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REVIEW

The Role and Regulation of mTOR in T-Lymphocyte Function

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Abstract The conversion of naïve T cells into effector T cells is initiated by stimulation through the T-cell receptor (TCR). Upon activation, T cells undergo significant morphological and functional changes, putting new metabolic demands on the cell. Past research has identified the mammalian target of rapamycin (mTOR) as a critical regulator of cell metabolism, and the development of new genetic models has begun to reveal an important role for this pathway in the homeostasis and function of T lymphocytes. In this review, we focus on the most recent findings that demonstrate the ability of mTOR to regulate T-cell activation, $CD8^+$ memory cell formation and function, and helper T lineage differentiation. Furthermore, we highlight the importance of tight control of mTOR signaling by tuberous sclerosis complex 1 for T-cell homeostasis, and the regulation of mTOR signaling by diacylglycerol kinases and the RasGRP1-Ras-Erk1/2 pathway in the context of TCR signaling.

Keywords Mammalian target of rapamycin · Tuberous sclerosis complex 1 · Diacylglycerol kinase · RasGPP1 · PI3K/Akt · T lymphocyte

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Introduction

The mammalian target of rapamycin (mTOR) is an evolutionarily conserved serine/threonine kinase that can simultaneously integrate multiple signaling inputs, including nutrients, amino acids, growth factors, and cytokines, to regulate cell metabolism, proliferation, and growth (Fingar and Blenis 2004; Stanfel et al. 2009; Wullschleger et al. 2006). The mTOR protein associates with a variety of proteins, and is a component of two unique signaling complexes referred to as mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). In mTORC1, mTOR associates with Raptor, and is sensitive to acute rapamycin treatment, while in mTORC2, mTOR is in complex with Rictor, and is less sensitive to acute rapamycin treatment (Kim and Sabatini 2004; Sarbassov et al. 2004). mTORC1 is activated by the GTP bound form of Ras homolog enriched in brain (Rheb). The tuberous sclerosis complex (TSC), a heterodimer consisting of hamartin (Tuberous sclerosis 1; TSC1) and tuberin (Tuberous sclerosis 2; TSC2), functions as an inhibitor of mTOR by inactivating Rheb (Huang and Manning 2009). At the present time, the relationship between the TSC complex and mTORC2 is not well understood.

The TSC complex was originally identified as a critical regulator of mammalian cell homeostasis in patients bearing disruptive genetic mutations in the TSC1 and/or TSC2 gene. Functioning as a tumor repressor, mutations in either gene often result in large non-malignant tumors that can affect the brain, eyes, kidneys, pancreas, and pulmonary system. While the tumors are benign, they often interfere with normal organ function due to their large size, which can encroach on and compact vital organs in the area. The most important mechanistic insight into how the TSC complex functions in normal cell homeostasis and

function came when Rheb was identified as the target of the GAP domain found in TSC2 (Garami et al. 2003; Inoki et al. 2002). Determining the structural motifs present on TSC1 and TSC2 has given researchers insight into the regulation and function of these molecules. Sequence analysis has revealed multiple phosphorylation sites on TSC2 in addition to its GAP domain. These target residues on TSC2 can be phosphorylated by a number of upstream kinases including Amp-activated protein kinase (AMPK), Akt, and Erk. The variety of kinases that target TSC2 indicate a central role for the TSC complex in the context of nutrient metabolism and signaling. While TSC2 has been the focus of significant structure–function research, the same cannot be said for TSC1. The most well-described domain within the TSC1 protein is its TSC2-binding motif. TSC1 has also been shown to be a binding partner of multiple proteins, and could serve as a protein scaffold that dictates the sub-cellular localization of its enzymatic partner TSC2.

Biochemical analysis has revealed that the TSC-mTOR signaling axis is downstream of several well-known signaling pathways. Work done primarily in cell lines has established the PI3K/Akt pathway as being a positive regulator of the mTOR pathway. In response to growth factors Akt phosphorylates TSC2, which leads to the disruption of the TSC1-TSC2 complex. The dissociation of the TSC complex leads to the activation of Rheb and mTORC1 (Cai et al. 2006; Inoki et al. 2002; Ma et al. 2005). TSC2 can also be phosphorylated by Erk, which inactivates the complex (Ma et al. 2005). Indeed, investigators have demonstrated that the mTOR pathway is robustly activated subsequent to T-cell receptor (TCR) and diacylglycerol (DAG) stimulation (Gorentla et al. 2011). In addition to the mTOR pathway being activated by the presence of extracellular stimuli, it is also regulated by intracellular energy reserves. AMPK is activated in response to low levels of intracellular ATP. During times of nutrient deprivation, AMPK directly phosphorylates TSC2, which promotes the GAP activity of the TSC complex, ultimately resulting in the inhibition of mTORC1 (Inoki et al. 2003).

The consideration of mTOR as a master regulator of cell metabolism comes from its capacity to control protein translation at a very fundamental level. As opposed to activating a few transcription factors that regulate a very specific set of genes, mTOR phosphorylates proteins that regulate the cell's translational machinery itself. As a component of two distinct signaling complexes, mTOR signaling proceeds through a number of downstream effector molecules. mTORC1 phosphorylates both the 70-kDa ribosomal S6 kinase (S6K1) and the translational repressor 4 elongation factor-binding protein 1 (4E-BP1) (Fingar et al. 2002). Once activated, S6K1 phosphorylates S6 (Jeno et al. 1988). It has been traditionally thought that phosphorylated S6 serves to promote mRNA translation, which includes elongation factors and elements of the ribosomal translation machinery. In this way, signaling through S6K is considered to promote protein translation, in general, by regulating the construction of the cell's translational machinery (Jefferies et al. 1994, 1997; Terada et al. 1994). Like S6K1, 4E-BP1 is a direct target of phosphorylation by mTORC1 and controls protein translation. 4E-BP1 acts as a repressor of translation by inhibiting the translation initiation factor eIF4E. Once phosphorylated by mTORC1, 4E-BP1 dissociates from eIF4E, where it can then initiate CAP-dependant translation (Gingras et al. 2001).

While the identification of rapamycin as a potent inhibitor of mTORC1 signaling has provided us with an invaluable tool to study the mechanisms that control mTORC1 signaling, no such reagent has been available to study the regulation of signaling exclusively associated with mTORC2. As a result, identifying the upstream regulators of mTORC2 has proven more challenging. Recent findings have shown that the TSC complex can directly associate with and regulate mTORC2 in an mTORC1 independent manner (Huang et al. 2008). Investigators have also demonstrated that mTORC2 is regulated by mTORC1 activity. Specifically, S6K1 can phosphorylate Rictor in vitro and in vivo, which can then inhibit mTORC2 from phosphorylating its downstream target, Akt (Julien et al. 2010; Sarbassov et al. 2005). Phosphorylation of Akt at serine 473 has been used as the canonical readout of mTORC2 activity. It is interesting to note that Akt is not only a target of mTORC2 signaling but also potently activates mTORC1. This logical sequence might suggest that mTORC2 can activate mTORC1; however, S6K1 activity is not altered in an mTORC2 knockdown model (Delgoffe et al. 2011; Jacinto et al. 2004; Lee et al. 2010).

mTOR Kinase Activity Is Required for Helper T Lineage Differentiation

Because the differentiation of naïve $CD4^+$ T cells is dependent on multiple signaling inputs, including TCR and cytokine stimulation, investigators hypothesized that the mTOR pathway may serve to integrate these signals to regulate the appropriate differentiation program of naïve $CD4^+$ T cells. The development of cytokine skewing in vitro culture systems has allowed investigators to begin to define the extrinsic and intrinsic signaling determinants that are involved in T-helper lineage differentiation. To date, the T-helper cells can be broadly differentiated into four distinct cell types, each with unique functional properties. These cells have come to be known as the T_h1, T_h2,

T_h17, and T_{reg} lineages and are largely defined by the cytokines they secrete in response to antigen receptor stimulation.

The first two lineages identified were the T_h1 and T_h2 subsets. T_h1 $CD4^+$ T cells have been shown to be important for the control of pathogens, such as *Toxoplasma gondii* and *Leishmania major*. The ability of these T_h1 cells to control infection is, in part, reliant on their ability to produce large amounts of IFN-γ (Szabo et al. 2002). T_h2 cells are noted for their ability to produce IL-4, which mediates allergic responses and protection from parasitic infection. T-bet and GATA-3 ware identified as T_h1 and T_h2 lineage-specific transcription factors, respectively (Ouyang et al. 1998; Szabo et al. 2002). While the existence of the T_h1 and T_h2 lineages has been established for some time, the emergence of a new lineage became evident during studies of experimental autoimmune encephalitis (EAE). From this model, the T_h17 lineage emerged and was defined as IL-17-producing $CD4^+$ T cells (Cua et al. 2003; Murphy et al. 2003). Like the T-bet in T_h1 and GATA-3 in T_h2 differentiation, RORγt was identified as a lineage-specific transcription for T_h17 cells. The importance of RORγt in the differentiation of IL-17-producing $CD4^+$ T cells, and in the progression of autoimmune inflammation has been extensively studied. RORγt deficient mice exhibit impaired T_h17 differentiation and are resistant to the induction of EAE (Chen et al. 2007).

In a model of mTOR inactivation, Delgoffe and colleagues (2011) generated a conditional mTOR deletion model, where the mTOR protein was specifically ablated in the T-cell lineage. In vitro differentiation cultures and in vivo immunizations revealed that mTOR signaling has a profound influence on T-cell effector differentiation. Specifically, mTOR-deficient T cells fail to differentiate into the T_h1, T_h2, and T_h17 lineages, which are associated with the inability to up-regulate the lineage-specific transcription factors. Furthermore, the failure of mTOR-deficient T cells to differentiate into the effector lineages was balanced by their differentiation into $Foxp3^+$ regulatory T cells. In complementary studies, conditional deletion models of Rheb and Rictor in T cells defined the contributions of mTORC1 and mTORC2, respectively, in $CD4^+$ T-cell differentiation. In one study, mTORC1 proved to be crucial for the differentiation of the T_h1 and T_h17 lineages (Delgoffe et al. 2011), while another study demonstrated the importance of mTORC2 in T_h1 and T_h2 differentiation (Lee et al. 2010). The generation of new models designed to investigate the specific contributions of components of the TSC-mTOR pathway will undoubtedly provide us with a clearer picture of this pathway's importance in helper T-cell differentiation, and may help to pinpoint potential targets to improve T-cell mediated immunity in vivo.

Negative Regulation of $CD8^+$ Memory T-Cell Development by mTOR

A successful T-cell immune response is characterized by two distinct phases. The first is the exponential expansion of antigen-specific T cells after infection. This massive phase of expansion ensures that there are enough effector cells to efficiently combat a spreading infection. Once the infection is resolved, the antigen-specific T-cell pool undergoes a period of contraction, following which only 5–10 % of the antigen-specific T-cell pool will remain. These remaining cells are commonly referred to as the memory T-cell pool. The second phase of the T-cell immune response is the re-exposure of antigen to the resting memory T-cell pool. Upon re-exposure to antigen, the memory T-cell pool will re-expand, produce cytokines, and effector molecules more aggressively than a naive T-cell pool with the same antigenic specificity. Memory T cells represent a distinct T-cell population, and possesses properties not associated with conventional naïve T cells. They highly express the IL-7 receptor and the survival protein Bcl-2. The expression of the IL-7 receptor and Bcl-2 contributes to self-renewal potential unique to memory T cells (Williams and Bevan 2007).

The primary expansion of a pathogen reactive T-cell clone and its contraction into a functional memory pool places these cells under extreme metabolic pressures. Given the data indicating that the differentiation of $CD4^+$ T cells into effector and regulatory lineages is largely dependent on a functional mTOR signaling pathway, investigators hypothesized that the mTOR pathway may also play a crucial role in the formation of a functional $CD8^+$ T-cell memory pool. A recent report demonstrates that when used in low doses, rapamycin can enhance different aspects of memory T-cell formation (Araki et al. 2009). When rapamycin is administered during the primary expansion phase of the T-cell response, an increased $CD8^+$ T-cell memory pool was formed after lymphocytic choriomeningitis virus (LCMV) infection. When the mTOR inhibitor was administered during the contraction phase, the conversion from effector to memory cell was expedited. Furthermore, the memory $CD8^+$ T cells formed in the presence of rapamycin were more effective at controlling viral replication. In the same study, the investigators established a cell intrinsic role for components of the mTOR in repressing memory T-cell formation by retrovirally delivering RNAi constructs against mTOR into LCMV-specific $P14^+$ $CD8^+$ T cells before adoptive transfer. In another study, IL-12 was shown to activate the mTOR pathway and the T_h1 transcription factor T-bet, leading to the generation of effector T cells. However, when stimulated in the presence of rapamycin, OT-I $CD8^+$ T cells exhibited curtailed expression of T-bet, and

enhanced expression of eomesodermin (Rao et al. 2010). Previous reports have shown a positive correlation between eomesodermin expression and precursor memory $CD8^+$ T-cell formation (Intlekofer et al. 2005), suggesting that mTOR signaling may directly influence the expression of transcription factors that dictate memory versus effector lineage decisions. In addition, expression of CCR7 and CD62L in central memory T cells is important for homing of these cells to lymph nodes. Since mTOR inhibits CCR7 and CD62L expression, it is possible that mTOR may also control memory T cells by influencing their migratory potential (Sinclair et al. 2008). Finally, TRAF-6 deficient T cells can develop into effector T cells, but fail to differentiate into memory T cells (Pearce et al. 2009). This study demonstrated that TRAF-6 deficient T cells exhibited lower AMP kinase activity, an inhibitor of mTOR. They showed, however, that the formation of memory T cells could be restored by treating the cells with metformin, an AMPK agonist. Treating the T cells with metformin activated AMP kinase and inhibited mTOR pathway activation.

Cumulatively, these results strongly suggest that the development of memory T cells is strongly inhibited by mTOR signaling. Presently, the mechanism by which mTOR inhibits memory T-cell differentiation remains undefined. These studies also revealed a previously unappreciated function for rapamycin. Generally considered an immunosuppressant, rapamycin's ability to promote memory T-cell formation and function may provide clinicians a new tool to enhance the effectiveness of vaccines.

Dys-Regulation of the TSC-mTOR Pathway Impacts T-Cell Homeostasis

In general, T cells can be thought to exist in two different states: naïve and activated. Naïve T cells are quiescent and are characterized by minimal metabolic activity. T cells become activated in response to TCR antigens and cytokines, which require an anabolic metabolism program that will support T-cell proliferation and protein synthesis. In this way, T-cell metabolism and homeostasis are intimately related. As an established regulator of cell metabolism, investigators have hypothesized that mTOR kinase activity may play a critical role for T-cell homeostasis and function.

The original insights into the importance of the mTOR pathway in T-cell biology emerged from in vitro cell cultures that included rapamycin. While rapamycin had been identified as a potent immunosuppressant, the mechanisms by which it imposed its inhibitory capacity on mammalian T cells remained unclear for many years. Initial findings showed that rapamycin inhibited the ability of T cells to proliferate in response to IL-2, but not their ability to produce IL-2. These studies also showed that rapamycin can induce anergy in $CD4^+$ T cells even in the presence of co-stimulation. T cells anergized in the presence of rapamycin exhibit decreased MAPK pathway activity as well as a block from the G1 to S phase of cell cycle by inhibiting cyclin D3 expression (Dumont et al. 1990; Powell et al. 1999; Zheng et al. 2007). These data led investigators to believe that entry into cell cycle was largely responsible for the prevention of T-cell anergy. Recent studies have shown, however, that the activation of the mTOR pathway itself can initiate the reversal of anergy, as opposed to entry into cell cycle (Allen et al. 2004; Colombetti et al. 2002; Zheng et al. 2007).

While experiments using rapamycin represent loss-of-function studies, only recently have gain-of-function models emerged to assess the consequence of hyper-activation of the TSC-mTOR pathway in T cells. In particular, the generation of a mouse model where TSC1 is genetically deleted exclusively in the T-cell lineage. Using this model, our group and others have demonstrated that TSC1 inhibits mTORC1 activity, but promotes mTORC2 signaling in T cells. TSC1-deficient T cells exhibit highly phosphorylated S6K1 and decreased phosphorylation of Akt and its downstream targets (O'Brien et al. 2011; Yang et al. 2011). In HEK293 cells, it has been demonstrated that Rictor, a component of mTORC2, is directly phosphorylated at Thr1135 by S6K1 after growth factor stimulation, and that this phosphorylation is sensitive to rapamycin. In cells that express a Rictor T1135A mutant, which cannot be phosphorylated by S6K1, mTORC2 dependant Akt phosphorylation was markedly increased, strongly suggesting that mTORC1 activation can directly suppress mTORC2 activity (Julien et al. 2010). Whether or not the regulation of mTORC2 by mTORC1, through S6K1 and Rictor, is physiologically relevant during the activation of primary T cells remains to be determined.

In addition to the signaling abnormalities in TSC1-deficient T cells, our research revealed that constitutive mTOR activation via TSC1-deficiency results in a smaller peripheral T-cell pool and impaired T-cell survival. The impaired phosphorylation of Akt by mTORC2 after TCR stimulation in TSC1-deficient T cells led us to hypothesize that ectopic expression of a constitutively active Akt construct in the absence of TSC1 may rescue the apoptotic phenotype. Expression of Akt-DD, a construct that mimics an Akt molecule that has been phosphorylated by PDK1 and mTORC2, could rescue the increased cell death observed in TSC1-deficient $CD4^+$ and $CD8^+$ T cells. Interestingly, the increased cell death cannot be rescued when TSC1-deficient T cells are retrovirally transfected with Akt-S473D, which mimics the activity of Akt phosphorylated exclusively by mTORC2 (O'Brien et al. 2011).

Since only expression of Akt-DD but not Akt-S473D can rescue these cells from death, it suggests that the increased death of TSC1-deficient T cells could not be solely due to decreased mTORC2 activity. The lack of survival defects in Rictor-deficient T cells also supports that mTORC2 is not essential for T-cell survival (Lee et al. 2010). Aside from decreased mTORC2-Akt activity, increased mTORC1 signaling has been reported to promote cell death. In hepatocyte cell lines, S6K1-deficiency led to down-regulation of caspase-8, caspase-3 activation, cytochrome c release, and protected against the onset of apoptosis (Gonzalez-Rodriguez et al. 2009). Since rapamycin cannot rescue TSC1-deficient T cells from death, enhanced mTORC1 may not directly cause death of these cells. However, this result does not completely rule out a role of increased mTORC1 activity in the death of TSC1 deficient T cells. The death prone property of TSC1-deficient T cells could be caused by a chronic increase of mTORC1 signaling, which could program these cell to a pro-death fate that could not be reverted by acute rapamycin treatment.

It is known that reactive oxygen species (ROS) are a byproduct of cellular respiration and cause mitochondrial damage resulting in the death of activated T cells (Hildeman et al. 1999). TSC1-deficient T cells display increased ROS production, but decreased mitochondrial content, number, and membrane potential, all of which are coincident with their apoptotic phenotype. Evidence generated in our lab demonstrates that *N*-acetylcysteine, an ROS scavenger, can reduce the death of TSC1-deficient T cells and can increase mitochondrial membrane potential in vitro, suggesting that TSC1 may promote T-cell survival mainly through the inhibition of ROS production to maintain mitochondrial integrity. Of note, CD28 mediated co-stimulation, but not rapamycin treatment, can reduce TSC1-deficient T-cell death correlated with reduced ROS production and increased mitochondrial potential, but without obvious increase of Akt activity. Thus, TSC1 may inhibit ROS production in T cells and promote T-cell survival through mTOR-independent mechanisms. Further studies are needed to determine the mechanisms by which TSC1 regulates ROS production and mitochondrial homeostasis.

The conversion of a naïve T cell into an effector cell is coordinated with fundamental changes in T-cell metabolism. During times of activation, T cells rely on energy produced during glycolysis, even in oxygen-rich environments. In accordance with an activated T cell's need to grow in size, replicate, and produce cytokines, the cell switches from a catabolic to anabolic metabolism profile. Past research has established the TSC-mTOR pathway as a critical regulator of glycolysis and an important interpreter of extracellular signaling determinants. Since T-cell activation requires such a dramatic change in their metabolic profile, investigators have recently hypothesized that proper regulation of the TSC-mTOR pathway may play a crucial role in maintaining naïve T-cell quiescence in vivo. While it is an area of intense study, naïve T-cell quiescence is still poorly understood, with several transcription factors being implicated as active enforcers of T-cell quiescence (Kuo et al. 1997; Modiano et al. 2008; Yusuf and Fruman 2003). In order to address the relationship between the TSC-mTOR pathway activation and naïve T-cell quiescence in vivo, the conditional TSC1 T-cell model was utilized. Both our group and others found that the deletion of TSC1 in T cells resulted in enlarged cells. Furthermore, using the DNA incorporating nucleotide BrdU, TSC1-deficient T cells had actively entered cell cycle, whereas wild-type naïve T cells were not actively synthesizing new DNA (O'Brien et al. 2011; Yang et al. 2011; Wu et al. 2011). These TSC1-deficient T cells also displayed the effector memory T-cell phenotype of $CD44^{hi}CD62L^{lo}$, which was at the expense of the naïve quiescent $CD44^{lo}CD62L^{hi}$ population. It is not currently understood whether the up-regulated CD44 expression is controlled directly by the TSC-mTOR pathway, or if it is a secondary result of enhanced homeostatic T proliferation in the $TSC1^{f/f}$-CD4-Cre mice, which exhibit pronounced T-cell lymphopenia. This phenomenon could be addressed by adoptively transferring both wild-type and TSC1-deficient T cells into naïve wild-type recipient hosts, and in the complementary experiment, transferring wild-type and TSC1-deficient T cells into the $TSC1^{f/f}$-CD4-Cre mice.

In agreement with TSC1-deficient T cells entry into cell cycle as measured by BrdU incorporation ex vivo, a large scale genetic analysis revealed up-regulation of several cell cycle-associated genes including cyclin A2 and cyclin B2 (Yang et al. 2011). Interestingly, this observed phenotype of hyper-proliferation in TSC1-deficient T cells somewhat conflicts with the reduced peripheral T-cell pool. However, because of the enhanced cell death observed in these cells, investigators surmised that by crossing their TSC1 knockout lines to Bcl-2 transgenic mice may rescue the cell death phenotype. They observed that $TSC1^{f/f}$-CD4-Cre-$Bcl2^{TG}$ mice had normal numbers of peripheral T cells and underwent apoptosis comparable to wild-type mice (Yang et al. 2011). As an anti-apoptotic molecule, Bcl-2 antagonized the activity of the pro-apoptotic molecule Bim, which was substantially up-regulated in TSC1-deficient T cells. Notably, the loss of quiescence in the $TSC1^{f/f}$-CD4-Cre mice was not restored when crossed to the Bcl-2 transgenic mice, indicating that the apoptotic program in TSC1-deficient T cells is not a causative factor in their non-quiescent phenotype. Additional experiments will be able to determine whether or not loss of quiescence leads to T-cell apoptosis. While crossing the experimental mice to the Bcl-2 transgene rescued the apoptotic phenotype in

TSC1-deficient T cells, mechanistic insights are not readily revealed because the addition of Bcl-2 into any pro-death program is typically viewed as a "cure-all" because of its far downstream role in the intrinsic cell death pathway. In our studies, rapamycin was unable to rescue T-cell death, suggesting that the TSC complex inhibits cell death by a mechanism independent of mTORC1 activity. At the present time, very little work has been done to determine the activity of the TSC complex independent of the mTOR kinase. Biochemical studies including co-immunoprecipitation and mass spectrometry could determine additional signaling partners for TSC1 and TSC2, and may provide valuable clues that would broaden our understanding of this tumor suppressor complex.

Given the aforementioned phenotype, investigators examined if the spontaneous conversion of quiescent TSC1-deficient T cells into cycling activated T cells affects their ability to mount an effective adaptive immune response in vivo. To investigate T-cell expansion and cytokine production in vivo, $TSC1^{f/f}$-CD4-Cre mice were infected with ovalbumin expressing *Listeria monocytogenes*. In response to bacterial infection, TSC1-deficient T cells showed impaired expansion and cytokine production, which could not be rescued by the introduction of the Bcl-2 transgene (Yang et al. 2011). We have shown that TSC1-deficiency in T cells does not globally affects TCR signaling. However, the heterogeneous expression of early T-cell activation markers after in vitro stimulation with α-CD3 suggests that TSC1 modulates T-cell activation, and that the enhanced mTORC1 activity in TSC1-deficient T cells regulates gene transcription associated with cytokine production. Because the TSC complex is an inhibitor of mTOR kinase activity, the $TSC1^{f/f}$-CD4-Cre model should serve as the complementary model to the $mTOR^{f/f}$-CD4-Cre line. Interestingly, both models exhibit an impaired ability to respond to invading pathogens, suggesting that mTOR activity is neither "all good" nor "all bad," but that an intermediate amount of activity is appropriate for normal T-cell responses in vivo. Determining the optimal window of mTOR kinase activity to mount a T-cell immune response will be a critical focus in this particular area of study.

mTOR Kinase Activity is Regulated by Signaling Events Proximal to the TCR

The engagement of the TCR initiates a variety of changes within a T cell. Among those changes are increase in size, proliferative capacity, and new protein synthesis. TCR signaling results in the production of secondary messengers, which serve to amplify and direct unique signaling pathways in activated T cells. As one of those secondary messengers, DAG binds to and activates both RasGRP1 and protein kinase C θ (PKCθ) via their cysteine-rich C1 domains. The result of DAG binding to RasGRP1 and PKCθ is the activation of the RasGRP-Ras-Mek-Erk and PKCθ-IKK-NF-κB pathways, respectively (Coudronniere et al. 2000; Dower et al. 2000; Ebinu et al. 2000; Isakov and Altman 2002; Sun et al. 2000; Tognon et al. 1998). The proximity of DAG production to the signaling events immediately downstream of the TCR, and its ability to activate multiple signaling pathways simultaneously, led investigators to hypothesize that the dys-regulation of DAG signaling and metabolism might perturb normal T-cell homeostasis and function.

As a potent positive regulator of T-cell activation, the termination of DAG signaling is necessary to limit damage mediated by perpetually activated T cells or the development of an autoreactive T-cell pool. A family of enzymes, the DAG kinases (DGKs), converts DAG to phosphatidic acid (PA), effectively terminating DAG-mediated signaling (Kazanietz 2002; Topham and Prescott 1999; van Blitterswijk and Houssa 2000; Zhong et al. 2008, 2011). In order to assess the functional relevance of DAG signaling in T cells in vivo, DGK-deficient mice were created, and analysis of DGK-deficient T cells revealed a variety of abnormalities. In vitro, DGK-deficient T cells show enhanced expression of the early T-cell activation markers and are hyper-proliferative. In vivo, DGK-deficient T cells are resistant to anergy induction and respond more vigorously to LCMV infection (Olenchock et al. 2006a; Zhong et al. 2003). In addition, DGKα and ζ synergistically promote T-cell maturation at least partially via generating PA (Guo et al. 2008). These observations demonstrate that DGKs serve to as signal switches by dampening DAG-mediated signaling and by initiating PA-mediated signaling. Such regulatory functions of DGKs are not limited to T cells as DGKζ has been shown to regulate the function of other lineages in the immune system (Liu et al. 2007; Olenchock et al. 2006b; Zhong et al. 2011).

In non-T cell line models, both DGK- and phospholipase D-derived PA have been reported to bind to and increase mTOR signaling (Avila-Flores et al. 2005; Fang et al. 2001). In T cells, we surprisingly found that deficiency of both DGKα and ζ enhances both mTORC1 and mTORC2 signaling following TCR stimulation. Phorbol 12-myristate 13-acetate, a DAG mimic, stimulation induces strong mTORC1 and mTORC2 activation. Together these observations indicate that DGKs predominantly inhibit mTOR activation by terminating DAG. At present, it is unclear whether DGK-derived PA participates in mTOR activation in T cells. Studies using both loss- and gain-of-function studies on each molecule within the RasGRP-Ras-Mek1/2 pathway have demonstrated further that the RasGRP-Ras-

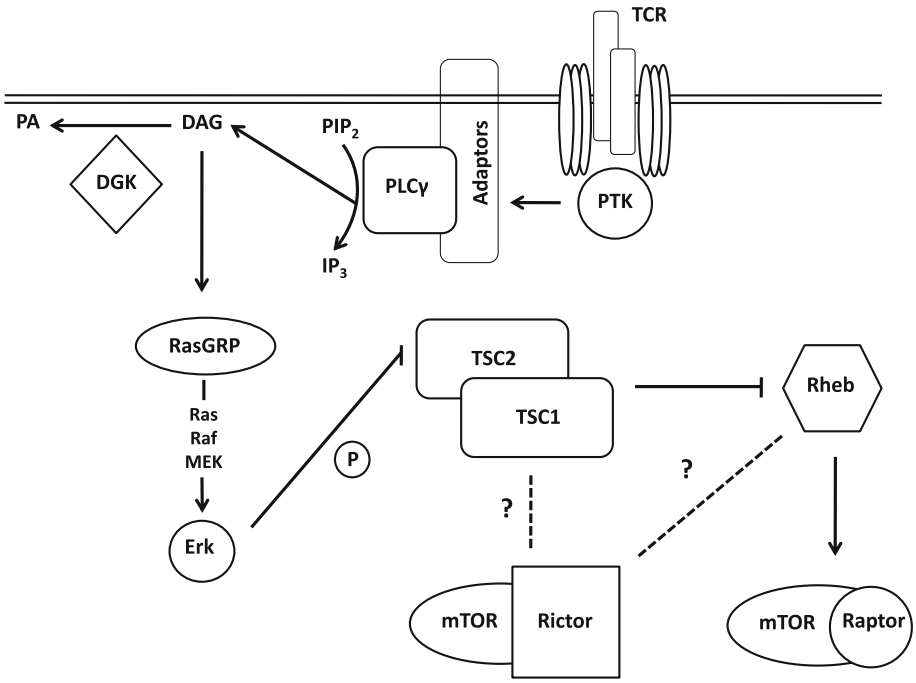

Fig. 1 mTOR signaling and its regulation by DGKs. mTOR signaling is mediated by two signaling complexes, raptor-containing mTORC1 and rictor containing mTORC2. DAG, generated by PLCγ1 following TCR engagement, activates the RasGRP1-Ras-Erk1/2 pathway. The pathway plays important roles for mTORC1 and mTORC2 activation in T cells. DGKs negatively control both mTORC1 and mTORC2 signaling by terminating DAG in T cells

Mek-Erk1/2 cascade functions as the downstream effector of DAG to activate both mTORC1 and mTORC2 in T cells (Gorentla et al. 2011). In contrast to mTORC1, the lack of a specific mTORC2 inhibitor has made the identification of upstream regulators of mTORC2 signaling more challenging. Work from our group has demonstrated, for the first time in T cells, that RasGRP1, Ras, and Mek function to promote mTORC2 signaling in response to TCR stimulation. Specifically, RasGRP-deficient thymocytes exhibit impaired mTORC2 signaling, while the phosphorylation of Akt (S473) was enhanced in thymocytes expressing a constitutively active Ras allele after TCR stimulation (Gorentla et al. 2011). In non-T cell lines, Erk1/2 can directly phosphorylate TSC2, leading to the dissociation of the TSC complex, and the activation of the mTORC1 pathway (Ma et al. 2005). It remains to be determined whether the RasGRP1-Ras-Erk1/2 pathway functions in a similar manner to promote mTORC1 activation in T cells and how this pathway leads to mTORC2 activation. These studies highlight a crucial role for DGK-mediated DAG metabolism in the activation of the mTOR pathway in response to TCR stimulation and has allowed for the construction of a conceptual signaling model connecting DAG metabolism and mTOR kinase activity (Fig. 1). The identification of the RasGRP1-Ras-Erk1/2 pathway as a critical activator of mTOR signaling also raises the possibility that abnormal mTOR signaling may contribute to the impairment of T-cell maturation and activation caused by the dys-regulation of this pathway (Dower et al. 2000; Shen et al. 2011a, b).

Concluding Remarks

The mechanisms underlying the T-cell receptor's ability to influence gene transcription has been the primary focus of T-cell biologists for the past 40 years. In physiological settings, T cells encounter antigen in secondary lymphoid organs that are replete with nutrients, growth factors, and cytokines. How the T cell integrates these environmental cues with the signals emanating from the TCR is not well understood, and has become an area of intense research. Recent work has demonstrated the ability of mTOR to translate the extracellular signals present in an activated T cell's microenvironment into a T-cell differentiation program specifically suited to control a burgeoning infection. To date, the most definitive studies examining the role of mTOR in T-cell function come from a conditional model of mTOR gene deletion in T cells. The generation of tissue-specific models that target other components of the TSC-mTOR pathway, and the identification of new mTOR kinase substrates will further serve to connect the TCR signal with T-cell metabolism and function. Finally, improving the understanding of mechanisms that regulate mTOR signaling should provide novel strategies to manipulate mTOR activity for treatment of autoimmune diseases and cancer.

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