Sodium chloride drives autoimmune disease by the induction of pathogenic TH17 cells. Nature. 2013; 496:518–522. [PubMed: 23467095]
56. Araki K, et al. mTOR regulates memory CD8 T-cell differentiation. Nature. 2009; 460:108–112. [PubMed: 19543266]
57. Rao PR, et al. The mTOR kinase determines effector versus memory CD8+ T cell fate by regulating the expression of transcription factors T-bet and Eomesodermin. Immunity. 2010; 32:67–78. [PubMed: 20060330]
58. Pearce EL, et al. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. Nature. 2009; 460:103–107. [PubMed: 19494812]
59. He S, et al. Characterization of the metabolic phenotype of rapamycin-treated CD8+ T cells with augmented ability to generate long-lasting memory cells. PLoS One. 2011; 6:e20107. [PubMed: 21611151]
60. van der Windt GJ, et al. Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development. Immunity. 2012; 36:68–78. [PubMed: 22206904]
61. Li Q, et al. A central role for mTOR kinase in homeostatic proliferation induced CD8+ T cell memory and tumor immunity. Immunity. 2011; 34:541–553. [PubMed: 21511183]
62. Diken M, et al. mTOR inhibition improves antitumor effects of vaccination with antigenen-coding RNA. Cancer Immunol Res. 2013; 1:386–392. [PubMed: 24778131]
63. Garcia K, et al. IL-12 is required for mTOR regulation of memory CTLs during viral infection. Genes Immun. 2014
64. Turner AP, et al. Sirolimus enhances the magnitude and quality of viral-specific CD8+ T-cell responses to vaccinia virus vaccination in rhesus macaques. Am J Transplant. 2011; 11:613–618. [PubMed: 21342450]
65. Araki K, et al. The role of mTOR in memory CD8 T-cell differentiation. Immunol Rev. 2010; 235:234–243. [PubMed: 20536567]
66. Li Q, et al. Regulating mammalian target of rapamycin to tune vaccination-induced CD8(+) T cell responses for tumor immunity. J Immunol. 2012; 188:3080–3087. [PubMed: 22379028]

67. Ferrer IR, et al. Cutting edge: Rapamycin augments pathogen-specific but not graft-reactive CD8+ T cell responses. J Immunol. 2010; 185:2004–2008. [PubMed: 20631309]

68. Ferrer IR, et al. Paradoxical aspects of rapamycin immunobiology in transplantation. Am J Transplant. 2011; 11:654–659. [PubMed: 21446969]

69. Macintyre AN, et al. Protein kinase B controls transcriptional programs that direct cytotoxic T cell fate but is dispensable for T cell metabolism. Immunity. 2011; 34:224–236. [PubMed: 21295499]

70. Sowell RT, et al. Cutting Edge: Generation of Effector Cells That Localize to Mucosal Tissues and Form Resident Memory CD8 T Cells Is Controlled by mTOR. J Immunol. 2014

71. Berezhnoy A, et al. Aptamer-targeted inhibition of mTOR in T cells enhances antitumor immunity. J Clin Invest. 2014; 124:188–197. [PubMed: 24292708]

72. Shrestha S, et al. Tsc1 promotes the differentiation of memory CD8+ T cells via orchestrating the transcriptional and metabolic programs. Proc Natl Acad Sci U S A. 2014

73. Park Y, et al. TSC1 regulates the balance between effector and regulatory T cells. J Clin Invest. 2013; 123:5165–5178. [PubMed: 24270422]

## Box 1

### A direct effect on mTOR or a secondary effect on Akt?

A complexity of studying mTOR signaling is the potential role of Akt in mediating observed effects. First, Akt activation is upstream of mTORC1 activity (Akt phosphorylates and inactivates TSC2 thus leading to enhanced mTORC1 activity). Second, Akt is activated in an mTORC2 dependent fashion. Finally, Akt activity is intimately regulated by feedback loops involving mTORC1. Deleting TSC1, leading to increased mTORC1 activity results in markedly diminished Akt activity. Alternatively, deletion of Rheb leading to decreased mTORC1 activity yields a slight increase in Akt activity, while deletion of Raptor results in a marked increase in Akt activity. Deletion of mTOR mitigates mTORC2 dependent Akt activation, but does not appear to inhibit phosphorylation of Akt upstream of TSC2. However, even the consequence of this observation is unclear since there is data to suggest that mTORC1 activation in T cells is Akt-independent. Finally, treatment with rapamycin at doses that solely inhibit mTORC1 can lead to enhanced Akt activity, while rapamycin at doses and duration that inhibit both mTORC1 and mTORC2 results in the abrogation of Akt phosphorylation.

## Box 2

**Outstanding questions**

What are the direct versus indirect pathways by which mTOR regulates T cell activation, differentiation and function?

What pathways link diverse upstream inputs (TCR engagement, costimulation, cytokines, metabolites) with mTORC1 and mTORC2 activation in T cells?

What is the role of Akt activation in these pathways?

How does mTOR signaling guide Treg cell differentiation and function, and is there a difference between natural Treg cells and induced Treg cells?

Do Rheb and Raptor have distinct roles in mTORC1 signaling, and likewise in T cell differentiation and function?

What are the specific targets downstream of mTORC1 and mTORC2 signaling that impact T cell fate and function, and what are the functions of these targets in metabolism and immune function?

## Highlights

- mTOR has emerged as a critical integrator of cues from the immune microenvironment
- By coordinating immunologic and metabolic programs, mTOR guides T cell fate decisions
- Mechanisms by which mTOR activity impacts T cell differentiation are not fully defined
- Contradictory findings highlight areas requiring further investigation

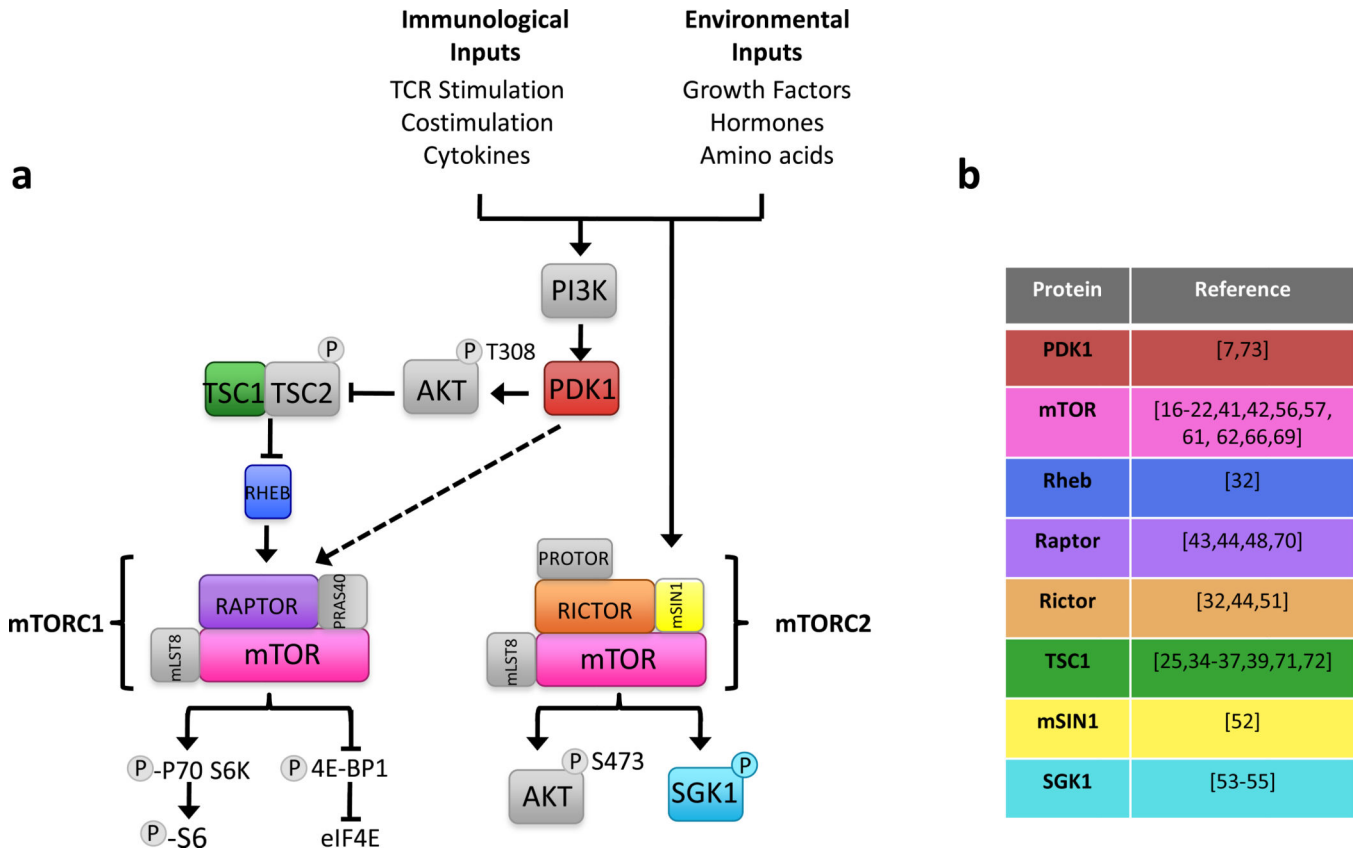


**Figure 1. A simplistic model of mTOR signaling**

a) mTOR signals via two distinct complexes, mTOR Complex 1 (mTORC1) and mTORC2. mTORC1 is characterized by the adaptor protein Raptor while mTORC2 is characterized by the adaptor protein Rictor. These differences in part contribute to the differences in downstream targets of the two complexes. mTORC1 is activated when Akt phosphorylates and inactivates TSC2, inhibiting the GAP activity of TSC1/TSC2 complex. This promotes the activation of the small GTPase Rheb and consequently the activation of mTORC1. The precise upstream pathways which contribute to the activation of mTORC2 are outside the scope of this review. The proteins shown in color indicate that they have been selectively genetically deleted in T cells and are discussed in this review. Proteins shown in gray have either not been specifically targeted, or as is the case for PI3K are outside the purview of this review. The dashed line signifies the non-canonical, Akt independent, activation of mTORC1 by PDK1 signaling as proposed by the Cantrell group [7]. b) A color coded reference table (the details of which are in Table 1) citing the references in which the specific component of mTOR signaling was deleted.

**Table 1**

Outcomes of selective deletion of mTOR specific proteins in T cells

| Protein | Function | Immunological Relevance/ opposing findings |
|---|---|---|
| PDK1 | A kinase critical for the activation of Akt, leading to mTORC1 activity | PDK1 is required for the activation of mTORC1. Finlay et al propose that the requirement of PDK1 induced mTORC1 activity is independent of Akt activity [7]<br>PDK1 is essential for IL-2 induced glucose uptake and T cell proliferation but not survival [69] |
| mTOR | The catalytic subunit of two distinct protein complexes: mTORC1 and mTORC2 | Specific loss of mTOR in CD4+ T cells results in loss of $T_H1$, $T_H2$ and $T_H17$ but enhanced Treg differentiation [22]<br>Use of mTOR activation inhibitor, Rapamycin, promotes the generation of $T_{REG}$ cells [16–21]<br>Rapamycin promotes generation of $T_H2$ CD4+ T cells in Balb/c mice and humans [41, 42]<br>Rapamycin treatment enhances quality and quantity of CD8+ T cell memory [56, 57, 61, 62]. However, prolonged high dose rapamycin treatment abrogates protective memory [66]<br>Rapamycin inhibits formation of resident memory cells in the intestinal mucosa [70] |
| RHEB | A small GTPase, a critical activator of mTORC1 | Loss of Rheb by CD4 Cre yields enhanced $T_H2$ but diminished $T_H1$ and $T_H17$ differentiation [32] |
| RAPTOR | The scaffold protein required for mTORC1 assembly | Lck Cre induced deletion of Raptor yields deficiency in $T_H17$ but not $T_H1$ phenotype [43]<br>Raptor deletion by CD4 Cre diminishes $T_H1$ and $T_H2$ generation [44]<br>Raptor deletion by Foxp3 YFP Cre or CD4 Cre abrogates $T_{REG}$ suppressive function, while simultaneous loss of both Raptor and Rictor partially rescues Treg function [48]<br>Loss of Raptor by CD4 Cre impairs CD8+ effector responses in vivo [44]<br>siRNA aptamer targeting of Raptor enhances CD8+ memory responses [71] |
| RICTOR | The scaffold protein necessary for mTORC2 assembly | Loss of Rictor by CD4 Cre impairs $T_H2$ but not $T_H1$ or $T_H17$ generation [32]<br>T cells with Lck Cre induced deletion of Rictor have diminished $T_H2$ and $T_H1$ but intact $T_H17$ differentiation [51]<br>Loss of Rictor by CD4 Cre only modestly impairs $T_H2$ generation in vitro, but addition of rapamycin to Rictor deficient T cells ablates IL-4 production [44] |
| TSC1 | Along with TSC2 acts as a negative regulator of Rheb/mTORC1 activity | TSC1 deficient CD8+ T cells have marked reduction in Bcl-2, elevated apoptosis, increased ROS production, and impairs CD8+ T cell survival prior to and upon activation [34, 36, 37, 39]. Data was generated through use of CD4 Cre [34, 37, 39], Lck Cre [36, 39], or Rosa26-Cre-$ER^{T2}$ [37]<br>TSC1 deficiency impairs CD8+ T cell homeostatic proliferation [36, 37, 39]<br>Loss of TSC1 ablates antigen specific anti bacterial responses and diminishes immunologic memory [35, 37, 72]<br>TSC1 restricts $T_H1$ and $T_H17$ differentiation, while Treg specific deletion of TSC1 diminishes suppressive function in vivo instead resulting in $T_H17$ effector like phenotype [73]<br>Lck Cre deletion of TSC1 enhances accumulation of thymic derived Foxp3+ Tregs, which may have reduced suppressive function [25] |
| mSIN1 | A component of mTORC2 | Deficiency of mSIN1 in hematopoietic stem cells resulted in normal T helper cell differentiation but enhanced generation of natural Tregs [52] |

| Protein | Function | Immunological Relevance/ opposing findings |
|---|---|---|
| SGK1 | An AGC kinase activated by mTORC2 | SGK1 regulates $T_H1$ and $T_H2$ differentiation [53]<br>SGK1 regulates IL-23R expression and induces pathogenic $T_H17$ generation in response to sodium chloride [54, 55] |