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Integrating canonical and metabolic signalling programmes in the regulation of T cell responses

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Abstract

Over the past decade, our understanding of T cell activation, differentiation and function has markedly expanded, providing a greater appreciation of the signals and pathways that regulate these processes. It has become clear that evolutionarily conserved pathways that regulate stress responses, metabolism, autophagy and survival have crucial and specific roles in regulating T cell responses. Recent studies suggest that the metabolic pathways involving MYC, hypoxia-inducible factor 1α (HIF1α), AMP-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) are activated upon antigen recognition and that they are required for directing the consequences of T cell receptor engagement. The purpose of this Review is to provide an integrated view of the role of these metabolic pathways and of canonical T cell signalling pathways in regulating the outcome of T cell responses.

T cell receptor (TCR) engagement by peptide–MHC complexes initiates a multitude of signalling programmes that prepare the cell for differentiation, proliferation and effector function. The canonical signalling pathways that lead to activation-induced transcription are mediated by nuclear factor-κB (NF-κB), activator protein 1 (AP-1) and nuclear factor of activated T cells (NFAT). These three pathways collaborate to promote the expression of effector molecules that are crucial for T cell function[1–7] (FIG. 1a). It is generally thought that TCR-induced signalling only leads to T cell activation when it occurs in the context of a second co-stimulatory signal, such as the ligation of CD28 (REF. 8). The precise pathways that mediate CD28-induced co-stimulation have not been completely elucidated. However, one such model posits that TCR-induced NFAT activation leads to T cell anergy, whereas in the context of co-stimulation, NFAT and AP-1 collaborate to promote full T cell activation[3]. Likewise, CD28 signalling leads to the activation of phosphoinositide 3-kinase (PI3K) and the subsequent activation of mammalian target of rapa-mycin (mTOR)[9]. In addition to co-stimulation, further signals from the microenvironment influence the outcome of TCR ligation. For example, specific cytokines are required to promote the differentiation of naive $CD4^+$ T cells into various T helper (T_H) cell subsets (FIG. 1b). Thus, immuno-logical inputs in the form of antigen recognition, co-stimulatory ligand engagement and cytokine stimulation guide the outcome of T cell activation and differentiation.

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Recently, the signalling pathways that control cellular metabolism have been shown to have a crucial role in dictating the outcome of T cell activation. Overall, this requirement for the coordination of T cell metabolism and T cell function reflects two important features of the T cell response: the ability of low frequency, antigen-specific naive T cells to rapidly increase in number in response to a pathogen, and their ability to generate long-lived memory T cells or regulatory T (T_{Reg}) cells that can modulate immune responses. In this Review, we aim to integrate the metabolic pathways with the canonical T cell signalling pathways to provide a comprehensive view of the pathways that regulate T cell immunity. This reveals potential new pharmacological targets for enhancing or inhibiting specific T cell responses.

Regulation of cellular metabolism

Cellular metabolism provides the means by which cells store and use macromolecules that are necessary for growth and for the generation of energy. Depending on nutrient availability and external or intracellular cues, cells can use different substrates and distinct pathways to produce energy. Likewise, cellular metabolism is dictated by the specific function of a cell. Glycolysis is a metabolic pathway by which the catabolism of six-carbon sugars (glucose) produces a net sum of two molecules of ATP and two of pyruvate from each molecule of glucose[10]. In the presence of oxygen, pyruvate derivatives enter the tricarboxylic acid cycle (TCA cycle) and promote the oxidative phosphorylation of energy inter mediates in the mitochondrial matrix to generate a total of ~30 ATP molecules (TABLE 1). If oxygen is unavailable, the two molecules of pyruvate that are generated from glyco lysis can be converted to lactate, which dramatically reduces the ATP yield but still provides an energy source for the cell[10]. In response to environmental cues, there are specific drivers of cellular metabolism that regulate the expression of enzymes that are crucial for various metabolic processes.

Glycolysis is promoted by the upregulation of MYC, which is a basic helix–loop–helix leucine zipper transcription factor (TABLE 2). MYC promotes the expression of `glucose transporter type 1` (GLUT1; also known as SLC2A1), pyruvate kinase, `lactate dehydrogenase` A (LDHA) and `hexokinase 2`, which are required for glucose uptake and for the rate-limiting steps of glycolysis[11,12]. In addition, MYC promotes the expression of both glutaminase and glutamine transporters[13], and further promotes glutaminolysis by transcriptionally repressing the microRNAs miR-23a and miR-23b, which allows for the increased expression of glutaminase[14]. Furthermore, MYC has also been found to have a role in promoting mitochondrial biogenesis[15].

Glycolysis is also regulated by hypoxia-inducible factor 1α (HIF1α), which is a heterodimeric basic helix–loop–helix and Per–Arnt–Sim (PAS) domain-containing transcription factor that, during hypoxia, binds to *cis*-acting hypoxia-response elements and leads to the transcription of numerous genes that are important for cell survival in low oxygen conditions[16] (TABLE 2). Not surprisingly, these genes include those encoding enzymes that are required for the glycolytic pathway[17]. In addition, HIF1α promotes the expression of GLUT1 (REF. 18) and enforces ATP synthesis by glycolysis, rather than

oxidative phosphorylation, by upregulating pyruvate dehydrogenase kinase 1 (PDK1), which is an enzyme that inhibits the entry of pyruvate into the TCA cycle[19,20].

HIF1α expression is not only regulated by oxygen levels but also depends on external cues that are integrated by mTOR activity[21]. mTOR is an evolutionarily conserved serine/threonine kinase that integrates a diverse array of environmental cues to regulate growth, survival and proliferation[22] (TABLE 2). mT O R is present in two distinct protein complexes — mTOR complex 1 (mTORC1) and mTORC2 — that each have unique downstream targets and functions. Activation of mTORC1 occurs by growth factor stimulation of PI3K, which initiates a signalling cascade that results in the inhibitory phosphorylation of the mTORC1 repressor tuberous sclerosis 2 (TSC2; also known as tuberin) by the kinase AKT[23]. In addition to growth factors, amino acids also activate mTORC1 and this leads to recruitment of mTOR to the lysosomal surface where it can interact with, and become activated by, its activator RAS homologue enriched in brain (RHEB)[24–26]. The mechanisms that regulate mTORC2 activation are less clear than those for mTORC1. However, it is known that growth factor stimulation enhances mTORC2 activity and recent studies have implicated a role for the association of the mTORC2 complex with ribosomes in promoting its activation[27].

The activity of mTORC1 enhances HIF1α expression at both the transcriptional and translational level, and thereby stimulates glycolysis and glucose transport[28]. The importance of HIF1α in mediating mTORC1-enhanced glycolysis is illustrated by the observation that small interfering RNA (siRNA)-mediated inhibition of *Hif1a* expression in cells that express constitutively active mTORC1 (*Tsc2*$^{-/-}$ cells) abrogates the expression of the glycolytic factors GLUT1, phosphofructokinase 1 and PDK1 (REF. 28). Interestingly, a recent report suggests that MYC activity is, in part, regulated by mTORC2 (REF. 29). It was observed that mTORC2 activity leads to the acetylation of forkhead box protein O1 (FOXO1), which initiates the release of MYC from a suppressive miR-34c–dependent network[29].

Although MYC, HIF1α and mTOR signalling promote an increased metabolic output by cells, other regulators promote energy conservation during times of limited resources. One such regulator is AMP-activated protein kinase (AMPK), which is a heterotrimeric serine/threonine kinase complex that monitors cellular energy levels (TABLE 2). The binding of AMP or ADP to AMPK induces its phosphorylation and activation by upstream kinases[30,31]. AMPK activation enhances glucose uptake and, at the same time, inhibits glucose, glycogen and fatty acid synthesis[31]. This occurs through the phosphoryl-ation and inhibition of acetyl-CoA carboxylase 1 (ACC1) and the inhibition of the lipogenic transcription factor sterol regulatory element-binding protein 1 (SREBP1; also known as SREBF1)[32]. In addition, AMPK promotes fatty acid oxidation through the phosphorylation and inhibition of ACC2. This results in the enhanced expression of carnitine palmitoyltransferase 1A (CPT1A), which is the rate-limiting factor in mitochondrial lipid uptake[10]. AMPK also enhances mitochondrial bio genesis and oxidative metabolism by promoting the transcriptional activity of peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1α; also known as PPARGC1A)[31]. Thus, AMPK regulates cell metabolism to limit energy expenditure and replenish ATP production. AMPK activity can

also diminish mTORC1 signalling through the phosphorylation of TSC2 and regulatory-associated protein of mTOR (RAPTOR; also known as RPTOR), which is a crucial component of mTORC1 (REFS 33,34). Under conditions of prolonged energy deprivation (starvation), AMPK promotes autophagy) by phos-phorylating and activating the serine/threonine protein kinase Unc-51-like kinase 1 (ULK1)[35]. Thus, AMPK shuts down energy-demanding synthetic pathways but promotes mechanisms that generate energy — such as glycolysis, oxidative phosphorylation and autophagy — as a means of deriving substrates from within the cell. By contrast, deficiency of the AMPK activator liver kinase 1 (LKB1; also known as STK11), and therefore loss of AMPK activation, promotes enhanced glucose and glutamine metabolism through the mTORC1-dependent upregulation of HIF1α during normoxic conditions[36].

Interestingly, although mTORC1 activity has been shown to increase glycolysis through regulation of HIF1α, mTORC1 activity can also promote oxidative phosphorylation. This occurs through the increased interaction of the transcriptional repres-sor yin and yang 1 (YY1) with PGC1α, which induces the expression of mitochondrial genes[37]. In addition, mTORC1 promotes lipid biosynthesis by enhancing the transcription and translation of SREBP1 (REF. 28) but limits fatty acid oxidation through the inhibition of CPT1A[38]. The activity of mTORC1 has also been shown to promote nucleotide synthesis. This process occurs through the activation of ribosomal protein S6 kinase β1, which post-translationally regulates *de novo* pyrimidine synthesis[39,40].

T cells have a specialized metabolism

Most cells use oxidative phosphorylation to maximize ATP production but activated T cells (and cancer cells) mainly generate ATP through glycolysis[41,42]. This use of glycolysis in the presence of oxygen was first described by Otto Warburg for cancer cells and it is therefore referred to as the Warburg effect[43]. Although glycolysis provides less ATP than oxidative phosphorylation, it has been proposed that avoiding oxidative phosphorylation allows for the generation of substrates that are required for the synthesis of amino acids, nucleic acids and lipids, all of which are vital for proliferation[44]. Of note, a recent report has challenged the necessity of Warburg physiology in T cell proliferation, instead suggesting that glycolysis is required to release translational inhibition of the mRNA that encodes the effector cytokine interferon-γ (IFNγ)[45]. Nonetheless, glucose uptake is essential for glyco lysis and enhanced cell surface expression of GLUT1 is a crucial aspect of TCR-induced T cell activation[46]. In this regard, it has been shown that CD28 signalling upregulates the expression of glucose transporters[47]. Similarly, the uptake and metabolism of the amino acid glutamine is essential for T cell activation, as glutamine deprivation blocks T cell proliferation and cytokine production[11,48]. Glutamine oxidation can lead to the generation of α-ketoglutarate, which is a key intermediate of the TCA cycle and which, in turn, provides substrates for the generation of various macromolecules[10]. Furthermore, T cells require fatty acid metabolism for their proliferation and function. Cholesterol synthesis is essential for membrane biogenesis and T cells that are deficient in SREBPs (owing to a T cell-specific deletion of SREBP cleavage-activating protein (SCAP)) have diminished proliferative capacity and reduced antiviral responses[49]. Along these lines, the expression of SREBP1 is enhanced following TCR activation through the suppression of the liver X receptor signalling

pathway[50]. Thus, T cell activation induces metabolic changes that allow for processes that are necessary to promote proliferation and cytokine secretion.

Although the considerations that are described above reflect the metabolic needs of T cells during activation and robust proliferation, it has become clear that different T cell subsets have unique metabolic needs. Thus, T cell activation and fate are linked to specific metabolic programmes that support selective T cell functions. In this regard, immunological signals such as co-stimulatory ligands, cytokines and antigen promote metabolism. Likewise, metabolic signals such as nutrient availability, hypoxia and growth factors regulate immune function. From a signalling perspective, central roles for the energy sensor AMPK and the evolutionarily conserved PI3K family member mTOR in integrating immuno-logical and metabolic pathways have emerged (TABLE 2). Similarly, the transcription factors MYC and HIF1α are central to promoting the expression of the genes that are required for metabolic programmes that support T cell responses (TABLE 2). Focusing on the metabolic and immunological roles of these molecules provides an integrative picture of the signalling pathways that regulate T cell activation, differentiation and function. In the following sections, we discuss the roles of these metabolic factors in influencing $CD4^+$ and $CD8^+$ effector T cell differentiation (FIG. 2), $CD8^+$ memory T cell generation and $CD4^+$ T_{Reg} cell function (FIG. 3).

Metabolic regulation of $CD4^+$ T_H cell lineages

$CD4^+$ T cells differentiate into distinct helper cell lineages following TCR engagement and cytokine stimulation[51] (FIG. 1b). To determine whether T_H cell subsets have distinct metabolic needs, the metabolic profiles of stimulated T_H1, T_H2 and T_H17 cells have been assessed[52]. This study showed that all $CD4^+$ T_H cell subsets upregu-late GLUT1 expression upon TCR activation and have elevated glycolytic rates[52]. Thus, T_H1, T_H2 and T_H17 cells all use glycolysis upon activation.

Recent studies have elucidated a role for MYC in establishing the metabolic profile that is required for effective T cell proliferation. T cell activation induces protein-level expression of both MYC and HIF1α within 2 hours of stimulation[11], and MYC expression levels are highest in proliferating lymphocytes[53]. However, MYC — but not HIF1α — is required for upregulating the expression of the glycolytic machinery and the substrates that are essential for glutamine metabolism. Deletion of *Myc* abrogates the ability of activated T cells to undergo glycolysis and to initiate the catabolism of glutamine[11]. Furthermore, MYC deficiency diminishes the expression of the glu-tamine exchanger CD98 (a heterodimer of SLC3A2 and SLC7A5), which reduces mTORC1 activity. The absence of MYC in T cells markedly inhibits activation-induced glutaminolysis, and the subsequent generation of nucleotides and polyamines that is necessary for proliferation[11].

Although HIF1α is not required for $CD4^+$ T cell proliferation or interleukin-2 (IL-2) production[11,54], several groups have shown that is has an important role in the generation and function of T_H17 cells[54,55]. HIF1α expression is highly induced under T_H17 cell-polarizing conditions during T cell activation. This upregulation of HIF1α expression is dependent on signal transducer and activator of transcription 3 (STAT3) and, importantly,

occurs even under normoxic conditions[55]. Furthermore, HIF1α promotes T_H17 cell differentiation by directly inducing the transcription of the gene that encodes retinoic acid receptor-related orphan receptor-γt (RORγt), and by cooperating with RORγt and the histone acetyltransferase p300 (also known as EP300) to drive the transcription of T_H17 cell-associated genes[55]. In addition, it was found that polarizing T cells *in vitro* under conditions of 5% oxygen promotes T_H17 cell differentiation in an mTORC1–HIF1α-dependent manner[56]. Under T_H17 cell-polarizing conditions, HIF1α promotes the transcription of the genes encoding the rate-limiting enzymes of glycolysis, such as hexo kinase 2, glucose-6-phosphate isomerase, pyruvate kinase and LDHA, as well as GLUT1 (REF. 54).

HIF1α expression has also been linked to the maintenance of T_H17 cells[57]. By studying T cells from patients with inflammation, it was observed that T_H17 cells resemble long-lived effector memory cells[57]. Indeed, HIF1α was shown to have an important role in maintaining the expression of high levels of anti-apoptotic genes in T_H17 cells[57]. In addition to HIF1α, the maintenance of T_H17 cells has been linked to the upregulation of T cell factor 7 (TCF7; also known as TCF1) and lymphoid enhancer-binding factor 1 (LEF1), which are targets of the WNT–β-catenin pathway that are expressed at high levels in stem cells[58]. Interestingly, work that has been carried out in neural stem cells has shown that HIF1α positively regulates the expression of TCF7 and LEF1 (REF. 59). Although further work will be necessary to support a role for HIF1α in promoting the expression of TCF7 and LEF1 in lymphocytes, these data suggest that HIF1α expression may induce stem cell-like properties in T_H17 cells. Thus, HIF1α coordinates immunological programmes — such as RORγt expression and forkhead box P3 (FOXP3) degradation (see below) — with metabolic programmes (for example, the upregulation of the glycolytic machinery and inhibitors of apoptosis) to promote the development of T_H17 cells.

Of note, one group has shown increased T cell activation and IFNγ production in T cells that are deficient for the alternatively spliced isoform of HIF1α known as I.1 (REF. 60). As IL-17 has been shown to inhibit IFNγ production, it has been proposed that the increase in IFNγ in these mice is due to decreased IL-17 production[55]. Nonetheless, follow-up studies have revealed that deletion of the HIF1α isoform I.1 in T cells enhances immunity in a model of bacterial infection[61]. Therefore, the precise role of HIF1α in T_H1 and T_H2 cell differentiation and function remains to be determined.

Dissecting the mTOR pathway has revealed a crucial role for mTOR in the regulation of $CD4^+$ T cell lineage differentiation. T cell-specific deletion of *Mtor* results in the abrogation of T_H1, T_H2 and T_H17 cell differentiation[62]. Instead, stimulation of mTOR-deficient $CD4^+$ T cells induces the accumulation of $FOXP3^+$ T_{Reg} cells[62]. Furthermore, a specific deletion of *Rheb* (leading to the loss of mTORC1) in T cells results in the loss of T_H1 and T_H17 cell differentiation, although T_H2 cell generation is unaffected[63]. By contrast, T cells that lack rapamycin-insensitive companion of mTOR (RICTOR), and thus lack mTORC2, are readily skewed towards T_H1 or T_H17 cell lineages (depending on which Cre recombinase is used) but they fail to differentiate into T_H2 cells[63,64]. In addition, RICTOR-deficient mice are resistant to T_H2 cell-mediated diseases[63,65]. Thus, mTORC1 is required for T_H1 and T_H17 cell differentiation, and mTORC2 is necessary for T_H2 cell development. Although the metabolic profiling of these cells is currently an area of active investigation, we propose that

mTOR regulates the metabolic potential of these cells to influence T cell differentiation. Of note, recent papers demonstrate that deletion of the mTORC1 component RAPTOR prevents the generation of T_H1, T_H2, T_H17 and T_{Reg} cells[66,67]. This is different from *Rheb*$^{-/-}$ mice, in which there are only defects in T_H1 and T_H17 cell differentiation[63]. Thus, RHEB-dependent mTORC1 signalling seems to have more selective effects on immune cells. We speculate that the differences that have been observed between *Raptor*$^{-/-}$T cells and *Rheb*$^{-/-}$ T cells are due to markedly enhanced or unopposed mTORC2 activity in *Raptor*$^{-/-}$ T cells, as many of the defects that are seen in these mice are not observed in mTOR-deficient T cells[62]. Nonetheless, the differences between the *Raptor*$^{-/-}$ and *Rheb*$^{-/-}$ T cells provide an opportunity to define mTORC1-dependent processes that selectively regulate T cell differentiation.

It should be pointed out that, in contrast to these two studies[63,64] on the role of RHEB and RAPTOR in T cells, there is a report suggesting that RAPTOR (and therefore mTORC1) is not required for T_H1 and T_H2 cell differentiation and is only crucial for T_H17 cell differentiation by enhancing the nuclear accumulation of RORγt[68]. An explanation for these discrepant findings remains to be elucidated. We speculate that the inconsistent results may be related to the enhanced expansion of a $CD4^{-}IFN\gamma^{+}$ cell population that can rapidly proliferate in cell cultures after magnetic isolation of RAPTOR-deficient $CD4^{+}$ T cells (J.D.P., unpublished observations).

Other studies have used LKB1-deficient $CD4^{+}$ T cells (which display a loss of AMPK activation) to investigate the roles of the AMPK and mTOR pathways in T_H cell differentiation. LKB1-deficient $CD4^{+}$ T cells show elevated production of IFNγ and IL-17, and have an enhanced propensity to differentiate into T_H1 or T_H17 cells[69]. LKB1 deficiency also results in enhanced glucose uptake with elevated protein-level expression of GLUT1 and hexokinase 2, which indicates that AMPK activation represses glycolysis[69]. In addition, LKB1-deficient T cells have increased expression of mTORC1 gene targets compared with wild-type cells, which further supports a crucial role for mTOR in T_H1 and T_H17 cell differentiation[69].

The metabolic link to CD8⁺ effector T cell function

$CD8^{+}$ effector T cells rely heavily on glycolysis to support their metabolic needs during their rapid proliferation in response to infection[70]. Inhibition of glycolysis during the activation of naive $CD8^{+}$ T cells abrogates effector cell generation[71]. For example, the glucose analogue 2-deoxy-d-glucose inhibits glycolysis and downregulates the expression of mRNAs encoding the $CD8^{+}$ T cell effector proteins IFNγ and perforin[72,73]. Interestingly, IL-2 production is unperturbed by 2-deoxy-d-glucose treatment.

MYC has been shown to be crucial for T cell proliferation and its requirement in T cell activation has been further highlighted by studies examining $CD8^{+}$ T cell function in *Myc*$^{+/-}$ mice. $CD8^{+}$ T cells that lack one copy of the *Myc* gene show impaired activation, as determined by the abrogated upregulation of CD44 (REF. 74). In addition, studies of HIF1β-deficient T cells have provided an insight into the role of HIF1β in $CD8^{+}$ T cell effector differentiation and function[75]. Upon initial activation, HIF1β-deficient T cells readily take

up glucose and initiate glycolysis. This is in contrast to MYC-deficient $CD8^+$ T cells, which lack the ability to initiate activation and glycolysis[11]. However, in response to IL-2, HIF1β-deficient $CD8^+$ T cells fail to sustain GLUT1 levels and they have reduced expression of the key rate-limiting glyco-lytic enzymes, such as hexokinase 2, pyruvate kinase, phospho fructokinase 1 and LDHA[75]. Concomitantly, the HIF1β-deficient $CD8^+$ T cells have reduced expression of effector molecules (namely, perforin and granzymes), but proliferation, IFNγ production and T-bet expression remain intact. Of note, these data indicate that the induction of HIF1β depends on mTORC1 activity and thus, mTOR is a crucial regulator of the glycolytic machinery that is upregulated by HIF1β expression[75]. Interestingly, this study also showed that mTOR activation in cytotoxic T lymphocytes (CTLs) occurs independently of AKT activation. This suggests that CTLs may use an alternative signalling pathway for the activation of mTORC1 (REF. 75).

Consistent with this study is a recent report that examines the function of Von Hippel–Lindau disease tumour suppressor (VHL)-deficient $CD8^+$ T cells, which have enhanced expression of HIF1α[76]. Compared with wild-type T cells, VHL-deficient T cells have enhanced effector activity and they more potently reject tumours. Interestingly, although such cells expressed increased levels of effector molecules — such as perforin and granzymes — the overexpression of HIF1α also resulted in the increased expression of inhibitory molecules, such as cytotoxic T lymphocyte antigen 4 (CTLA4) and lymphocyte activation gene 3 protein (LAG3)[76].

In addition, branched chain amino acids activate the mTOR pathway, as well as providing the building blocks for protein synthesis. A recently defined feature of T cell activation is the increased cell surface expression of the neutral amino acids transporter solute carrier family 7 member 5 (SLC7A5; also known as LAT1). Deletion of *SLC7A5* in T cells markedly inhibits clonal expansion and effector cell differentiation[77]. Similarly, T cell-specific deletion of *Raptor* abrogates $CD8^+$ T cell effector function (including IFNγ production) and proliferation in response to infection[66]. Furthermore, RAPTOR deficiency results in the downregulation of glycolytic transcripts and MYC protein, and the generation of transcripts that are important in lipid synthesis and oxidative phosphorylation[66]. Thus, the RAPTOR–mTORC1 pathway coordinates metabolic programmes that are important for T cell activation and function.

AMPK is activated by an increase in the AMP/ATP ratio, as well as following TCR engagement. Interestingly, the activation of AMPK following antigen recognition requires the activation of calcium/calmodulin-dependent protein kinase kinases (CaMKKs) but this is not necessary for the activation of AMPK by an increase in the AMP/ATP ratio. These results suggest that in lymphocytes, AMPK activation in response to antigen anticipates ATP depletion even in the presence of adequate nutrients[78]. Nonetheless, $CD8^+$ T cells that lack expression of the catalytic α1-subunit of AMPK (AMPKα1) are activated, proliferate and secrete cytokines to an extent that is similar to wild-type T cells[79,80]. Thus, AMPK activation is dispensable for T cell activation in the presence of adequate nutrients. However, metabolic stress due to glucose deprivation induces enhanced cell death in AMPKα1-deficient T cells[80]. Similarly, T cells that are deficient in tuberous sclerosis 1 protein homologue (TSC1; also known as hamartin) have increased mTOR activation and

show increased apoptosis as a result of abnormal mito-chondrial potential and the increased production of reactive oxygen species[81–83].

Memory and T_{Reg} cells are metabolically alike

It has been established that glucose uptake and a high glyco lytic rate are required for effective $CD4^+$ and $CD8^+$ T cell responses, but both peripherally derived T_{Reg} cells and $CD8^+$ memory T cells do not primarily use glycolysis for energy generation and instead rely on fatty acid metabolism[52,84]. Compared with effector T cells, $CD8^+$ memory T cells have enhanced mitochondrial spare respiratory capacity, which provides the extra energy storage that is necessary to promote survival[85]. Memory T cells must also respond rapidly following antigen re challenge. In this regard, it was found that memory T cells have a greater mitochondrial mass compared with naïve T cells[86]. Consequently, following antigen rechallenge, effector memory T cells more extensively use oxidative phosphorylation and glycolysis compared with activated naive T cells[86,87]. Furthermore, a recent study suggests that memory $CD8^+$ T cells use an AKT-dependent, rapa mycin-insensitive metabolic programme that facilitates rapid activation-induced glycolysis[87].

A role for mTOR in regulating the differentiation of $CD8^+$ effector and memory T cells was revealed by treating mice with low doses of rapamycin during infection with lymphocytic choriomeningitis virus (LCMV)[88]. It was found that mTOR inhibition markedly enhanced the generation of memory T cells. Given that rapamycin is used as an immunosuppressive agent, these results seem counterintuitive. However, it was shown that rapamycin treatment mitigates the expression of T-bet and enhances the expression of eomeso-dermin, which is a transcription factor that is associated with memory T cell differentiation[89]. Furthermore, in a model of homeostatic proliferation-induced memory, rapamycin administration abrogates the requirement of IL-15 signalling for the upregulation of eomesodermin to promote a memory response[90]. Consistent with these studies is a recent report showing that treatment of mice with a 4-1BB aptamer–*Raptor*-specific siRNA — which targets *Raptor* to an aptamer that binds the $CD8^+$ T cell co-stimulatory molecule 4-1BB (also known as CD137 and TNFRSF9) — led to diminished mTORC1 activity in $CD8^+$ T cells and the generation of an enhanced memory response[91].

In addition to coordinating transcription factors that are associated with effector and memory T cell generation, it is clear that mTOR regulates $CD8^+$ T cell differentiation by guiding metabolic programmes. Pearce *et al*.[84] defined the necessity of fatty acid metabolism in $CD8^+$ memory T cell generation. They observed that $CD8^+$ T cells that lack tumour necrosis factor (TNF) receptor-associated factor 6 (TRAF6) have impaired memory cell generation owing to defects in fatty acid metabolism. Treatment of $Traf6^{-/-}$ cells with metformin (which activates AMPK) or rapamycin restores fatty acid oxidation and consequently rescues memory T cell generation. In a similar system, culturing LCMV-specific T cells with rapamycin before adoptive transfer into infected mice leads to a marked increase in the frequency of long-lived memory cells[92]. However, the inhibition of oxidative phosphorylation using oligomycin reduced the survival advantage of the rapamycin-treated cells[92]. In addition, although AMPK deficiency does not affect $CD8^+$ CTL activity, it has been found that AMPK is required for $CD8^+$ memory T cell generation, as AMPKα1-

deficient $CD8^+$ T cells fail to mount an effective secondary response to an *in vivo* infection[80].

As discussed, HIF1α has been shown to be crucial for promoting T_H17 cell differentiation but some studies suggest that it has the opposite effect on T_{Reg} cell development. This inhibition of T_{Reg} cell development was shown to occur through HIF1α-mediated degradation of the FOXP3 protein during T_H17 cell development via proline hydroxylation and subsequent ubiquitylation[55]. Such studies are consistent with other work showing that HIF1α expression is strongly induced in $CD4^+$ T cells under T_H17 cell-polarizing conditions, whereas its expression is mitigated under conditions that promote $CD4^+$ T_{Reg} cell development[54]. Consistent with these findings, the deletion of *Hif1a* favours the generation of T_{Reg} cells[54,55]. Furthermore, blocking glycolysis inhibits T_H17 cell development but promotes T_{Reg} cell differentiation[54].

However, in contrast to these studies, other groups have suggested that HIF1α promotes T_{Reg} cell differen-tiation[93,94]. For example, it has been shown that hypoxia can enhance the expression of FOXP3 in Jurkat T cells in a HIF1α-dependent manner[93]. Similarly, another report showed a marked increase in FOXP3 expression under hypoxic conditions[94]. This upregulation was HIF1α dependent and was mediated by the direct binding of HIF1α to the *FOXP3* promoter. HIF1α also promotes optimal T_{Reg} cell function, as HIF1α-deficient T_{Reg} cells fail to provide protection in an *in vivo* colitis model[94]. Although the precise mechanisms that account for the positive and negative roles of HIF1α in T_{Reg} cells are yet to be delineated, it has been suggested that HIF1α — similarly to IFN-regulatory factor 4 (IRF4), B lymphocyte-induced maturation protein 1 (BLIMP1; also known as PRDM1), GATA-binding protein 3 (GATA3) and B cell lymphoma 6 (BCL-6) — might have an intrinsic role in both effector and T_{Reg} cell differentiation and function[94].

As indicated above, a crucial role for mTOR in $CD4^+$ T cell differentiation was identified by studying mice in which *Mtor* expression was selectively deleted in T cells[62]. It was found that under T_H1, T_H2 and T_H17 cell-polarizing conditions, the mTOR-deficient T cells failed to differentiate into the respective $CD4^+$ effector T cell subsets and instead became $FOXP3^+$ T_{Reg} cells[62]. These findings are consistent with previous studies showing that rapamycin promotes the generation of T_{Reg} cells both *in vitro* and *in vivo*[95–97], as well as other studies suggesting that activation of the AKT–mTOR pathway through sphingosine-1-phosphate impedes the development of thymus-derived T_{Reg} cells during thymic generation and instead favours the generation of T_H1 cells[98]. Furthermore, activation of the AKT–mTOR pathway by overexpression of a constitutively active form of AKT inhibits T_{Reg} cell genera-tion[99]. These findings are consistent with observations that T_{Reg} cells rely less on glycolysis and more on fatty acid metabolism[52].

Interestingly, several reports cite the necessity of mTORC1 activity in promoting T_{Reg} cell function[67,100]. However, this seems to be at odds with the findings that *Mtor* deletion and rapamycin treatment promote T_{Reg} cell function. To reconcile these observations, we have proposed that decreased mTOR activity promotes the generation of ‘memory’ T_{Reg} cells and that high mTOR activity may be necessary for promoting the function of ‘effector’ T_{Reg} cells[101]. Such a model is consistent with the observation that strong TCR engagement in the

presence of transforming growth factor-β (TGFβ) promotes T_{Reg} cell generation, even though it also promotes increased mTOR activity (J.D.P., unpublished observations).

Targeting metabolism for immunoregulation

It is well established that potent inhibition of T cells can be achieved by blocking the activation of NFAT, NF-κB and AP-1. Indeed, the robust ability of the cal-cineurin inhibitors cyclosporine A and FK506 to inhibit T cell responses has revolutionized transplantation[102]. However, blocking NFAT activation also inhibits tolerance and the activation of T_{Reg} cells[103]. Thus, more selective pharmacological agents are needed to specifically target effector T cell responses. As HIF1α, MYC, AMPK and mTOR have crucial and selective roles in defining T cell function and fate, they represent novel and specific targets for immune modulation.

As an example, a recent drug screen identified the cardiac glycoside digoxin as an inhibitor of HIF1α[104]. Interestingly, digoxin has been shown to be a potent inhibitor of T_H17 cell differentiation[105,106]. Digoxin treatment selectively inhibits T_H17 cell generation without affecting the differentiation of other effector T cell subsets. Furthermore, digoxin — as well as other RORγt-specific inhibitors — mitigates T_H17 cell-mediated autoimmune disease in mice[105,107]. These studies showed that digoxin blocks RORγt activity. However, in light of the ability of digoxin to inhibit HIF1α, it is possible that the abrogation of HIF1α-induced gene expression might also be contributing to the reduced T_H17 cell response. Similarly, direct targeting of MYC could be a potent immunosuppressive strategy. Indeed, it has been shown that inhibitors of bromo domain and extra-terminal domain (BET) protein and MYC protein can suppress $CD4^+$ T cell-mediated cytokine production and autoimmunity[108].

Targeting AMPK as a means of regulating T cell function has also been studied. The AMPK agonist 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) has been used to decrease disease severity in mouse models of acute and chronic dextran sulphate sodium-induced colitis[109]. This was associated with decreased T_H1 and T_H17 cell responses[109]. In another study, AICAR treatment mitigated disease severity in experimental autoimmune encephalomyelitis (EAE)[110]. This effect was associated with a reduction in the levels of T_H1 cell-associated cytokines (IFNγ and TNF) and an increase in the expression of IL-4 and IL-10 (REF. 110). Furthermore, compared with control-treated mice, treatment with another AMPK activator, metformin, reduced GLUT1 expression and increased the percentage of airway-infiltrating T_{Reg} cells in a mouse model of asthma[52]. Likewise, metformin has been shown to decrease the T_H17 cell response and mitigate disease severity in EAE[111]. Overall, these data suggest that targeted activation of AMPK may prove to be a potent strategy for treating inflammatory diseases. Additionally, metformin treatment enhances memory T cell generation in mice[84] and therefore AMPK activation might also have a role in potentiating vaccine efficacy.

Targeting mTOR has proven to be an effective means of suppressing immune responses. Indeed, the mTORC1 inhibitor rapamycin has been used to prevent transplant rejection[102,112]. Interestingly, the mechanism of action of rapamycin was initially thought to be related to its ability to inhibit T cell proliferation[9]. In fact, rapa mycin is a relatively poor

inhibitor of proliferation and the effectiveness of this agent is most probably owing to its ability to inhibit effector T cell metabolism, inhibit effector T cell differentiation and promote T_{Reg} cell differ-entiation[113]. Along these lines, it should be noted that whereas rapamycin was initially thought to only inhibit mTORC1 activity, it is clear that — particularly under conditions of prolonged exposure — rapamycin can also inhibit mTORC2 activity[114]. This might explain the ability of rapamycin to promote the generation of T_{Reg} cells. Alternatively, given that rapamycin can promote the generation of memory T cells, investigators are exploring the use of rapamycin to enhance vaccine responses. Indeed, this strategy has proven effective in a non-human primate model of vaccinia virus vaccination[115]. Whereas rapamycin (and other rapalogues) inhibit mTOR activity by sterically blocking the formation of the mTOR complex, mTOR kinase inhibitors have also been developed more recently. These agents are designed to inhibit the activity of both mTORC1 and mTORC2 (REFS. 116, 117). These inhibitors are designed to become new cancer therapies but their use in regulating immune responses should also be explored.

Future perspectives

In this Review, we have demonstrated that metabolic signalling programmes are integral to T cell activation, differentiation and function. Thus, whereas classical immunotherapies target ubiquitous pathways of T cell activation, we propose a more selective means of regulating immune responses by targeting specific metabolic signalling programmes. As such, we believe that selective metabolic inhibitors might prove to be clinically useful immunomodulators. In the case of immunosuppression, such agents would have the advantage of inhibiting effector T cell function but also enhancing T_{Reg} cell function. For example, whereas calcineurin inhibitors (such as cyclosporine A and FK506) block the generation of T_{Reg} cells, mTOR inhibition does not[118]. To this end, treating liver transplant recipients with siro-limus (an mTOR inhibitor), rather than FK506, increases the number of liver $FOXP3^+$ T_{Reg} cells[119]. Likewise, a calci neurin inhibitor-free regimen (using sirolimus) has been developed to promote stable mixed donor chimer-ism after non-myeloablative allogeneic haematopoietic stem cell transplantation for adult sickle cell disease[120]. Furthermore, there seems to be promise in the strategy of directly inhibiting glycolysis. In mouse models, 2-deoxy-d-glucose has been shown to prevent the development of EAE and to promote T_{Reg} cell generation[52,54], as well as promoting $CD8^+$ memory T cell generation[71]. Likewise, inhibitors of glutamine metabolism — which are being developed as anticancer agents — might turn out to be potent inhibitors of effector T cell responses[121]. Therefore, future work should not be focused on indiscriminate switching on or off of T cell responses but rather on modulating T cell responses depending on what immune mechanism is required. This therapeutic approach may harness the most potent response and minimize undesired effects.

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Glossary

Glucose transporter type 1 (GLUT1)	A unidirectional transporter that facilitates the transport of glucose across the plasma membrane
Lactate dehydrogenase A (LDHA)	An enzyme that catalyses the conversion of pyruvate to lactate
Hexokinase 2	An enzyme that initiates the first reaction of glycolysis by phosphorylating glucose to produce glucose-6-phosphate
Pyruvate dehydrogenase kinase 1 (PDK1)	An enzyme that phosphorylates and inactivates pyruvate dehydrogenase, thereby inhibiting the catalysis of pyruvate to acetyl-CoA and preventing the initiation of the tricarboxylic acid cycle
Phosphofructokinase 1	A rate-limiting enzyme of glycolysis that requires ATP to convert fructose-6-phosphate into fructose-1,6-bisphosphate
Carnitine palmitoyltransferase 1A (CPT1A)	A rate-limiting mitochondrial enzyme that is necessary for fatty acid oxidation. CPT1A catalyses the transfer of the acyl group of long-chain fatty acids to acylcarnitine, which allows for its transport from the cytosol to the mitochondria
Autophagy	An evolutionarily conserved process in which acidic double-membrane-bound vacuoles sequester intracellular contents (such as damaged organelles and macromolecules) and target them for degradation through fusion with secondary lysosomes
α-ketoglutarate	A key intermediate of the tricarboxylic acid cycle that can be derived from glutaminolysis

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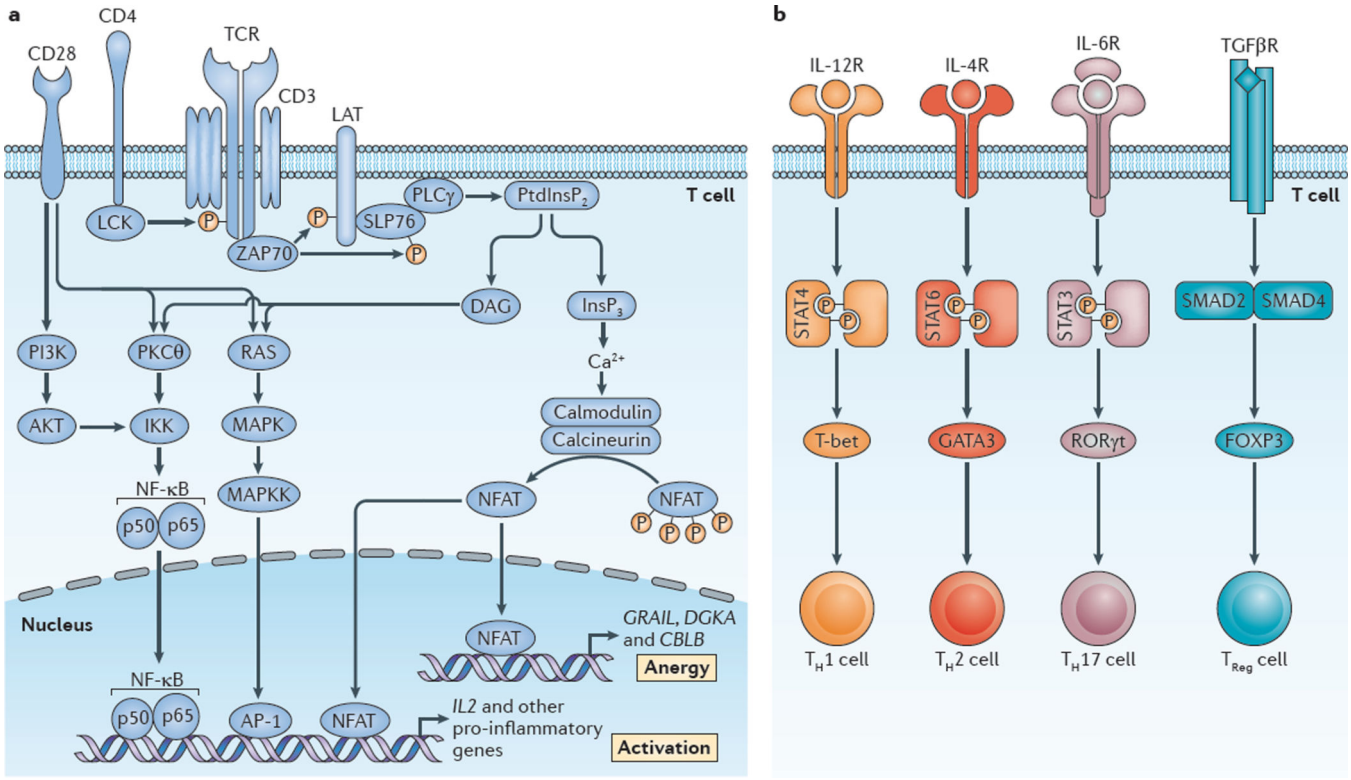

Figure 1. Canonical T cell signalling pathways: signal 1 and signal 2

a | Signal 1 (T cell receptor (TCR) engagement) in the setting of signal 2 (co-stimulation; depicted as CD28) leads to full T cell activation[122]. This is facilitated by the activation of three canonical transcription factors — nuclear factor-κB (NF-κB), activator protein 1 (AP-1) and nuclear factor of activated T cells (NFAT)[6,7,123,124]. This, in turn, leads to the expression of multiple cytokines, chemokines and cell surface receptors, all of which promote T cell activation and proliferation[3]. Alternatively, TCR recognition alone (in the absence of co-stimulation) leads to an 'off' signal in the form of T cell anergy[5,125]. Under these conditions, NFAT is activated in the absence of full AP-1 activation, which leads to the expression of genes such as diacylglycerol kinase-α (*DGKA*) and the E3 ubiquitin-protein ligases *CBLB* and *GRAIL* (which encodes gene related to anergy in lymphocytes; also known as *RNF128*), which inhibit full T cell activation[3]. **b** | Upon T cell activation, cytokines in the T cell microenvironment determine the outcome of antigen recognition with regard to effector T cell differentiation[51]. As shown for $CD4^+$ T cells, interleukin-12 (IL-12), IL-4 and IL-6 activate signal transducer and activator of transcription 4 (STAT4), STAT6 and STAT3, respectively. This leads to the expression of T-bet, GATA-binding protein 3 (GATA3) and retinoic acid receptor-related orphan receptor-γt (RORγt), which facilitates the generation of T helper 1 (T_H1), T_H2 and T_H17 cells. Alternatively, transforming growth factor-β (TGFβ) signalling through SMAD2-SMAD4 promotes the expression of forkhead box P3 (FOXP3) and the generation of regulatory T (T_{Reg}) cells. DAG, diacylglycerol; IKK, inhibitor of NF-κB kinase; $InsP_3$, inositol-1,4,5-trisphosphate; LAT, linker for activation of T cells; MAPK, mitogen-activated protein kinase; MAPKK, MAPK kinase; PI3K, phosphoinositide 3-kinase; PKCθ, protein kinase Cθ; PLCγ,

phospholipase Cγ; $PtdInsP_2$, phosphatidylinositol-4,5-bisphosphate; SLP76, SH2 domain-containing leukocyte protein of 76 kDa (also known as LCP2); ZAP70, ζ-chain-associated protein kinase of 70 kDa.

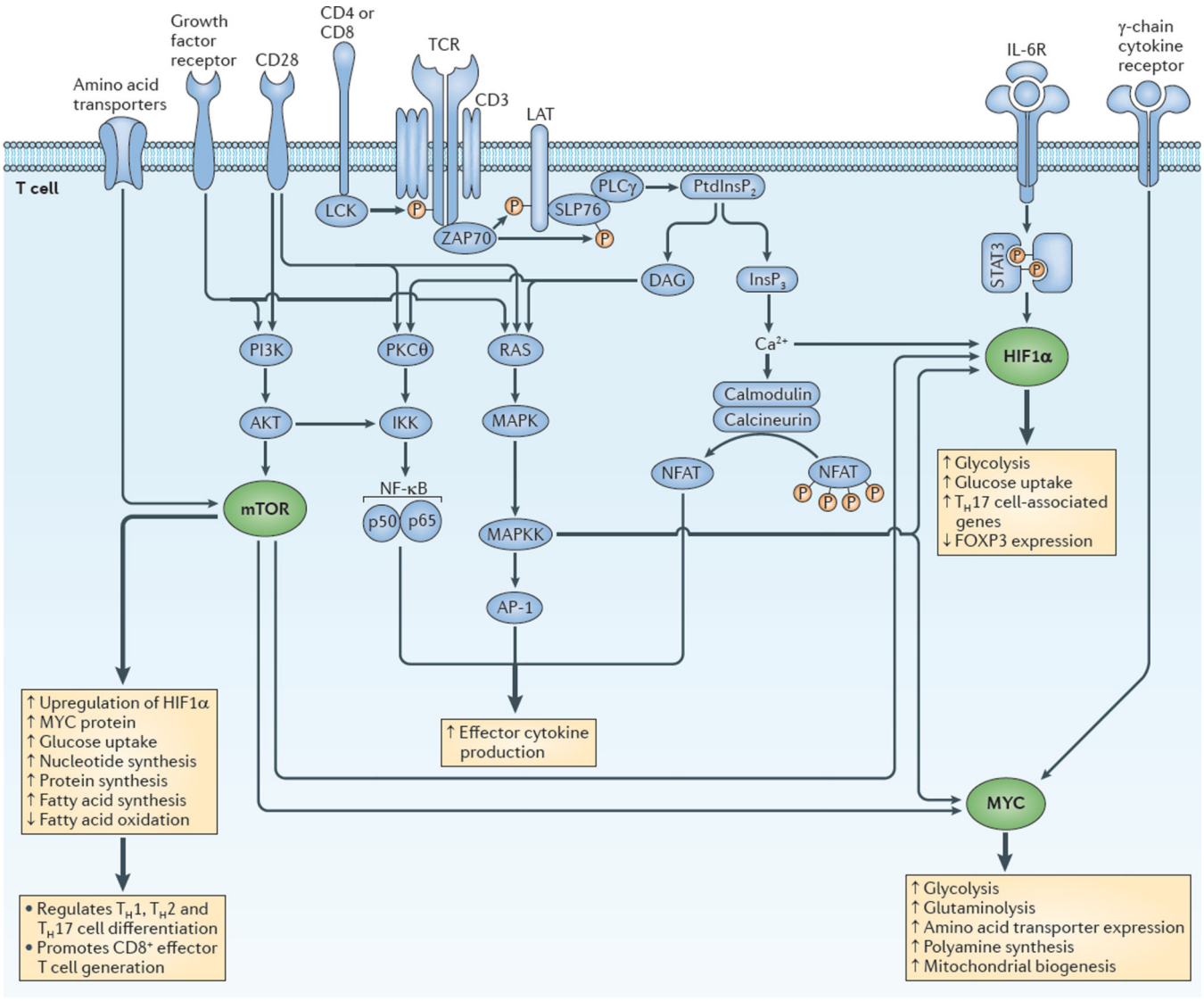

Figure 2. Integrating immunological and metabolic signalling programmes to promote effector T cell generation and function

The figure shows the coordinated integration of canonical T cell signalling (blue) and metabolic regulators (green) to promote the generation and function of effector T cells. In this perspective, hypoxia-inducible factor 1α (HIF1α) and MYC are just as integral to T cell effector generation as nuclear factor of activated T cells (NFAT), activator protein 1 (AP-1) and nuclear factor-κB (NF-κB). Similarly, mammalian target of rapamycin (mTOR) signalling is as crucial in effector T cell activation and differentiation as the activation of mitogen-activated protein kinase (MAPK), protein kinase Cθ (PKCθ) and calcineurin. Thicker arrows indicate the activation of metabolic programmes. Thinner arrows indicate signalling cascades. DAG, diacylglycerol; FOXP3, forkhead box P3; GLUT, glucose transporter; IKK, inhibitor of NF-κB kinase; IL-6R, interleukin-6 receptor; $InsP_3$, inositol-1,4,5-trisphosphate; LAT, linker for activation of T cells; MAPKK, MAPK kinase; PI3K, phosphoinositide 3-kinase; PKCθ, protein kinase Cθ; PLCγ, phospholipase Cγ; $PtdInsP_2$, phosphatidylinositol-4,5-bisphosphate; SLP76, SH2 domain-containing leukocyte

protein of 76 kDa (also known as LCP2); STAT, signal transducer and activator of transcription; TCR, T cell receptor; T_H cell, T helper cell; ZAP70, ζ-chain-associated protein kinase of 70 kDa.

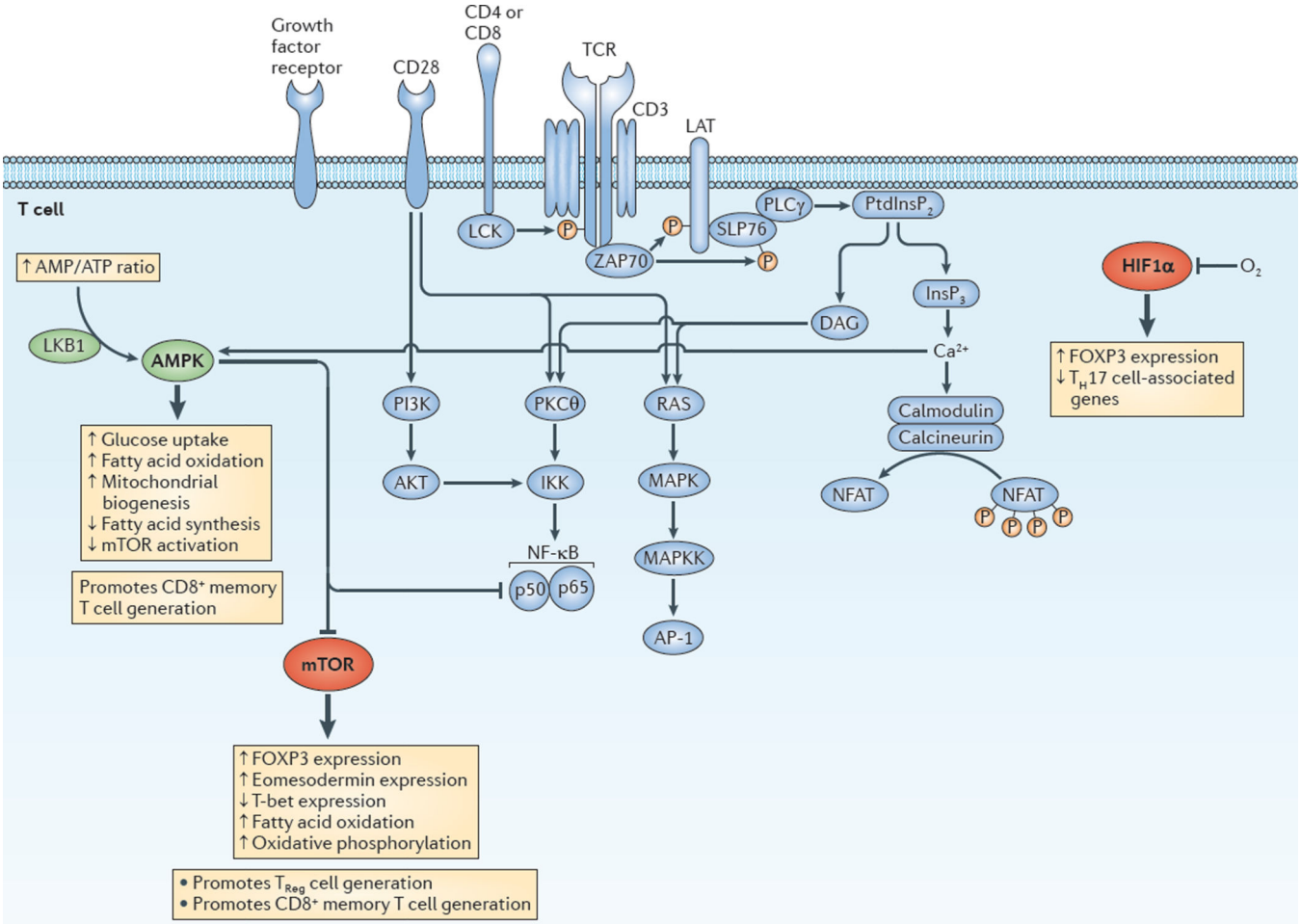

Figure 3. Integrating immunological and metabolic signalling programmes to promote CD8$^+$ memory and CD4$^+$ regulatory T cell generation

This figure depicts the integration of the canonical T cell signalling pathways (blue) and metabolic regulators (green for activated and red for inhibited). AMP-activated protein kinase (AMPK) activation promotes metabolic programmes that enhance the generation of memory and regulatory T (T_{Reg}) cells. Alternatively, it is the inhibition of mammalian target of rapamycin (mTOR) and hypoxia-inducible factor 1α (HIF1α) activation that promotes the generation of CD8$^+$ memory or CD4$^+$ regulatory T_{Reg} cells. From this perspective, memory T cells and T_{Reg} cells share similar metabolic requirements. Thicker arrows indicate the downstream consequences of AMPK activation, and of the inhibition of mTOR and HIF1α. AP-1, activator protein 1; DAG, diacylglycerol; FOXP3, forkhead box P3; IKK, inhibitor of NF-κB kinase; $InsP_3$, inositol-1,4,5-trisphosphate; LAT, linker for activation of T cells; LKB1, liver kinase B1; MAPK, mitogen-activated protein kinase; MAPKK, MAPK kinase; NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor-κB; PI3K, phosphoinositide 3-kinase; PKCθ, protein kinase Cθ; PLCγ, phospholipase Cγ; $PtdInsP_2$, phosphatidylinositol-4,5-bisphosphate; SLP76, SH2 domain-containing leukocyte protein of 76 kDa (also known as LCP2); TCR, T cell receptor; T_H cell, T helper cell; ZAP70, ζ-chain-associated protein kinase of 70 kDa.

Table 1

A summary of metabolic pathways and molecules

Metabolic process	Description	Substrate(s)	Crucial components
Glycolysis	• A metabolic process that results in the catabolism of six-carbon sugars into two molecules of pyruvate • The pyruvate can be converted into lactate or further catabolized in the TCA cycle	Glucose	• GLUT1, GLUT3 or GLUT4 (for glucose uptake) • Hexokinase 2 • Phosphofructokinase 1 • Pyruvate kinase • Lactate dehydrogenase A • PDK1
TCA cycle	A series of enzyme-catalysed chemical reactions that result in the reduction of NAD^+ molecules, which can be substrates of the electron transport chain during oxidative phosphorylation	Acetyl-CoA (synthesized from sugars, lipids or amino acids)	• Pyruvate dehydrogenase • Citrate synthase • α-ketoglutarate dehydrogenase
Oxidative phosphorylation	Oxidation of energy intermediates during the electron transport chain establishes a proton gradient across the mitochondrial inner membrane, which drives ATP synthesis	• NADH • $FADH_2$	• Oxygen
Fatty acid oxidation	Catabolism of fatty acids into acetyl-CoA, which can be further broken down in the TCA cycle for ATP synthesis in the electron transport chain	Fatty acids	• CPT1A
Glutaminolysis	• Catabolism of the amino acid glutamine for energy generation • Yields pyruvate or intermediates of the TCA cycle	Glutamine	• CD98 (for glutamine uptake) • Glutaminase • α-ketoglutarate dehydrogenase
Fatty acid synthesis	Anabolic process leading to the generation of fatty acids from acetyl-CoA and malonyl-CoA precursors	• Acetyl-CoA • Malonyl-CoA	• ACC1 • SREBPs

ACC1, acetyl-CoA carboxylase 1; CD98, a heterodimeric glutamine exchanger comprising SLC3A2 and SLC7A5; CoA, coenzyme A; CPT1A, carnitine palmitoyl-transferase 1A; GLUT, glucose transporter; PDK1, pyruvate dehydrogenase kinase 1; SREBPs, sterol regulatory element-binding proteins; TCA cycle, tricarboxylic acid cycle.

Table 2

Regulators of metabolism are also regulators of T cell differentiation and function

Regulator	Description	Metabolic function	T cell function
MYC	Transcription factor	• Drives cell proliferation, cell growth and apoptosis • Enhances glycolytic and glutaminolytic metabolism	• Activated upon TCR stimulation[11] • Required for TCR-induced proliferation and activation of T cells[11]
HIF1α	Transcription factor	Under hypoxic conditions, regulates gene expression necessary for survival in low oxygen primarily by promoting metabolic switch to glycolysis	• Activated upon TCR stimulation[11] • Necessary for T_H17 cell differentiation and survival[54–57] • Induces transcription of *Rorc* (which encodes RORγt) and mediates FOXP3 degradation[54,55] • Sustains cytotoxic response in $CD8^+$ T cells[75,76]
AMPK	Serine/threonine kinase	• Senses the intracellular AMP/ATP ratio • During low ATP levels, promotes ATP conservation by inhibiting cell cycle progression and mitochondrial biogenesis, and also regulates metabolic switch to catabolism	• Activated upon TCR stimulation[78] • Required for $CD8^+$ memory T cell generation[80,92]
mTOR	Serine/threonine kinase	Regulates cell growth, proliferation, survival and metabolic gene expression, resulting in enhanced glycolysis and lipid biosynthesis	• Activated upon TCR stimulation[75] • Regulates T_H1, T_H2, T_H17 and T_{Reg} cell differentiation[62–67] • Necessary for $CD8^+$ effector T cell generation[66,75]

AMPK, AMP-activated protein kinase; FOXP3, forkhead box P3; HIF1α, hypoxia-inducible factor 1α; mTOR, mammalian target of rapamycin; RORγt, retinoic acid receptor-related orphan receptor-γt; TCR, T cell receptor; T_H cell, T helper cell; T_{Reg} cell, regulatory T cell.