Regulation and function of mTOR signalling in T cell fate decisions

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Abstract | The evolutionarily conserved kinase mTOR (mammalian target of rapamycin) couples cell growth and metabolism to environmental inputs in eukaryotes. T cells depend on mTOR signalling to integrate immune signals and metabolic cues for their proper maintenance and activation. Under steady-state conditions, mTOR is actively controlled by multiple inhibitory mechanisms, and this enforces normal T cell homeostasis. Antigen recognition by naive $CD4^+$ and $CD8^+$ T cells triggers mTOR activation, which in turn programmes the differentiation of these cells into functionally distinct lineages. This Review focuses on the signalling mechanisms of mTOR in T cell homeostatic and functional fates, and discusses the therapeutic implications of targeting mTOR in T cells.

Metabolism
Intracellular chemical reactions that convert nutrients and endogenous molecules into energy and biomass (proteins, nucleic acids and lipids). Naive T cells have a catabolic metabolism through which they use glucose, fatty acids and amino acids for ATP generation via the tricarboxylic acid cycle and oxidative phosphorylation. On antigen stimulation, the bioenergetic demands of a T cell increase dramatically and the cells transition into anabolic metabolism mediated by glycolysis and glutaminolysis.

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The mammalian target of rapamycin (mTOR; now officially known as the mechanistic target of rapamycin) is a conserved serine/threonine kinase that has a central role in the regulation of cell growth and metabolism[1]. mTOR senses and integrates diverse environmental signals, including nutrients and growth factors, many of which deliver their inputs to the phosphoinositide 3-kinase (PI3K)–AKT pathway that ultimately activates mTOR. mTOR exists in two multiprotein complexes in metazoans. mTOR complex 1 (mTORC1) contains a scaffolding protein, regulatory associated protein of mTOR (RAPTOR), and is sensitive to the immunosuppressant rapamycin (FIG. 1a). mTORC2 has a distinct scaffolding protein, rapamycin-insensitive companion of mTOR (RICTOR), and is relatively resistant to rapamycin except under prolonged periods of treatment. Aberrantly elevated mTOR activity is frequently associated with human malignancies, and consequently mTOR has been the subject of extensive investigation by cancer biologists[1]. However, emerging evidence highlights a crucial role of mTOR signalling in both the innate and adaptive immune systems.

The outcome of an adaptive immune response depends on the sensing of antigenic and inflammatory signals by T cells. T cells have evolved to perceive these immune stimuli and further coordinate them with diverse environmental and metabolic cues through the evolutionarily conserved mTOR pathway. Thus, mTOR endows T cells with the ability to properly integrate a multitude of signals to determine the outcome of adaptive immunity. The first major function ascribed to mTOR in T cells was the promotion of cell cycle progression, but more recent studies have established mTOR signalling as a fundamental determinant of cell fate decisions both under steady-state conditions and following cognate antigen recognition. It is likely that mTOR affects these diverse processes in T cells by serving as a signalling node to coordinately regulate immune receptor signalling pathways, metabolic programmes and migratory activity. As several excellent reviews have covered the immune functions of mTOR[2–4], this Review mainly focuses on the most recent genetic studies that have identified new roles for mTOR signalling in T cell fate decisions and on the therapeutic implications of modulating mTOR functions in T cells. Following the hierarchy of signal transduction, I first discuss the extracellular inputs that feed into the mTOR pathway in T cells. Then I describe the mechanisms through which negative and positive components of mTOR signalling impinge on cell fate decisions in T cells, with a special focus on T cell homeostasis and T helper (T_H) cell differentiation (TABLE 1). Finally, I discuss mTOR downstream effector pathways that are involved in T cell metabolism, as well as the implications of targeting mTOR and metabolic pathways for disease therapeutics.

mTOR activating signals

In T cells, mTOR senses three broad categories of instructive signals. The first category comprises the immune activation signals transduced from dendritic cells (DCs) through antigens, co-stimulatory molecules and pro-inflammatory cytokines (known as signals 1, 2 and 3); these signals are essential for directing proper T cell activation and differentiation[5]. The other two types of instructive signals are mediated by environmental

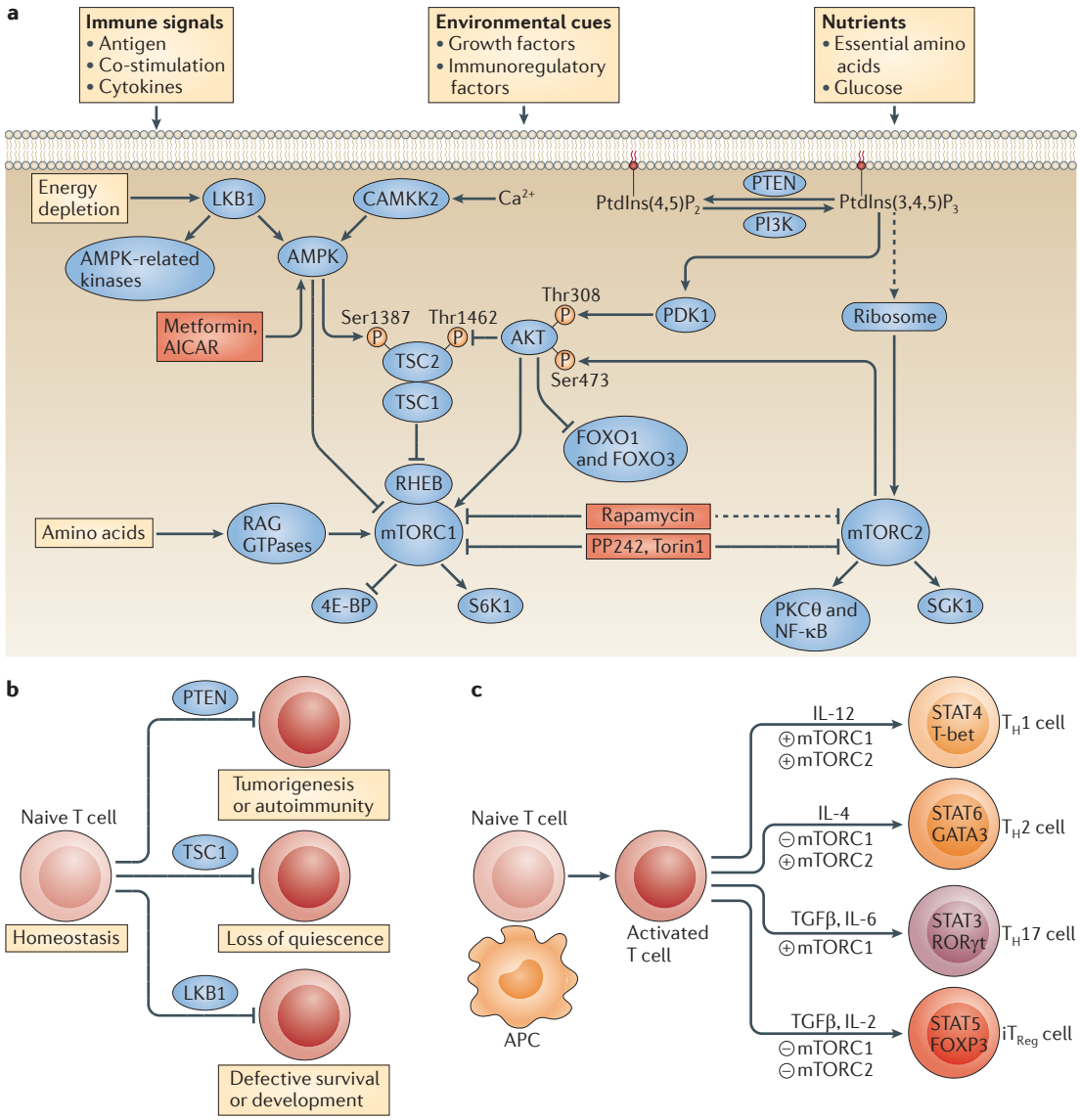


Figure 1 | **Regulation and function of mTOR signalling pathways in T cells. a** | In T cells, mammalian target of rapamycin (mTOR) can be activated by multiple signals. These include the conventionally defined immune signals 1, 2 and 3 (antigenic stimulation, co-stimulation and cytokines); growth factors and immunomodulatory factors (such as leptin and sphingosine-1-phosphate (S1P)); and nutrients. The tuberous sclerosis 1 (TSC1)–TSC2 complex integrates signals from the phosphoinositide 3-kinase (PI3K)–AKT and liver kinase B1 (LKB1)–AMP-activated protein kinase (AMPK) pathways. These pathways reciprocally regulate TSC2 activity through AKT-dependent phosphorylation of threonine 1462 (Thr1462) and AMPK-dependent phosphorylation of serine 1387 (Ser1387). Following antigenic stimulation, the TSC complex is inactivated by T cell receptor (TCR) signals, resulting in the activation of mTOR complex 1 (mTORC1), whereas TSC function is maintained in naive T cells to keep mTORC1 activation in check. In addition, AKT and AMPK can directly modulate mTORC1 functions independently of TSC and the small GTPase RHEB, and amino acids can activate mTORC1 via the RAG family of small GTPases. mTORC1 is best known for its function in promoting translation initiation and protein synthesis by directly phosphorylating ribosomal protein S6 kinases (S6Ks) and eIF4E-binding proteins (4E-BPs). Additional mTORC1 targets include regulatory proteins with roles in cell signalling, metabolism and autophagy. mTORC2 is important for the full activation of AKT through the phosphorylation of Ser473, and thus AKT can be both upstream of mTORC1 and downstream of mTORC2. Moreover, mTORC2 phosphorylates various protein kinase C (PKC) isoforms — including PKCθ, which activates nuclear factor-κB (NF-κB) in T cells — as well as serum- and glucocorticoid-regulated kinase 1 (SGK1). **b** | Under steady-state conditions, molecules that inhibit mTOR actively maintain the homeostasis of T cells in the thymus and periphery by preventing the cells from engaging alternative fates. Although phosphatase and tensin homologue (PTEN), TSC1 and LKB1 have a shared capacity to inactivate mTOR, they have distinct effects in enforcing T cell homeostasis. **c** | Following antigenic stimulation, mTOR signalling promotes the differentiation of T helper 1 (T_H1), T_H2 and T_H17 cells, and inhibits the generation of induced regulatory T (iT_{Reg}) cells. AICAR, 5-aminoimidazole-4-carboxamide riboside; APC, antigen-presenting cell; CAMKK2, calcium/calmodulin-dependent protein kinase kinase 2; FOXO, forkhead box O; IL, interleukin; PDK1, 3-phosphoinositide-dependent protein kinase 1; $PtdIns(4,5)P_2$, phosphatidylinositol-4,5-bisphosphate; $PtdIns(3,4,5)P_3$, phosphatidylinositol-3,4,5-trisphosphate; STAT, signal transducer and activator of transcription; TGFβ, transforming growth factor-β.

Table 1 | **Genetic models involving T cell-specific deletion of mTOR signalling components and negative regulators**

Target gene	Cre expression system	Biochemical defects	Thymic phenotypes	Peripheral phenotypes	Refs
Mtor	*Cd4*-Cre	Abrogated mTORC1 and mTORC2 activities	Increased CD4/CD8 ratio	Diminished T_H1, T_H2 and T_H17 cell differentiation; spontaneous induction of T_{Reg} cell differentiation	6,30
Rheb	*Cd4*-Cre	Abrogated mTORC1 activity	None	Diminished T_H1 and T_H17 cell differentiation; increased T_H2 cell differentiation	30
Rictor	*Cd4*-Cre; *dLck*-iCre	Abrogated mTORC2 activity	None	Diminished T_H2 cell differentiation (in both mutants); decreased T_H1 cell differentiation (in *dLck*-iCre mutants)	30,77
Pten	*Cd4*-Cre; *Lck*-Cre	Increased AKT and mTOR activities	Lymphoma; minor defects in development	Autoimmune disease	44–47, 49–51
Tsc1	*Cd4*-Cre; *Lck*-Cre	Increased mTORC1 activity and decreased mTORC2 activity	None (in *Cd4*-Cre mutants) or a minor reduction in cell numbers (in *Lck*-Cre mutants)	Loss of T cell quiescence and survival; diminished antigen-specific responses	7–9
Lkb1	*Cd4*-Cre; *Lck*-Cre	Diminished AMPK activity and increased mTORC1 activity	Reduced survival; defective β-selection and positive selection	Reduced survival and TCR-induced proliferation; increased metabolism and cytokine production	57–60

AMPK, AMP-activated protein kinase; *dLck*, distal *Lck* promoter; *Lkb1*, liver kinase B1; mTOR, mammalian target of rapamycin; mTORC, mTOR complex; *Pten*, phosphatase and tensin homologue; *Rictor*, rapamycin-insensitive companion of mTOR; TCR, T cell receptor; T_H, T helper; T_{Reg}, regulatory T; *Tsc1*, tuberous sclerosis 1.

stimuli, such as growth factors and immunomodulatory factors, and by metabolic cues, which are derived mainly from nutrients (FIG. 1a). Whereas the immune activation signals are unique to T cells, the environmental and metabolic stimuli act on all eukaryotic cells. Many of the upstream signals activate mTORC1 through the GTP-loaded form of the small GTPase RHEB (FIG. 1a). RHEB is tightly regulated by the tuberous sclerosis 1 (TSC1)–TSC2 complex, which, through its GTPase-activating protein (GAP) activity towards RHEB, inactivates RHEB and mTORC1. The TSC complex serves as a molecular switch to integrate upstream signals and, in particular, the positive and negative signals transduced from the PI3K–AKT and AMP-activated protein kinase (AMPK) pathways, respectively[1]. RHEB deficiency in T cells reduces mTORC1 activation in response to T cell receptor (TCR) stimulation[6], whereas loss of TSC1 disrupts the entire TSC complex and enhances basal and TCR-induced mTORC1 activity[7–9]. These results highlight a crucial role for the TSC–RHEB axis in T cell responses.

TSC-independent pathways also engage mTORC1, although the importance of these mechanisms in T cells remains to be determined. Activation of mTORC1 in response to amino acids requires the RAG family of GTPases[10,11], whereas direct phosphorylation of RAPTOR by AMPK inhibits mTORC1 during energy stress[12]. Furthermore, the mitogen-activated protein kinase (MAPK) p38β targets different components of mTORC1 to either positively or negatively regulate the activity of this complex under different environmental stresses[13,14]. Upstream pathways that activate mTORC2 are just beginning to be identified, with recent studies showing a role for ribosomes in linking PI3K to mTORC2 activation[15,16]. Moreover, another small GTPase, RAC1, binds directly to mTOR to activate mTORC1 and mTORC2, which provides a means to regulate both mTOR complexes simultaneously[17]. The convergence of multiple signals on mTOR suggests that its basic function is as a signal integrator.

AMP-activated protein kinase
(AMPK). A group of serine/threonine kinases that are activated in response to energy depletion. AMPK is an important activator of fatty acid oxidation and a potent inhibitor of mTORC1.

Immune activation signals. Both mTORC1 and mTORC2 are activated within minutes of TCR stimulation. The magnitude of mTOR activation is directly correlated with the duration of the T cell–DC interaction and the dose of the cognate antigen[18,19]. The activity of mTOR is further shaped by co-stimulatory signals. CD28-mediated co-stimulation is a classic activating signal for the PI3K–AKT pathway, which in turn upregulates the mTOR activity induced by the TCR to facilitate productive T cell activation[20–22]. Another co-stimulatory receptor, OX40, which is a member of the tumour necrosis factor receptor (TNFR) family, assembles a signalling complex by recruiting PI3K–AKT to augment TCR-dependent AKT signalling[23]. By contrast, the PDL1 (PD1 ligand 1)–PD1 (programmed cell death protein 1) axis, a negative co-stimulatory pathway in T cells, downregulates mTOR signalling to mediate immune tolerance[24].

Compared with the rapid activation of mTOR by the TCR, the homeostatic cytokine interleukin-7 (IL-7) induces delayed yet sustained PI3K–AKT signalling and mTOR activation. This activation depends on the transcriptional activity of signal transducer and activator of transcription 5 (STAT5) and contributes to IL-7-mediated glucose uptake and trophic effects in T cells[25,26]. However, increased mTORC1 activity as a result of TSC1 deficiency impairs IL-7-dependent survival responses in naive T cells[7], suggesting that a defined level of mTOR activity is important to mediate the IL-7 response. In antigen-stimulated $CD8^+$ T cells, IL-12 enhances and prolongs TCR-dependent mTOR activation to programme the functional maturation of effector T cells. Similarly to IL-7-mediated stimulation of naive T cells, IL-12-induced mTOR activation in antigen-stimulated $CD8^+$ T cells is indirect and depends on STAT signalling (in this case, through STAT4)[27]. In T_H2 and T_H17 cells, mTOR is activated by IL-4 and IL-1, respectively, to facilitate cell cycle progression[28,29]. Furthermore, there is extensive interplay between mTOR and STAT signalling in T cells[6,30] and other cells[31].

The leptin receptor. Leptin — an adipose tissue-derived hormone that regulates energy metabolism — has long been known to directly regulate T cell proliferation and cytokine production, thereby linking nutritional status and pro-inflammatory immune responses[32]. More recently, regulatory T cells (T_{Reg} cells) were shown to express leptin and its receptor and to have greater mTOR activity than conventional T cells[33]. Neutralization of leptin or deletion of the gene encoding the leptin receptor considerably diminishes mTOR activity in T_{Reg} cells, which suggests a link between autocrine secretion of leptin and mTOR activation in T_{Reg} cells. The mTOR activity that results from leptin signalling maintains the anergic state of T_{Reg} cells, because transient inhibition of mTOR or neutralization of leptin reverses the hyporesponsiveness of T_{Reg} cells to TCR stimulation, resulting in their robust proliferation[33,34]. Leptin also activates mTOR in autoreactive $CD4^+$ T cells to promote their survival and mediate autoimmune neuroinflammation[35]. As the leptin level is directly correlated with nutrient status, the leptin–mTOR axis has been proposed to bridge metabolism and immunity[33].

S1PR1. Sphingosine-1-phosphate receptor 1 (S1PR1) — a G protein-coupled receptor for the bioactive lipid sphingosine-1-phosphate (S1P) — is a crucial regulator of T cell egress from the thymus and secondary lymphoid organs. In T_{Reg} cells, S1PR1 activates AKT and mTOR, and this delivers a cell-intrinsic negative signal to restrain T_{Reg} cell suppressive activity[36]. In conventional $CD4^+$ T cells, S1PR1 is dispensable for immediate mTOR activation but is important to sustain mTOR activity during the differentiation of these cells into effector T cells[37]. S1PR1-dependent activation of mTOR inhibits the generation of T_{Reg} cells while driving T_H1 cell development in a reciprocal manner. These studies identify an S1PR1–mTOR axis that controls T_{Reg} cell function and T cell lineage choices[36,37].

TLRs. When activated by their ligands, Toll-like receptors (TLRs) expressed by innate immune cells induce the release of large amounts of pro-inflammatory molecules that are important for immediate immune defence. In addition, both $CD4^+$ and $CD8^+$ T cells express functional TLRs, and recent results suggest a T cell-intrinsic role for TLRs in immune responses. TLR2 signals through the adaptor molecule myeloid differentiation primary-response protein 88 (MYD88) to activate mTOR and promote the expression of T-bet in effector $CD8^+$ T cells[38]. As mTOR is also downstream of TCR signalling, the activation of mTOR bridges TCR and TLR signals and thereby promotes effector $CD8^+$ T cell function[38] and contributes to the generation of T cell memory[39]. However, MYD88 deficiency in T cells diminishes initial T cell clonal expansion, but not the subsequent generation of the memory population[40]. Further study is required to clarify how TLR2–MYD88 signalling affects mTOR activation in T cells.

mTOR is also activated by additional extracellular signals in T cells. Insulin induces the activation of mTORC1 and promotes mTOR-dependent expression of T-bet in antigen-activated $CD8^+$ T cells[27]. Also, Notch 1 activates the AKT–mTOR pathway during early thymocyte development and relies on AKT-dependent metabolic and trophic effects to regulate thymocyte differentiation and survival[41]. Finally, mTOR is intimately linked to nutrient sensing in all eukaryotic cells[1], and the physiological relevance of the metabolic regulation of mTOR is discussed in further detail below.

mTOR in immune homeostasis

T cells develop in the thymus through a stepwise differentiation process. Once mature T cells are released into the periphery, they circulate through the blood and peripheral lymphoid organs in a quiescent state that is characterized by small cell size and low metabolic activity. The survival of naive T cells relies on the engagement of TCRs by self-peptide–MHC complexes and on the availability of IL-7, and is further shaped by growth factors and nutrients for metabolic fitness[42,43]. Given the central role of mTOR as an environmental sensor, it might be predicted that mTOR activity is required for the homeostasis of thymocytes and peripheral T cells. Surprisingly, deletion of the *Mtor* gene after initial thymocyte development (using the *Cd4*-Cre system) had minimal effects on the homeostasis of peripheral T cells under steady-state conditions[6], although the requirement for mTOR in early thymic development has yet to be determined. By contrast, several negative regulators of mTOR signalling were found to enforce normal homeostasis of T cells, as manifested by the disrupted development and maintenance in T cells lacking these inhibitory molecules (FIG. 1b).

PTEN suppresses lymphoma and autoimmunity. Phosphatase and tensin homologue (PTEN) mainly functions as a lipid phosphatase and hydrolyses phosphatidylinositol-3,4,5-trisphosphate, thereby mediating the reverse reaction to PI3K (FIG. 1a). Deletion of *Pten* in T cells causes lymphomas that originate in the thymus and are largely driven by MYC overexpression[44–49]. $Pten^{-/-}$ thymocytes exhibit markedly elevated AKT and mTOR activities even before oncogenic transformation[47,50]. Importantly, tumour development is blocked by the inhibition of mTOR through rapamycin treatment or by the deletion of 3-phosphoinositide-dependent protein kinase 1 (*Pdk1*), indicating a crucial role for AKT and mTOR signalling in this process[47,48]. Somewhat unexpectedly, PTEN deficiency does not overtly disrupt the homeostasis of premalignant thymocytes, except for some minor defects in cell size and in the generation of double-positive thymocytes during ontogeny and of invariant natural killer T cells (iNKT cells)[47,50,51].

$Pten^{+/-}$ mice develop a late-onset autoimmune disease that is associated with resistance to apoptosis mediated by CD95 (also known as FAS)[52]. Consistent with this observation, complete loss of PTEN in T cells impairs central tolerance as well as peripheral tolerance[44] and disrupts the induction of forkhead box P3 (FOXP3) expression[53]. Recent studies demonstrate that the development of autoimmunity in $Pten^{-/-}$ mice can be exclusively mediated by peripheral

Regulatory T cells
(T_{Reg} cells). A subset of $CD4^+$ T cells that expresses FOXP3 and is crucial for the maintenance of immune tolerance. Although T_{Reg} cells mainly develop in the thymus as a separate lineage of $CD4^+$ T cells, known as naturally occurring T_{Reg} cells, a second subset of T_{Reg} cells (known as induced T_{Reg} cells) arises *de novo* from conventional T cells in the periphery following antigen stimulation in the presence of TGFβ.

Invariant natural killer T cells
(iNKT cells). A subset of immune cells that shares properties with both T cells and natural killer cells.

Central tolerance
A process that eliminates self-reactive lymphocytes during their development. For T cells, this occurs mainly through clonal deletion in the thymus.

Peripheral tolerance
A process that downregulates the activation of self-reactive T cells in secondary lymphoid organs. Two of the most important mechanisms are suppression by regulatory T cells and the induction of T cell anergy.

T cells. Therefore, the two main defects that result from the loss of PTEN function — lymphoma and autoimmunity — are separable and are mediated by T cells at distinct developmental stages[49]. Because of the pivotal roles for PTEN in immune homeostasis and function, molecular signals that regulate PTEN have received considerable interest. Work from several independent groups has revealed that the microRNA cluster *mir-17-92* represses the expression of PTEN to promote AKT–mTOR activity in lymphocytes, and consequently controls PTEN-dependent autoimmune and oncogenic functions[54–56].

TSC1 maintains the quiescence of naive T cells. TSC1 and TSC2 function as an integral complex to stringently control mTORC1 activity (FIG. 1a). We and others have recently found that TSC1-mediated control of mTOR signalling enforces a programme of quiescence in naive T cells by controlling cell size, cell cycle entry and metabolic machinery[7–9]. The abrogation of quiescence predisposes TSC1-deficient T cells to apoptosis, and this results in the loss of conventional T cells and iNKT cells. The remaining *Tsc1*$^{-/-}$ T cells exist in a unique 'semi-activated' ($CD44^{+}CD122^{-}$) state *in vivo* and exhibit increased levels of activation, cell cycle entry and cytokine expression after acute TCR stimulation compared with activated wild-type T cells. Despite this, TSC1-deficient mice fail to generate effective antibacterial responses, even when the excessive apoptosis is largely blocked, which suggests that the maintenance of naive T cell quiescence is important for a productive immune response[7]. The precise mechanism by which TSC1 deficiency dampens the immune response is uncertain, but it may involve premature activation of the cell cycle and metabolic machineries and of transcriptional responses in *Tsc1*$^{-/-}$ naive T cells before they receive proper TCR signals[7]. These studies identify TSC1 as a bona fide factor in establishing naive T cell quiescence to facilitate immune homeostasis and function.

Tsc1$^{-/-}$ peripheral T cells exhibit increased mTORC1 activity but diminished mTORC2 activity compared with wild-type peripheral T cells[7–9]. Treatment of *Tsc1*$^{-/-}$ mice with rapamycin partly rectifies the altered T cell homeostasis and survival, whereas loss of mTORC2 alone has no apparent effect. These results indicate that mTORC1 activation makes a crucial contribution to T cell homeostasis[7]. By contrast, in mature thymocytes lacking TSC1, mTORC1 activity is increased but cell survival is not affected[7]. Moreover, although PTEN-deficient T cells upregulate mTORC1 activity, they largely maintain their quiescence before tumour development[47,50], and this suggests a crucial but cell context-dependent effect of mTORC1 on T cell homeostasis. Collectively, these data illustrate that the active control of mTORC1 by TSC1 is a key checkpoint that enforces the quiescence of naive T cells in the periphery[7–9].

LKB1 promotes T cell development and survival. Liver kinase B1 (LKB1; also known as STK11) phosphorylates and activates AMPK subfamily members in response to energy depletion. Deletion of *Lkb1* in T cells results in profound defects in multiple compartments, including extensive apoptosis of T cells, impaired thymic selection and dysregulated T cell metabolism and proliferation[57–60]. Despite the well-documented role of the LKB1–AMPK axis in mTORC1 inhibition[61] (FIG. 1a), the signalling mechanisms downstream of LKB1 in T cells are not fully understood. The increased mTORC1 activity in *Lkb1*$^{-/-}$ T cells contributes to excessive cytokine production[60], but whether it leads to the reduced cell survival or other defects is unclear. Also, although AMPK activity is diminished in *Lkb1*$^{-/-}$ T cells[57–60] and AMPK is activated by TCR stimulation[62], loss of AMPKα1 (the predominant AMPK isoform in T cells) causes only a slight disturbance of T cell homeostasis[60,63]. Therefore, T cell homeostasis probably requires additional AMPK-related kinases that are regulated by LKB1 (REF. 61).

Together, analyses of T cells deficient in the tumour suppressors PTEN, TSC1 and LKB1 have provided new insights into mechanisms of immune homeostasis. Notably, haematopoietic stem cells (HSCs) that lack these molecules exhibit some analogous defects to the mutant T cells in the control of proliferation, survival and function[64–70], suggesting that HSCs and naive T cells have a common requirement for these pathways for their proper maintenance. The defects in PTEN- or TSC1-deficient HSCs can largely be rescued by rapamycin[64,66,67], whereas LKB1 functions independently of mTOR in HSCs[68–70]. Therefore, despite their shared ability to inhibit mTOR signalling, these molecules use distinct pathways for the homeostatic control of HSCs and possibly T cells. Aside from these well-characterized mTOR inhibitory molecules, recent studies have identified additional negative regulators of mTOR with important roles in cell signalling and disease regulation. These regulators include DEPTOR (DEP domain-containing mTOR-interacting protein)[71], sestrin 1 and sestrin 2 (REF. 72), and the mTORC1 component PRAS40 (40 kDa proline-rich AKT substrate)[73,74]. However, the roles of these molecules in T cell immunity have not been addressed.

Moreover, mTOR may further interact and crosstalk with transcriptional and immune signalling pathways to mediate T cell homeostasis. In particular, forkhead box O1 (FOXO1) — a crucial transcription factor for the survival and trafficking of naive T cells[75,76] — is under the stringent control of AKT-mediated phosphorylation and nuclear exclusion and thus could serve as an important downstream target of mTORC2. Consistent with this idea, transcriptional targets of FOXO1 — including IL-7 receptor subunit-α (IL-7Rα) and trafficking molecules such as CD62L — have altered expression levels in T cells that are treated with mTOR or AKT inhibitors, as well as in PTEN-, RICTOR- or PDK1-deficient T cells[75,77–79]. The functional link and physiological relevance of these interactions remain to be fully defined. Finally, diacylglycerol kinases have been shown to inhibit TCR-induced mTOR activity by downregulating diacylglycerol-mediated RAS signalling[80], which may contribute to T cell homeostasis and function.

Box 1 | **mTOR in peripheral tolerance**

Induced regulatory T (T_{Reg}) cells act in synergy with naturally occurring T_{Reg} cells to establish immune tolerance[139]. Inhibition of the activity of mammalian target of rapamycin (mTOR) has been shown to induce *de novo* expression of forkhead box P3 (FOXP3)[140,141], or to expand pre-existing natural T_{Reg} cell populations[113]. Conversely, increasing AKT activity — either through deletion of phosphatase and tensin homologue (*Pten*) or through enforced expression of constitutively active AKT — leads to mTOR-dependent inhibition of induced T_{Reg} cell differentiation[53,142]. Moreover, several upstream receptors, including PD1 ligand 1 (PDL1) and sphingosine-1-phosphate receptor 1 (S1PR1), regulate the generation of induced T_{Reg} cells by modulating mTOR activity[24,37]. Two downstream pathways mediate the inhibitory effects of mTOR on induced T_{Reg} cell differentiation (FIG. 2a). First, SMAD3 — a key transcription factor that functions downstream of transforming growth factor-β (TGFβ) signalling in the induction of FOXP3 — is antagonized by mTOR signalling in multiple cell types, including T cells[6,37,143]. Second, forkhead box O1 (FOXO1) and FOXO3, which induce FOXP3 expression[144–146], are inactivated by AKT-dependent phosphorylation, although how this is controlled by mTOR complex 2 (mTORC2) signalling requires further studies[147]. Interestingly, FOXO proteins have been shown to form a complex with SMAD3 to control neuroepithelial and glioblastoma cell proliferation[148], and the integration of SMAD and FOXO signalling by mTOR in T cells will be an interesting topic to explore.

Aside from immune suppression mediated by T_{Reg} cells, another important mechanism of peripheral tolerance is the induction of T cell anergy. T cell anergy is usually induced by T cell receptor signalling (signal 1) alone, in the absence of co-stimulation (signal 2). Among the molecules that are strongly activated by signal 2 are AKT and mTOR[21,22]. Indeed, blocking mTOR activity using rapamycin induces T cell anergy *in vitro*, even in the presence of both signal 1 and signal 2. *In vivo*, mTOR inhibition promotes T cell anergy under conditions that would normally induce active priming, indicating that mTOR has a central role in determining whether a T cell becomes activated or anergic[21,22]. Although rapamycin treatment inhibits the G1 phase of the cell cycle, blocking T cell proliferation alone does not induce anergy[4]. Instead, blocking metabolic pathways that are necessary for mTOR activation promotes T cell anergy, suggesting that mTOR-dependent metabolic control is a key determinant of T cell activation and anergy[21,22].

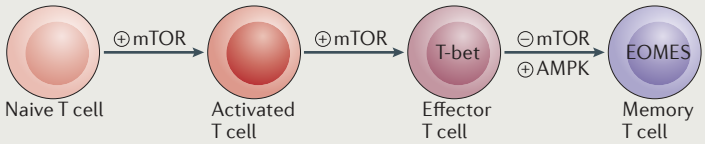
mTOR in T helper cell differentiation

Early studies of mTOR signalling in T cell responses centred on the antiproliferative effect of the inhibitor rapamycin. Analyses of *Mtor*$^{-/-}$ T cells have confirmed a role for mTOR in cell cycle progression, although the proliferation of these T cells is delayed but not abolished[6]. Accumulating evidence, however, highlights a central role for mTOR as a fundamental determinant of cell fate in antigen-activated $CD4^+$ and $CD8^+$ T cells. The inhibition of mTOR with rapamycin facilitates the induction of anergic and regulatory $CD4^+$ T cells — two crucial components of peripheral tolerance (BOX 1) — as well as the differentiation of memory $CD8^+$ T cells (BOX 2). Excellent reviews discuss the role of mTOR signalling in the differentiation of regulatory, effector and memory T cells[4,81–83], so here I focus mainly on more recent genetic evidence that establishes a crucial role for mTOR in T_H cell differentiation.

Decision making between effector T cells and T_{Reg} cells. Naive $CD4^+$ T cells respond to antigen stimulation by developing into distinct effector cell lineages (such as T_H1, T_H2 and T_H17 cells), which have specialized properties and effector functions (FIG. 1c), or into T_{Reg} cells, which prevent excessive immune reactions[84]. Powell and colleagues revealed that mTOR promotes the differentiation of effector T cells[6]. *Mtor*$^{-/-}$ T cells have normal activation markers and levels of IL-2 production in response to TCR stimulation, but fail to differentiate into T_H1, T_H2 and T_H17 cells. Furthermore, they are unable to activate lineage-selective STAT proteins or express the lineage-selective master transcription factors (these are STAT4 and T-bet for T_H1 cells; STAT6 and GATA3 for T_H2 cells; and STAT3 and RORγt for T_H17 cells). These results identify an indispensable role for mTOR in the differentiation of $CD4^+$ effector T cells[6]. Conversely, TCR stimulation of *Mtor*$^{-/-}$ T cells results in the spontaneous induction of FOXP3 expression, even in the absence of exogenous cytokines[6]. This phenotype is not observed in either RHEB- or RICTOR-deficient T cells, indicating that both mTOR complexes contribute to the inhibition of T_{Reg} cell induction, possibly through distinct downstream mechanisms[6,30,77] (FIG. 2a).

T_H1 and T_H17 cell differentiation. The two mTOR complexes have disparate effects on effector T cell differentiation. Deficiency of RHEB, and consequently loss of mTORC1 activity, largely recapitulates the impaired T_H1 and T_H17 cell differentiation in *Mtor*$^{-/-}$ T cells, suggesting that mTORC1 mediates the mTOR-dependent differentiation of T_H1 and T_H17 cells[30]. *Rheb*$^{-/-}$ T cells express increased levels of suppressor of cytokine signalling 3 (SOCS3), which is a negative regulator of STAT signalling, and silencing of SOCS3 expression restores T_H1 cell differentiation in these cells (FIG. 2b). Thus, mTORC1 signalling promotes T_H1 cell differentiation by modulating cytokine signalling[30]. As mTORC1 activity can also be regulated by upstream signals other than RHEB[12], how such RHEB-independent pathways contribute to T cell differentiation awaits future investigation. In addition, deficiency of RICTOR, and thus loss of mTORC2 activity, reduces T_H1 cell differentiation through the downregulation of AKT signalling[77] (FIG. 2b), although another independent study shows a dispensable role for mTORC2 in T_H1 cell differentiation[30]. How mTOR affects T_H17 cell differentiation remains to be fully established, but the mechanism could involve, in part, the upregulation of hypoxia-inducible factor 1α (HIF1α)[85,86] (see below for details).

Box 2 | **mTOR in memory CD8⁺ T cell differentiation**



CD8⁺ T cells constitute an important arm of adaptive immunity owing to their response to pathogens, which involves clonal expansion and differentiation into cytotoxic T cells[149].

During the contraction phase that follows clonal expansion, most CD8⁺ effector T cells die by apoptosis, while the remaining small subset of antigen-specific T cells develops into a long-lived population of memory T cells. Recent results have revealed a crucial role of mammalian target of rapamycin (mTOR) in the lineage decisions between short-lived effector T cells and memory T cell precursors. Araki *et al.* showed that mTOR modulates memory CD8⁺ T cell formation in a manner dependent on the kinetics and strength of mTOR activity[119]. In their model of lymphocytic choriomeningitis virus infection, rapamycin treatment during the T cell expansion phase diminished the apoptotic death of effector T cells, leading to an increase in the quantity of memory T cells. By contrast, rapamycin treatment during the contraction phase promoted the protective capacity of memory T cells and thus the quality of T cell memory. These effects were dose dependent, as the expansion of CD8⁺ T cell populations was blocked by a high dose of rapamycin. Silencing of regulatory associated protein of mTOR (RAPTOR) expression in T cells recapitulated the effects of rapamycin, thereby establishing a cell-intrinsic role of mTOR complex 1 (mTORC1) in memory CD8⁺ T cell formation[119]. However, it is unclear how mTORC1 is activated by upstream signals in CD8⁺ T cells, as this process seems to require phosphoinositide 3-kinase (PI3K)[27] but occurs largely independently of AKT[79].

Pearce *et al.* independently identified an inhibitory role for mTOR in the generation of T cell memory and further linked this function to an upstream regulator, TNFR-associated factor 6 (TRAF6)[96]. In a model of *Listeria monocytogenes* infection, *Traf6*$^{-/-}$ CD8⁺ T cells showed normal effector T cell differentiation but could not develop into memory T cells. This defect was associated with a failure to upregulate fatty acid oxidation, and it was rectified by pharmacological activation of AMP-activated protein kinase (AMPK) or inhibition of mTOR[96]. More recently, fatty acid oxidation has been shown to promote mitochondrial respiratory capacity selectively in memory T cells as an important mechanism to enhance their survival[150]. In another related study, Rao *et al.* found that rapamycin treatment diminished CD8⁺ effector T cell function, but promoted memory T cell formation and tumour immunity[27]. Inhibition of mTOR decreased T-bet levels while inducing the expression of eomesodermin (EOMES), and these mechanisms were important for regulating T cell differentiation[27] (see the figure). However, deletion of phosphatase and tensin homologue (*Pten*) does not strongly affect the formation of CD8⁺ memory T cells[151], and thus the underlying molecular mechanisms remain to be identified. These studies collectively support a central role for mTOR in dictating the effector or memory fate of CD8⁺ T cells in infection and tumour immunity.

T_H2 cell differentiation. In contrast to RHEB–mTORC1 signalling, mTORC2 signalling is required for T_H2 cell differentiation (FIG. 2c). Two groups have independently demonstrated that loss of RICTOR impairs T_H2 cell differentiation *in vitro* and *in vivo*, without appreciably affecting the development of T_H17 cells[30,77]. In the study by Lee *et al.*, the inability of *Rictor*$^{-/-}$ T cells to differentiate into T_H2 cells was attributed to decreased protein kinase Cθ (PKCθ) activity and nuclear factor-κB (NF-κB)-mediated transcription, as complementation with activated PKCθ restored the T_H2 cell defect[77]. In a separate study, Delgoffe *et al.* described an increase in SOCS5 expression in *Rictor*$^{-/-}$ T cells that accounted for the diminished T_H2 cell response[30]. Collectively, these results establish mTORC2 as a crucial regulator of T_H2 cell differentiation[30,77], and future studies will determine whether PKCθ, NF-κB and SOCS5 act in separate pathways or are components of the same signalling cascade. By contrast, mTORC1 negatively controls T_H2 cell differentiation, as indicated by the increased activation of STAT6 and expression of GATA3 in *Rheb*$^{-/-}$ T cells[30] (FIG. 2c).

In summary, mTOR dictates cell fate decisions in effector and regulatory T cells, with mTORC1 and mTORC2 having distinct effects on immune receptor signalling. These effects are further shaped by metabolic pathways, which are discussed below. Moreover, loss of mTORC1 or mTORC2 activity impairs T cell proliferation, with a stronger effect observed in *Rheb*$^{-/-}$ T cells[30,77]. Cell cycle progression is known to be a prerequisite for T cell differentiation, in part because it allows for the epigenetic remodelling of cytokine loci[87]. It would be informative to examine how mTORC1 and mTORC2 regulate cell cycle progression, and whether this affects epigenetic regulation or lineage differentiation.

Effector pathways and metabolic regulation

To orchestrate T cell homeostasis and differentiation, mTOR regulates several downstream pathways, including those involved in immune receptor signalling, metabolic programming and T cell trafficking (BOX 3). Whereas the effects of mTOR on immune receptor signalling molecules, as described above, are easy to appreciate, the relative contribution of mTOR to T cell metabolism and trafficking and how this influences cell fate decisions *in vivo* remain under debate[4,81,83]. However, evidence is emerging that mTOR serves as a signalling node to regulate both the metabolism and the migration of T cells and to further link these processes to immune signalling and transcriptional networks. This would ensure that the metabolic programme and migratory activity of a T cell match its cell fate decision. In particular, recent studies have revealed exciting new findings regarding how mTOR-dependent metabolism controls T cell fate, an area with notable therapeutic implications.

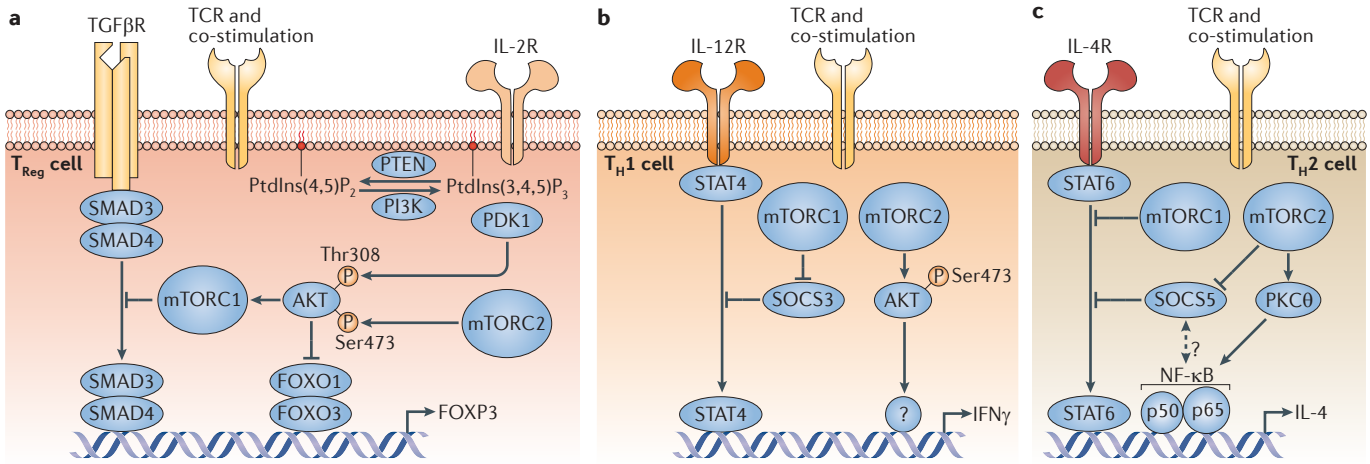


Figure 2 | **mTOR-dependent signalling in CD4⁺ T cell differentiation. a** | For the differentiation of regulatory T (T_{Reg}) cells, the induction of forkhead box P3 (FOXP3) expression depends on the transcription factors SMAD3, SMAD4, forkhead box O1 (FOXO1) and FOXO3. Mammalian target of rapamycin (mTOR) inhibits the generation of induced T_{Reg} cells by antagonizing the function of SMAD3 and SMAD4 downstream of transforming growth factor-β receptor (TGFβR) signalling, and by inducing the nuclear exclusion of FOXO1 and FOXO3. These two effects are probably mediated by mTOR complex 1 (mTORC1) and mTORC2, respectively. **b** | In the differentiation of T helper 1 (T_H1) cells, mTORC1 inhibits the induction of suppressor of cytokine signalling 3 (SOCS3) — a crucial negative regulator of signal transducer and activator of transcription 4 (STAT4) — to promote interleukin-12 receptor (IL-12R) signalling and T_H1 cell differentiation. In addition, mTORC2 is required for the activation of AKT, which also contributes to interferon-γ (IFNγ) production. **c** | mTORC2 promotes T_H2 cell differentiation via two mechanisms: by preventing the expression of SOCS5, which is a negative regulator of IL-4R and STAT6 signalling; and by activating protein kinase Cθ (PKCθ) and thereby promoting nuclear factor-κB (NF-κB)-mediated transcription. By contrast, mTORC1 negatively regulates STAT6 signalling and T_H2 cell differentiation. PDK1, 3-phosphoinositide-dependent protein kinase 1; PI3K, phosphoinositide 3-kinase; PtdIns(4,5)P_2, phosphatidylinositol-4,5-bisphosphate; PtdIns(3,4,5)P_3, phosphatidylinositol-3,4,5-trisphosphate; PTEN, phosphatase and tensin homologue; TCR, T cell receptor; TGFβR, TGFβ receptor.

Catabolic metabolism
The breakdown of complex substances into simpler ones, which is often accompanied by ATP production. Examples include the oxidation of fatty acids and amino acids.

Oxidative phosphorylation
A metabolic pathway that produces ATP from the oxidation of nutrients and the transfer of electrons in a two-step process in mitochondria. The first reaction involves the conversion of intermediate molecules (pyruvate and fatty acids) into acetyl-CoA, which enters the tricarboxylic acid cycle, yielding free electrons that are carried by NADH and $FADH_2$. In the second reaction, electrons from NADH and $FADH_2$ are transferred to the electron-transport chain, resulting in the movement of protons out of the mitochondrial matrix and the generation of an electrochemical potential for ATP synthesis.

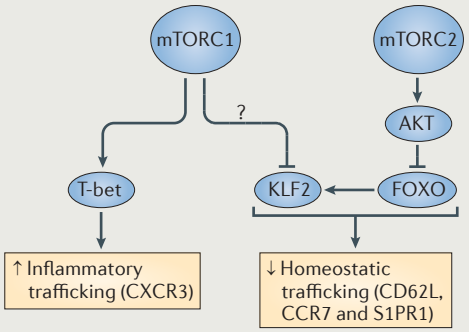
Fatty acid oxidation
An important metabolic process used to derive energy through the mobilization and oxidation of fatty acids, mainly in the mitochondrial matrix. Fatty acid oxidation is positively and negatively regulated by AMPK and mTOR, respectively.

Metabolism in T cell activation and differentiation. Naive T cells have a catabolic metabolism through which they generate ATP via the tricarboxylic acid (TCA) cycle and oxidative phosphorylation. T cell activation markedly increases the uptake and consumption of glucose and glutamine, with a concomitant suppression of fatty acid oxidation[43,88–90] (FIG. 3a). Among the central regulators of this metabolic reprogramming induced by TCR stimulation are PI3K–AKT signalling and MYC, which regulate glucose metabolism and the global metabolic transcriptome, respectively[88,91,92]. Additional transcription factors such as oestrogen-related receptor-α (ERRα) also contribute[93], but HIF1α — which has overlapping functions with MYC in cancer cell metabolism[94] — is not required for TCR activation-induced metabolic reprogramming[88]. The PI3K–AKT pathway signals through mTOR, which engages in extensive crosstalk with MYC in many cellular contexts[95]. In activated T cells, rapamycin inhibits the expression of MYC and the induction of glycolysis[85,88], whereas T cells lacking MYC fail to fully activate mTORC1 in response to TCR signals[88]. Consistent with a pivotal role of mTOR in T cell metabolism, *Tsc2*$^{-/-}$ T cells, which have constitutive mTOR activity, are highly glycolytic after TCR stimulation[93]. Moreover, mTOR orchestrates the metabolic programme of naive T cells under steady-state conditions, as gene expression programmes for the metabolism of glucose, nucleotides and amino acids are abnormally upregulated in *Tsc1*$^{-/-}$ naive T cells, which have increased mTORC1 activity[7]. In summary, mTOR has a crucial role in orchestrating the metabolic programmes of both naive and activated T cells.

Recent studies demonstrate that a metabolic switch to AMPK-dependent fatty acid oxidation is required for the differentiation of CD8⁺ memory T cells and CD4⁺ T_{Reg} cells[96,97], both of which are less anabolic than their effector cell counterparts[4]. The defective memory formation in CD8⁺ T cells lacking TNFR-associated factor 6 (TRAF6) is associated with a failure to upregulate fatty acid oxidation, and enhancement of fatty acid oxidation by treatment with metformin (which activates AMPK) or rapamycin restores memory formation[96]. Notably, AKT-independent metabolic responses have been identified in CD8⁺ T cells[79,83], although such findings do not necessarily exclude a role for mTOR in these processes because AKT inhibition has only a weak effect on mTOR activity in CD8⁺ T cells[79]. In CD4⁺ T cells, elevated levels of fatty acid oxidation and AMPK activity are associated with differentiation into T_{Reg} cells but not effector T_H cells, and inhibition of fatty acid oxidation prevents rapamycin-induced T_{Reg} cell generation[97]. Therefore, a common component for the differentiation of memory T cells and T_{Reg} cells is the selective requirement for fatty acid oxidation, in a process reciprocally regulated by mTOR and AMPK.

Box 3 | **mTOR in T cell trafficking**

The trafficking of naive and memory T cells requires signals that are transduced through chemokine receptors and trafficking molecules, such as CC-chemokine receptor 7 (CCR7), CD62L and sphingosine-1-phosphate receptor 1 (S1PR1). Antigen-stimulated effector T cells downregulate these surface molecules, but upregulate pro-inflammatory chemokine receptors and adhesion molecules that endow these cells with an increased ability to traffic to sites of inflammation instead of entering secondary lymphoid organs[152]. Therefore, quiescent and activated T cells are characterized by differential migratory activities, and proper regulation of T cell migration is essential for a productive immune response. A role for mTOR in T cell migration was revealed by the observation that rapamycin treatment prevents the downregulation of CCR7, CD62L and S1PR1 in $CD8^+$ T cells responding to T cell receptor or cytokine signals[78]. Rapamycin-treated effector T cells tend to migrate to the lymph nodes and spleen rather than to non-lymphoid tissues[27,78]. Conversely, deficiency of phosphatase and tensin homologue (PTEN) or tuberous sclerosis 1 (TSC1) enhances the downregulation of these cell-surface receptors[7,75,78]. mTOR-induced effects may involve both the AKT–mTORC1 (mTOR complex 1) pathway and the mTORC2–AKT–FOXO (forkhead box O) pathway (see the figure), and depend on KLF2 (Krüppel-like factor 2), a crucial transcription factor for CCR7, CD62L and S1PR1 expression[83]. This is further supported by the observations that inhibition of AKT activity or deficiency of 3-phosphoinositide-dependent protein kinase 1 (PDK1) enhances KLF2 expression and the expression of the downstream trafficking molecules[79]. In addition to controlling KLF2 expression, mTOR is required for the expression of T-bet[27]. Among its many downstream effects, T-bet directs the expression of pro-inflammatory chemokine receptors — such as CXC-chemokine receptor 3 (CXCR3) — to coordinate T cell migration with effector and regulatory activities[153,154]. These results highlight that regulation of T cell migration is an important mechanism by which AKT–mTOR signalling modulates immune function *in vivo*[83].

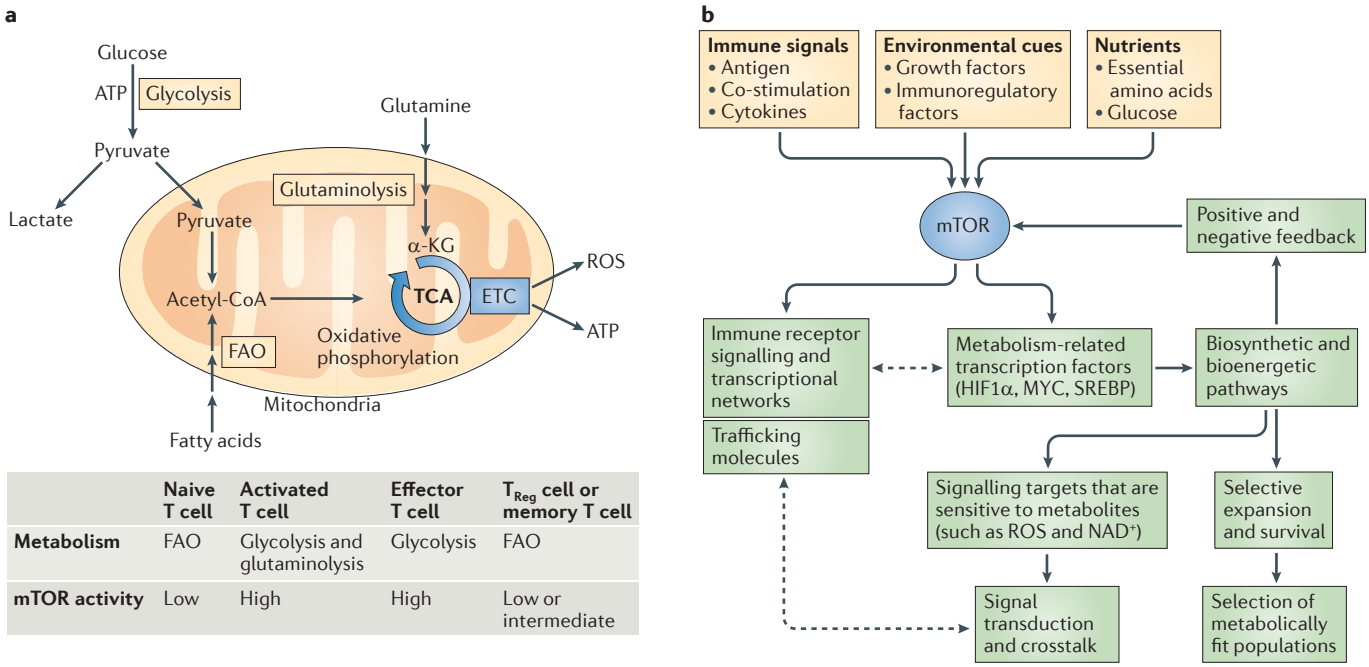


In contrast to the differentiation of T_{Reg} cells and memory $CD8^+$ T cells, effector T cell differentiation is accompanied by strong upregulation of glycolysis. Pharmacological blocking of mTOR or glycolysis reduces the differentiation of T_H1, T_H2 and T_H17 cells[85,97]. HIF1α is selectively expressed in T cells undergoing T_H17 cell differentiation, and its induction requires signalling through mTOR[85,86]. HIF1α is required for mediating glycolysis during T_H17 cell differentiation and contributes to lineage choices between T_H17 cells and induced T_{Reg} cells[85]. Therefore, the mTOR-dependent induction of the transcription factors MYC and HIF1α orchestrates a metabolic checkpoint in TCR-activated and T_H17-polarized cells, respectively[85,88]. HIF1α has also been shown to have direct effects in promoting the degradation of FOXP3 and the transcriptional activity of RORγt[86]. Interestingly, similarly to the induction of T_{Reg} cells, T_H17 cell differentiation seems to be particularly sensitive to metabolic perturbations. Impairments in T_H17 cell differentiation result not only from the blockade of glycolysis[85], but also from the depletion of specific amino acids[98] and from increases in lipid metabolism[99]. As mTORC1 is a key regulator of amino acid and lipid metabolism[100], it will be interesting to examine whether these additional metabolic processes in T_H17 cells are regulated by mTOR.

Sensing and propagating metabolic cues. As a central environmental sensor, mTOR links growth factor signalling with the availability of nutrients, especially amino acids[1,101]. The ancient pathways of amino acid metabolism and mTOR-mediated metabolic control are exploited by the immune system as important mechanisms for immune regulation[102]. For example, in response to T_{Reg} cell-mediated suppression, DCs upregulate enzymes that consume multiple essential amino acids that are present in the tissue microenvironment. Consequently, T cells respond to nutrient starvation by downregulating the activity of mTORC1 and inducing FOXP3 expression[103]. Although other nutrient sensors, such as the kinase GCN2 (also known as EIF2AK4), have been identified in T cells[104], mTORC1 seems to have a dominant role in sensing amino acid availability[103,105]. Consistent with this notion, the inhibition of mTOR-dependent amino acid or glucose metabolism attenuates T cell activation and instead induces T cell anergy[21,22] (BOX 1).

Despite these advances, the extent to which mTOR-dependent metabolic pathways regulate T cell differentiation remains a contentious issue[4,81,83]. As T cell fate is ultimately manifested as lineage-specific gene expression programmes, how do the metabolic intermediates downstream of mTOR activation dictate signalling and transcriptional events? Here, I discuss three potential mechanisms (FIG. 3b). First, direct signalling activities have been identified for certain metabolic intermediates, such as reactive oxygen species (ROS) and nicotinamide adenine dinucleotide (NAD^+), the production of which during the metabolic flux depends on mTOR. These intermediates signal by serving as substrates or modifiers of enzymes and other regulators, and thereby function as metabolic checkpoints[106]. Given the recently identified roles of ROS and the NAD^+-dependent deacetylase sirtuin 1 in T cell function and differentiation[107,108], mTOR-dependent metabolic flux may directly engage signal transduction and crosstalk.

T cell anergy
A state of T cell unresponsiveness to antigen stimulation in which T cells fail to proliferate and produce interleukin-2.



	Naive T cell	Activated T cell	Effector T cell	T_{Reg} cell or memory T cell
Metabolism	FAO	Glycolysis and glutaminolysis	Glycolysis	FAO
mTOR activity	Low	High	High	Low or intermediate

Figure 3 | **mTOR in T cell metabolism. a** | This schematic shows the main metabolic programmes that occur in different T cell subsets. **b** | This scheme shows proposed mechanisms through which metabolic pathways mediated by mammalian target of rapamycin (mTOR) affect T cell differentiation. An important upstream signal for mTOR activation is nutrients, in particular essential amino acids, the levels of which are actively controlled by dendritic cells. Once activated, mTOR serves as a platform to engage several downstream effector pathways, including immune receptor signalling, metabolic programmes and migratory activity. mTOR promotes metabolism by activating a gene expression programme that consists of metabolic gene targets of the transcription factors hypoxia-inducible factor 1α (HIF1α), MYC and sterol regulatory element-binding protein (SREBP). The expressed target proteins in turn affect biosynthetic and bioenergetic pathways. To explain how these metabolic pathways regulate T cell differentiation, three potential downstream mechanisms are proposed: signal crosstalk through metabolite-sensitive signalling targets; feedback control; and selective expansion and survival. Among metabolites with signalling activities, reactive oxygen species (ROS) oxidize the catalytic cysteine residues of phosphatases to cause the inactivation of these enzymes, whereas NAD^+ is required for the activity of sirtuin family deacetylases. α-KG, α-ketoglutarate; ETC, electron-transport chain; FAO, fatty acid oxidation; ROS, reactive oxygen species; TCA, tricarboxylic acid cycle.

Second, nutrient or energetic signals can mediate positive and negative feedback on mTOR activity itself, which may affect not only cell metabolism but also other mTOR-dependent events, such as immune receptor signalling. In particular, the activation of mTORC1 by amino acids and the crosstalk of mTOR with MYC upregulate the expression of the amino acid transporter CD98, which establishes a positive loop that amplifies mTOR signalling in T cells[88,101]. Considering the classic role of mTOR in protein translation, which consumes amino acids, it is perhaps appropriate that mTOR activity is regulated by such a positive loop. Consistent with this notion, inhibition of translation by cycloheximide considerably increases mTORC1 activity, presumably by elevating the levels of intracellular amino acids[109]. However, excessive mTORC1 activation may deplete cellular ATP and cause energetic stress, which activates AMPK to negatively control mTOR activity[110].

Third, metabolism is intricately linked to the cell cycle and apoptotic machineries[111,112]. T cells with suitable metabolism are more likely to survive and/or proliferate and, consequently, on a population level, are selected to develop into a particular lineage. In support of this notion, rapamycin differentially affects the proliferation and survival of effector T cells and T_{Reg} cells[113–116]. Future efforts to identify the molecular components that orchestrate these processes should provide exciting insights into the interface between metabolism and immunity.

Therapeutic targeting of mTOR

The immunosuppressive effect of rapamycin was recognized in the 1970s, before the identification of its molecular target. Recent studies suggest that blocking mTOR not only mediates immunosuppression to reduce transplant rejection and autoimmune disorders, but also boosts immunity under selective conditions and affects other aspects of T cell homeostasis and function.

mTOR inhibition for immunosuppression. In the late 1990s, the US Food and Drug Administration approved the use of rapamycin to prevent rejection in kidney transplantation. A major mechanism of action for rapamycin is the induction and expansion of T_{Reg} cell populations and the inhibition of effector T cell differentiation, as described above. This mechanism is distinct from that of other immunosuppressants (such as cyclosporine A

and FK506), which mainly block Ca^{2+} signalling and calcineurin activation downstream of TCR stimulation, and thus allows for the design of combinatorial therapies with rapamycin and other immunosuppressive agents. This is important because rapamycin monotherapy has a rather weak effect in preventing graft rejection, despite its multiple immunomodulatory functions.

The immunosuppressive effect of rapamycin is also apparent in patients with and experimental models of systemic autoimmune diseases, such as systemic lupus erythematosus and rheumatoid arthritis, as well as organ-specific autoimmune disorders[117,118]. Rapamycin probably establishes long-term immune tolerance in these models by expanding T_{Reg} cell populations and inhibiting the differentiation and function of effector T cells. Notably, rapamycin also has potent effects on DCs and other immune components[2], and the effects of rapamycin *in vivo* might be attributed to these cells, in addition to T cells.

Rapamycin for vaccine development to boost immunity. Despite the well-established immunosuppressive effects of mTOR inhibition, rapamycin and metformin promote the generation of protective T cell memory in models of infection with lymphocytic choriomeningitis virus and *Listeria monocytogenes*. Mechanistically, this has been associated with the induction of a metabolic switch from glycolysis to fatty acid oxidation in the presence of rapamycin and metformin[96,119]. The immunostimulatory effect of rapamycin for $CD8^+$ T cell responses has been extended to additional infectious models[120,121] and to antitumour immune responses[122,123]. Therefore, mTOR inhibitors may serve as novel adjuvants for the development of vaccines against pathogens and tumours. In particular, this strategy of tumour immunotherapy is promising owing to its synergy with the direct suppression of tumour growth by mTOR inhibitors. Notably, the effect of mTOR inhibition depends on the dose range and kinetics of the treatment. Administration of a very high dose of rapamycin prevents the expansion of $CD8^+$ T cell populations, whereas the duration and timing of rapamycin treatment affect the quantity and quality of memory T cell responses[82,119]. To explore the basis for rapamycin-mediated immunosuppressive and immunostimulatory effects on $CD8^+$ T cell responses, Ferrer *et al.* compared the effects of rapamycin on immune responses induced by *L. monocytogenes* and by a skin transplant against the same antigen[120]. Treatment with rapamycin augmented antigen-specific T cell responses to the pathogen but not to the transplant. Thus, the environment in which an antigen is presented influences the effects of rapamycin on T cell responses[120].

T cell malignancy and metabolic dysregulation. A common haematological malignancy is T cell acute lymphoblastic leukaemia (T-ALL), which frequently harbours activating mutations of *NOTCH1* and/or loss-of-function mutations of *PTEN*. Interestingly, both types of mutation activate mTOR signalling, suggesting a pivotal role for mTOR in the development of T-ALL. Consistent with this notion, in a PTEN-deficient mouse model of T-ALL, rapamycin was effective in blocking T-ALL initiation and development. However, following rapamycin withdrawal, most of the PTEN-deficient mice rapidly became ill and died. These results indicate that leukaemia stem cells and leukaemia cell blasts have differential responses to rapamycin, which could contribute to the limited clinical efficacy of rapamycin in cancer[48].

Chronic inflammation in adipose tissues has been established as a major mechanism that causes insulin resistance and the subsequent development of type 2 diabetes. Although earlier studies mostly implicated macrophages as the main inflammatory cell infiltrates[124], recent reports have identified the recruitment of effector $CD8^+$ T cells and $FOXP3^+$ T_{Reg} cells to adipose tissues, where they function to exacerbate and ameliorate inflammation, respectively[125–127]. Given the prominent immunomodulatory role of mTOR in T cells, it will be interesting to explore whether mTOR signalling in T cells contributes to the pathogenesis of metabolic diseases. This raises the notion that therapeutic targeting of mTOR in metabolic diseases may have a dual effect, through the regulation of T cell metabolism and effector function as well as through the direct modulation of the function of metabolic tissues (such as adipose tissues, skeletal muscles and liver), in which mTOR signalling has a central role.

Targeting mTOR: beyond rapamycin. Rapamycin inhibits mTOR through an unusual allosteric mechanism that involves the interaction with its intracellular partner FKBP12. Whereas rapamycin strongly inhibits the functions of ribosomal protein S6 kinases (S6Ks) downstream of mTORC1, it has a surprisingly weak effect on the phosphorylation of the other major mTORC1 targets, eIF4E-binding proteins (4E-BPs)[128]. Moreover, rapamycin treatment frequently abrogates mTORC1-mediated feedback inhibition of PI3K–AKT signalling, leading to a paradoxical enhancement of AKT activity[129]. The incomplete inhibition of mTORC1 and the reversal of the feedback loop by rapamycin attenuate its therapeutic effects, as reflected by the disappointing therapeutic outcomes of rapamycin and other first-generation mTOR inhibitors in patients with cancer[130]. Therefore, selective ATP-competitive mTOR inhibitors (including Torin1 and PP242) were recently developed, and these molecules completely inhibit mTORC1 activity, including the rapamycin-resistant phosphorylation of 4E-BPs[131–133]. Compared with rapamycin, these second-generation inhibitors exert stronger inhibition of mTORC2 and thus are less likely to activate the feedback loop[131–133]. Indeed, these new inhibitors have greater antitumour effects than rapamycin[134,135], and some of them have entered clinical trials as new cancer therapeutics[130]. Their immunomodulatory function is expected to be extensively investigated in the near future.

Because of the pleiotropic effects of mTOR in T cells, targeting the upstream and downstream components of mTOR signalling, rather than mTOR itself, offers an alternative strategy for added specificity. In particular, modulation of mTOR-dependent metabolic pathways is efficacious for modulating T cell-mediated diseases.

Autophagy
A recycling process in which the cell degrades cytoplasmic organelles and proteins in lysosomes.

A classic example is provided by the AMPK activators metformin and AICAR (5-aminoimidazole-4-carboxamide riboside), which block energy-mediated activation of mTORC1 without interfering with PI3K–AKT-mediated growth factor signalling. Aside from modulating T cell memory, as described above[96], these AMPK activators have both prophylactic and therapeutic effects on experimental autoimmune encephalomyelitis by attenuating the severity of disease[136,137]. Similarly, blocking glycolysis protects mice from autoimmune neuroinflammation mediated by T_H17 cells[85]. Moreover, blocking upstream activators of mTOR in T cells, such as S1PR1 and the leptin receptor, has similar effects to rapamycin in modulating T cell responses in selective models[33,37]. Further studies of mTOR-dependent signalling axes will provide more opportunities to specifically target mTOR-associated pathways.

Concluding remarks

Cell fate decisions mediated by mTOR are of particular importance to T cells because of the unique features of these cells. Such features include: their constant exposure to unpredictable pathogen threats; their extensive proliferation following antigenic stimulation; and their continuous migration in a variety of tissues. Recent advances have established mTOR as a fundamental determinant of T cell homeostatic and functional fates, but many questions remain. Further studies are needed to elucidate the upstream signals and downstream effectors that mediate T cell homeostasis and antigen-specific immune responses. Also, we have yet to define the emerging roles of mTOR in certain cell biological processes such as autophagy, a process that is crucial for T cell homeostasis and function[138]. Much of our current knowledge of the mTOR-controlled signalling networks is derived from the use of *in vitro* systems and cell lines. However, the identification of T cell-specific and context-dependent signals promises to provide more insight into the regulation of adaptive immunity. From this perspective, the use of sophisticated genetic systems offers the ultimate molecular and cellular specificities to dissect the underlying processes. Moreover, although research in this area has benefited from the use of rapamycin over the past two decades, the development and application of more potent and selective inhibitors of mTOR is essential to advance both research and clinical applications. The continued expansion of our understanding of mTOR signalling will provide legitimate therapeutic opportunities for T cell-mediated disorders.

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Competing interests statement

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