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REVIEW

Metabolic checkpoints in activated T cells

Ruoning Wang & Douglas R Green

The immunological process of clonal selection requires a rapid burst in lymphocyte proliferation, and this involves a metabolic shift to provide energy and the building blocks of new cells. After activation, naive and memory T cells switch from the oxidation of free fatty acids to glycolysis and glutaminolysis to meet these demands. Beyond this, however, the availability of specific metabolites and the pathways that process them interconnect with signaling events in the cell to influence cell cycle, differentiation, cell death and immunological function. Here we define 'metabolic checkpoints' that represent such interconnections and provide examples of how these checkpoints sense metabolic status and transduce signals to affect T lymphocyte responses.

As one of the most ancient functional properties of cells, metabolism is not only required for the fulfillment of all bioenergetic and biosynthetic demands but also actively integrated into the signaling cascades that dictate cellular fate. Understanding the dynamic interplay between the metabolic machinery and cellular signaling has emerged as a focus in the study of metabolic disorders, cancer and, most recently, the immune response. In this Review, we consider how signaling via the immune system integrates with metabolic programs to control immunological functions. This has been explored mainly in the context of metabolic reprogramming during the activation of T lymphocytes and, to a lesser extent, in other cell types of the immune system. We discuss how such changes in metabolism occur and their potential consequences in terms of 'metabolic checkpoints', which we define as molecular mechanisms that sense metabolic status and, in turn, regulate cellular functions. Understanding of such checkpoints holds the promise of novel manipulation of immune responses and therapeutic intervention under conditions in which metabolic dysfunction, such as metabolic disease, nutritional imbalance and cancer, affect immunological function.

Metabolic demands in T cells

As the central players in the adaptive immune response, T lymphocytes have evolved to rapidly respond to invading pathogens. This response occurs through several characteristic phases: a period of initial cell growth, followed by massive clonal expansion and differentiation, a contraction or death phase, and the establishment and maintenance of immune memory[1,2]. The T cell metabolic machinery is regulated for coordination of the transitions between these different phases[3].

During the initial growth phase, T cells undergo an activation-induced reprogramming of their metabolism, switching from the β-oxidation of fatty acids in naive T cells to the glycolytic, pentose-phosphate and glutaminolytic pathways in activated T cells[3–5] (**Fig. 1**). This phase, which lasts approximately 24 h after activation and precedes the first cell division, represents the engagement of biosynthetic machineries for the production of proteins, nucleic acids, lipids, carbohydrates and other 'building blocks' for the generation of new cells. The metabolic reprogramming associated with this growth phase is controlled mainly by the functions of the transcription factor c-Myc and the nuclear receptor ERRα[4,6,7]. In addition, pharmacological inhibition of phosphatidylinositol-3-OH kinase (PI(3)K) impairs the upregulation of glycolysis after CD28-mediated costimulation *in vitro*. This effect is probably due to the inhibition of cell-surface expression of the glucose transporter Glut1 dependent on the kinase Akt[6]. However, p85α and p110δ, the regulatory and dominant catalytic subunits of PI(3)K in T cells, seem dispensable for activation-induced proliferation of T cells *in vitro*, although *in vivo* proliferation in p110δ-defective T cells is impaired[8,9]. Finally, Akt signaling, a major downstream effector of PI(3)K, is dispensable for the maintenance of glucose uptake in proliferating cytotoxic $CD8^+$ T cells *in vitro*[10]. Such observations suggest that the PI(3)K-Akt pathway may not be generally essential in the metabolic reprogramming of T cells, although this has not been formally tested in T cells lacking all isoforms of PI(3)K.

As activated T lymphocytes begin to proliferate, the cells engage distinct transcriptional programs that drive them into functional subsets depending on the context (cytokines and other extracellular signals) in which they were activated. These subsets determine the nature of the immune response. Whereas $CD8^+$ T cells differentiate into cytotoxic T lymphocytes that kill host cells infected with pathogens, $CD4^+$ T cells differentiate into either induced regulatory T cells (iT_{reg} cells) that suppress uncontrolled immune responses or cells of the T_H1, T_H2 or T_H17 subset of helper T cells (effector T cells) that mediate appropriate immune responses[11,12]. After the clearance of pathogens, most clonally expanded and differentiated T cells undergo apoptosis in an abrupt contraction phase. The remaining antigen-specific T cells (memory T cells) are responsible for enhanced immunity after re-exposure to the pathogen[13]. Of these various T cell subsets, the iT_{reg} cells and memory T cells rely on lipid oxidation as a major source of energy, whereas cytotoxic T lymphocytes and effector T cells sustain high glycolytic activity and glutaminolytic activity[14–16]. However, the detailed metabolic profiles of differentiated and memory T cells remain to be explored.

Department of Immunology, St. Jude Children's Research Hospital, Memphis, Tennessee, USA. Correspondence should be addressed to R.W. (ruoning.wang@stjude.org) or D.R.G. (douglas.green@stjude.org).

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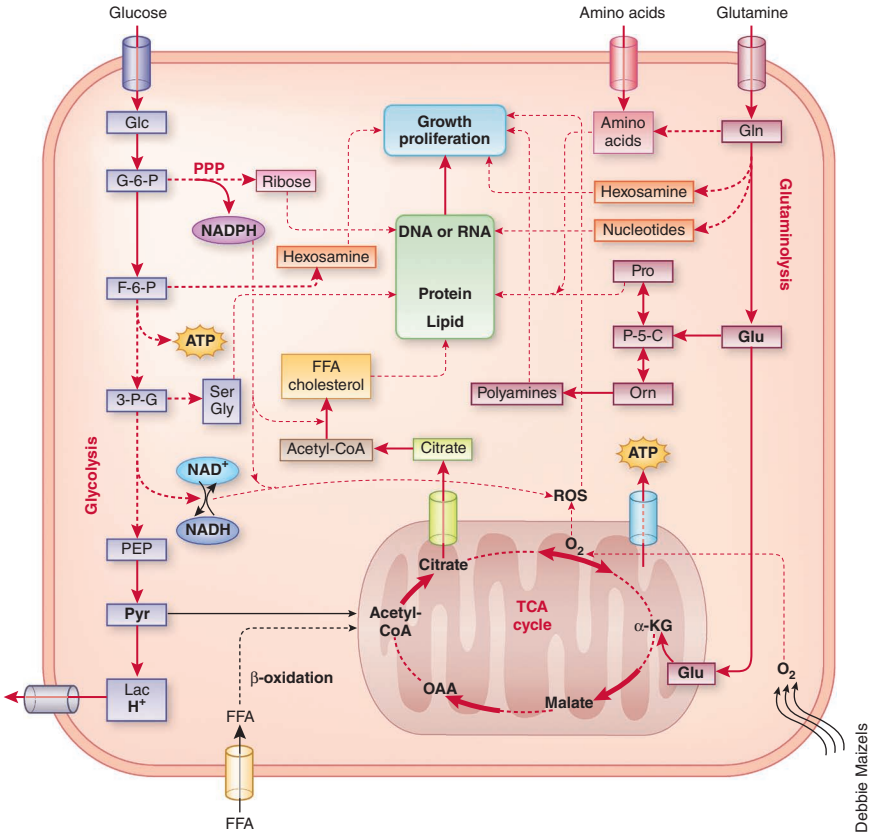

Figure 1 T cell metabolic reprogramming. In naive and memory T cells, mitochondria-dependent catabolic pathways, including glucose oxidation through the tricarboxylic acid (TCA) cycle and β-oxidation of fatty acids, provide most of the metabolic support for basic cellular functions. After T cell activation, β-oxidation rapidly decreases and other metabolic pathways (red), including glycolysis and glutaminolysis, increase. The glucose (Glc) catabolic pathway branches toward the production of NADPH and 5-carbon ribose (via the pentose phosphate pathway (PPP)) at glucose-6-phosphate (G-6-P) and detours toward lactate production (aerobic glycolysis) at pyruvate. The carbons of glucose are further diverted into various synthetic pathways to generate the precursors of hexosamines, amino acids (such as serine (Ser) and glycine (Gly)) and lipids via various metabolic interconnections. Meanwhile, mitochondria are fueled by the anapleurotic substrate α-ketoglutarate (α-KG), generated via glutaminolysis. Depending on the oxygen supply and the abundance of HIF-1α, α-ketoglutarate metabolizes in either a clockwise or counterclockwise manner through the tricarboxylic acid cycle (as presented here) to provide energy and a carbon resource for lipids, respectively. In addition, glutamine (Gln) serves as an important donor of carbon and nitrogen for the biosynthesis of hexosamines, nucleotides, amino acids and polyamines. Collectively, the metabolic reprogramming after T cell activation is optimized to support cell growth and proliferation by providing carbons and ATP. In functionally differentiated T cells, both $CD8^+$ cytotoxic T cells and $CD4^+$ effector T cells sustain high glycolytic activity, whereas $CD8^+$ memory T cells and $CD4^+$ T regulatory cells rely on the β-oxidation of fatty acids as a source of energy. F-6-P, fructose-6-phosphate; 3-P-G, glycerate-3-phosphate; Pyr, pyruvate; Lac, lactate; FFA, free fatty acids; OAA, oxaloacetate; PEP, phosphoenolpyruvate; P-5-C, 1-pyrroline-5-carboxylate; Glu, glutamate; Orn, ornithine.

The concept of metabolic checkpoints

The effective adaptive immune response requires T cells to function in various microenvironments, including hostile metabolic conditions. Meanwhile, immunological signals actively instruct the intracellular metabolic programs and adjust the metabolic state of T cells to adapt to changes in extracellular oxygen and nutrient supply or disruption of the intracellular metabolic machinery. By analogy to the concept of cell-cycle and DNA-damage checkpoints[17,18], we consider such adaptations as consequences of 'metabolic checkpoints'. These are composed of the following four components: metabolic signals, sensors of those signals, signal transducers and molecular effectors of the checkpoint (**Fig. 2** and **Table 1**). The biological consequences of engaging such checkpoints in the immune system include not only changes in metabolic function but also effects on cell cycle, differentiation, cell death and immunological functions.

Metabolic signals reflect changes in the extracellular nutrient environment or intracellular metabolic status. Such signals include metabolites involved in cell metabolic pathways or metabolic products, byproducts and cofactors such as ATP, $NADP^+$-NADPH, acetyl-CoA and reactive oxygen species (ROS). This is fundamentally different from the concept of second messengers, such as cAMP-cGMP and phosphoinositides, which are not primary signals but instead are products of upstream signaling events.

The sensors of a metabolic checkpoint are proteins that physically interact with and respond to metabolic signals by changes in their biological status and consequently initiate downstream signaling events. Of note, the Michaelis constant (K_m) of any proposed metabolic sensor for its sensed biomolecules must be in the physiological range of the bioavailability of those biomolecules. However, direct experimental evidence in support of such a requirement has in many cases been largely absent because of the difficulties in quantifying the biomolecules. Unicellular organisms such as bacteria and yeast sense and respond to extracellular nutrients through cell-surface receptors and transporters[19–21]. The physical interactions among nutrients, receptors and transporters trigger a series of intracellular signaling events that result in adaptive cellular responses. Although it is possible that higher organisms use similar mechanisms to sense metabolic status and mediate signaling events, direct evidence for this is lacking. Concrete examples of true metabolic sensors are discussed below (summary in **Table 1**).

The subsequent stage of a metabolic-checkpoint response involves the engagement of components of signal-transduction pathways and their downstream effectors that elicit the appropriate cellular responses, including metabolic 'rewiring', cell growth, proliferation, death and differentiation. Although many examples of translating metabolic signaling to cellular response have been described in other cellular systems and are reviewed elsewhere[22], here we focus on the mechanisms that have been demonstrated to be relevant to T cell function and adaptive immune responses.

The HIF-1α checkpoint

The cellular and physiological responses to changes in oxygen concentrations involve an immediate adaptive response to regulate oxygen homeostasis, followed by a signaling response to modulate various

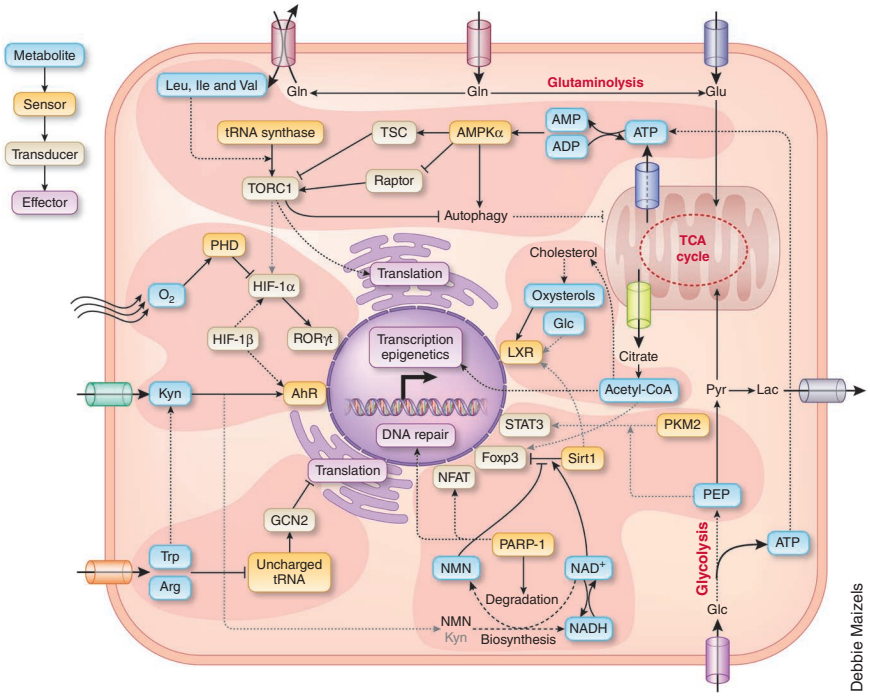

Figure 2 Metabolic checkpoints in T cell function. Metabolic checkpoints are cellular mechanisms that ensure the accurate 'translation' of a cell's metabolic status into a proper cellular response and are composed of metabolic signals, sensors, transducers and effectors. AMPK and TORC1 coordinate the sensing of intracellular amino acids and ATP, and regulate autophagy, protein translation and probably HIF-1. GCN2 represents another amino-acid checkpoint and directly controls protein translation. The tryptophan-derived metabolite Kyn serves as an endogenous ligand of AhR, which may interact with HIF-1 and coordinately direct T_H17 differentiation. Acetyl-CoA, the precursor of cholesterol, indirectly 'instructs' LXR activity and directly regulates epigenetics via protein acetylation. As an NAD-dependent deacetylase, Sirt1 may suppress the differentiation of T_{reg} cells by modifying Foxp3. PARP-1, an NAD-consuming enzyme, may also interact with Sirt1 and serve as an NAD checkpoint. Finally, the glycolytic enzyme PKM2 may use PEP, which is also its glycolytic substrate, as a phosphate donor to modify its putative substrate STAT3, thus potentially acting as a checkpoint that responds to PEP concentrations. NMN, nicotinamide mononucleotide.

cellular processes required for cell survival and specific functions. The former effect probably depends on cellular oxygen-sensing mechanisms mediated through NADPH oxidase and the electron carriers of the respiratory chain, whereas the latter involves prolyl-4-hydroxylase (PHD) proteins as sensors that connect oxygen concentration to downstream cell signaling events[23]. Under conditions of sufficient oxygen, PHD hydroxylates hypoxia-induced factor 1α (HIF-1α), which leads to its degradation. Under conditions of low oxygen, HIF-1α is stabilized. This allows it to associate with HIF-1β to generate the transcription factor HIF-1 and the transcription of HIF-1-targeted genes. HIF-1β also interacts with the transcription factor AhR, as outlined below, which adds further complexity to the regulation of HIF-1. The targets of HIF-1 include genes encoding effectors that enhance glycolysis and promote angiogenesis and thus remodel both intrinsic cellular metabolic programs and extrinsic microenvironments[24,25].

Given its relatively high K_m for oxygen[26,27], PHD is an excellent sensor of oxygen. However, PHD-mediated hydroxylation also consumes α-ketoglutarate to produce succinate, both of which are intermediate metabolites in the tricarboxylic acid (TCA) cycle[24]. Therefore, the oxygen-sensing mechanism may also be under the influence of the mitochondria-dependent carbohydrate catabolic pathway. The activity of PHD is inhibited by higher intracellular concentrations of succinate and also by mitochondrial production of ROS[28,29]. It is therefore conceivable that the HIF-1α checkpoint represents a signaling hub that is 'instructed' by many metabolic inputs and contributes to different types of metabolic responses.

T cells differentiate and function in various microenvironments, in which they are exposed to a wide range of local oxygen tension from high (normoxia) to low (hypoxia)[30,31]. Therefore, it is likely that the function of T cells in hypoxic environments is dependent on HIF-1α. During T cell differentiation, HIF-1α promotes glycolysis in differentiating T_H17 cells and reciprocally increases T_H17 differentiation and decreases iT_{reg} differentiation *in vitro* and *in vivo*[14,32]. In addition, HIF-1α also directly enhances activity of the transcription

Table 1 Metabolic checkpoints in T cell differentiation

Metabolic perturbation	Nutrient-sparse microenvironment (such as tumors or inflamed sites) or low-protein diet	Low-oxygen microenvironment (such as secondary lymphoid organs)	IDO-, TDO- and Arg-1–expressing microenvironment (such as tumors or other immunosuppressive environments)	Calorie restriction and/or fasting
Metabolic signals	Amino acids (–) and AMP/ATP (+)	Oxygen (–)	Kyn (+) Arg (–) Trp (–)	NAD^+/NADH (+)
Sensors	Leucyl tRNA synthase (–) and AMPK (+)	PHD (–)	AhR (+) Uncharged tRNA (+)	Sirt1 (+)
Transducers	TORC1 (–)	HIF-1α (+)	AhR (+) and GCN2 (+)	Sirt1 (+)
Effectors	HIF-1α (–), autophagy (+), protein translation (–), glycolysis (–) and FAO (+)	RORγt (+), Foxp3 (–) and glycolysis (+)	IL-17 (+), protein translation (–) and metabolic effectors (?)	Foxp3 (+) and metabolic effectors (?)
T cell fate (differentiation)	Memory T cell differentiation (+?) and T_{reg} cell differentiation (+)[16,62]	T_H17 cell differentiation (+) and T_{reg} cell differentiation (–)[14,32]	T cell activation (–), T_{reg} cell and T_H17 cell differentiation (– or +?)[68–72,74–76,78]	T_{reg} cell differentiation (+)[114,115]

Metabolic checkpoints influence T cell function and differentiation. (+) or (–) indicate positive or negative influences, respectively, on metabolites, enzyme activities and cellular processes. FAO, β-oxidation of fatty acids.

factor RORγt and represses activity of the transcription factor Foxp3 as a direct molecular effector mechanism of this metabolic checkpoint[32] (**Table 1**). In addition to hypoxia, antigen stimulation or T_H17-polarizing cytokines substantially enhance HIF-1α expression even under conditions of normoxia[14]. This regulation may be achieved either through a mechanism dependent on the TORC1 protein complex (discussed below) or through the action of PHD, via ROS and intermediate metabolites of the tricarboxylic acid cycle, such as succinate and α-ketoglutarate (as discussed above). However, HIF-1α is dispensable for T cell development[14,33]. In addition, HIF-1α-deficient T cells produce more proinflammatory cytokines after T cell activation for reasons that are unclear at present[34].

The AMPK-TORC1 checkpoint

Two evolutionary conserved signaling molecules, AMPK and mTOR, are central players in the coordinated sensing of cellular metabolic state and dictation of cell fate[35–38]. AMPK is an αβγ heterotrimer whose activation requires both the binding of AMP-ADP to the γ-subunit and phosphorylation of the α-catalytic subunit by the upstream signaling kinases LKB1 and CaMKKβ. Whereas AMP serves as a potent allosteric activator of AMPK, both AMP and ADP promote and stabilize the activating phosphorylation of AMPK. Given its relatively high affinity for AMP-ADP, AMPK is generally considered a sensor of the intracellular concentration of AMP and ADP, which is indicative of bioenergetic status[37,38].

Extracellular growth factors and nutrients converge on the regulation of mTOR, a component of two functional multicomponent protein complexes, TORC1 and TORC2. Activation of the protein-kinase activity of TORC1 requires the derepression of TSC1-TSC2, a heterodimeric inhibitory component of the complex, and the recruitment of GTPases of the Rag family. A leucyl-tRNA synthetase–dependent amino acid–sensing mechanism determines the activation of TORC1 via Rag GTPases[39,145]. Growth factor signals upstream of TORC1 converge on the TSC1-TSC2 complex, which is phosphorylated and inhibited largely through a PI(3)K-Akt–dependent mechanism, thereby promoting TORC1 activity[36]. In addition, mTOR has been postulated to be a sensor of ATP because of its reported high millimolar K_m for ATP, which is at odds with the fact that most protein kinases have a micromolar K_m for ATP[40]. A concentration of ATP in the micromolar range has been suggested to be sufficient for mTOR-mediated phosphorylation of its substrates[41,42]. Nevertheless, when ATP is limiting (and in the presence of high concentrations of AMP and ADP), AMPK directly phosphorylates essential components of TORC1, such as TSC2 and raptor[43,44]. This generally leads to inhibition of TORC1 activity. Therefore, TORC1 is known as a central signal transducer that functions in metabolic checkpoints by integrating both amino acid– and ATP-sensing pathways to determine cell fate[36].

Emerging evidence demonstrates that immunological signals actively regulate AMPK and TORC1 and consequently direct T cell–mediated immune responses. After T cells are activated, Ca^{2+} signaling quickly engages activation of AMPK[45,46]. AMPKα-deficient $CD8^+$ T cells have higher glycolytic activity and produce more inflammatory cytokines than wild-type T cells do *in vitro*, but AMPKα-deficient $CD4^+$ T cells do not, which indicates AMPK is a negative regulator of T cell activation, presumably through inhibition of TORC1 (ref. 47). Intriguingly, iT_{reg} cells have enhanced phosphorylation of AMPK, indicative of its activation, and pharmacological activation of AMPK promotes the development of iT_{reg} cells in an asthma model *in vivo*[15]. However, AMPK is dispensable for the proliferation of T cells and the cytotoxic effector function of $CD8^+$ T cells *in vivo*[46]. This suggests that the function of AMPK in T cell is dependent on the cellular context. Although CaMKK has been suggested to be the upstream activating kinase of AMPK[45], T cell–specific deletion of LKB1 also results in a defect in AMPK activation after T cell activation[47]. However, T cell–specific deletion of LKB1 results in the impairment of thymocyte development and fewer peripheral T cells, but T cell–specific deletion of AMPK does not. This phenotypic discrepancy indicates an AMPK-independent function for LKB1 in T cells[47]. Mechanistically, the enhanced TORC1 activity in LKB1- or AMPKα-deficient T cells suggests that LKB1-AMPK signaling may negatively regulate the effector function of T cells through inhibition of TORC1 signaling[47]. Consistent with that, functional T cell immune responses require intact TORC1 signaling, and the inhibition of mTOR activity by rapamycin leads to T cell anergy after activation[48,49].

Nevertheless, it may be that hyperactive TORC1 also alters T cell activation and function. T cells that lack TSC1 have enhanced TORC1 activity, as expected, but do not generate effective immune responses[50]. This effect, however, seems to manifest only slowly after ablation of TSC1 and may reflect more complex events as a consequence of constitutive TORC1 activity.

Downstream of the AMPK-mTOR pathway, macroautophagy has an essential role in the maintenance of cellular metabolic homeostasis by degrading cytoplasmic material to provide internal nutrients and clearing damaged mitochondrial to control mitochondrial quality[51]. AMPK and TORC1 directly phosphorylate the mammalian autophagy-initiating kinase Ulk1 at different sites, which results in the activation and inhibition of macroautophagy, respectively[52–54]. Moreover, mTOR also targets the autophagy regulator Atg13 to suppress macroautophagy[55,56]. Consistent with rapid activation of AMPK, macroautophagy is rapidly engaged in T lymphocytes after antigenic stimulation[57–59]. T cell–specific deletion of any of the autophagy-related molecules Atg3, Atg5 or Atg7 results in defects in survival and proliferation after antigenic stimulation of T cells. These defects may be due to the accumulation of damaged intracellular organelles such as mitochondria and endoplasmic reticulum and may also be related to the defects associated with the ablation of TSC1 noted above[59,60].

However, the requirement for AMPK and macroautophagy activity is at odds with the concomitant requirement for mTOR activity in T cells after antigenic stimulation. This discrepancy suggests that the AMPK-mTOR-macroautophagy axis is regulated in a dynamic and temporal manner after antigenic stimulation. In support of that idea, initially transient inhibition of mTOR activity followed by an increase in mTOR activity is necessary for the population expansion of iT_{reg} cells *in vivo*[61]. Notably, either pharmacological activation of AMPK or T cell–specific deletion of mTOR is sufficient to drive T cell differentiation toward iT_{reg} cells after antigen stimulation[47,62]. However, mTOR activity is absolutely required for the differentiation of effector T cells. In particular, TORC1 promotes T_H1 and T_H17 differentiation, whereas TORC2, which differs from TORC1 in both regulation and effects, promotes T_H2 differentiation[63,64]. Finally, restraining TORC1 activity enhances the differentiation of memory T cells and is required for the maintenance of T cell quiescence, possibly through enhancement of the oxidation of fatty acids[50,65].

Additional downstream effectors of TORC1 include regulators of cell metabolism, cell growth, cell differentiation, cell proliferation and death. TORC1 controls the translation of proteins through regulation of the translation-initiation factor eIF4E and S6 kinase[36]. Another effector is HIF-1α (discussed above), which is stabilized in a TORC1-dependent manner during T_H17 differentiation[14]. The sustained upregulation of c-Myc is also dependent on TORC1 after T cell activation[4]. The metabolic processes controlled by c-Myc and HIF-1α via a transcriptional increase in metabolic enzymes in the glycolytic

and glutaminolytic pathway can further influence AMPK-mTOR signaling, forming feed-forward regulatory loops[4,14]. Collectively, the metabolic checkpoint imposed by AMPK-TORC1 has an instructive role in integrating immunological signals and many metabolic inputs to direct T cell fate and immunological function (**Table 1**).

The GCN2 checkpoint

One of the earliest events after amino-acid starvation is the accumulation of uncharged tRNA, which binds the serine-threonine kinase GCN2 and activates its kinase activity. Subsequently, phosphorylation of the translation-initiation factor eIF2 suppresses global protein synthesis and limits the consumption of amino acids while enhancing translation of the gene encoding GCN4, which results in the transcription of genes encoding metabolic molecules required for the biosynthesis of amino acids[66,67].

Under some conditions, tumor cells and cells of the immune system, such as dendritic cells and macrophages, express the amino acid–catabolic enzymes IDO, TDO and Arg-1. As a result, the depletion of extracellular tryptophan and arginine leads to the activation of GCN2 and consequently inhibits T cell function[68–70]. In addition to regulation of amino-acid homeostasis by the adaptive response, the activation of GCN2 by amino-acid deprivation in T cells inhibits T_H17 differentiation and promotes T_{reg} cell development and T cell anergy[71,72]. Intriguingly, a low-protein diet, which would potentially diminish the circulating pool of amino acids, results in less homeostatic proliferation of $CD8^+$ memory T cells and an impaired recall response[73]. However, the downstream molecular mechanism for these effects remains unclear.

Nuclear receptor–mediated metabolic checkpoints

The nuclear-receptor superfamily is a group of transcription factors critically involved in the regulation of metabolic and inflammatory programs in T cells. Many of their endogenous ligands have been identified as metabolites and, therefore, this superfamily may direct the immunological responses of T cells by integrating both local metabolic signals and immunological signals.

One example of this is the aryl hydrocarbon receptor (AhR), which is an important ligand-dependent regulator of the differentiation of T_H17 and T_{reg} cells[74–76]. Its endogenous ligand has been identified[77,78]. As described above, tumor cells, macrophages and dendritic cells can have relatively high expression of the tryptophan catabolic enzymes IDO and TDO. This can result in the depletion of tryptophan and accumulation of the tryptophan catabolite kynurenine (Kyn) in T cell microenviroments. Kyn is an endogenous ligand of human AhR that is produced by human tumor cells through the tryptophan catabolic reaction mediated by TDO[78]. Tumor-derived Kyn directly suppresses T cell–mediated antitumor immune responses and consequently promotes tumor progression. Given that finding, it remains to be determined whether dendritic cells and macrophages that express IDO and TDO may 'instruct' the differentiation of T_H17 and T_{reg} cells by regulating T cell–intrinsic AhR signaling (**Table 1**).

LXR is another member of the nuclear-receptor superfamily; it has important roles in regulating lipid and cholesterol metabolism[79,80]. The cholesterol derivatives oxysterols represent a major group of its endogenous ligands that act in the metabolic feedback regulation of LXR[81,82]. In addition, glucose and its derivative glucose-6-phosphate have been shown to directly interact with and activate LXR to an extent similar to that of other known LXR ligands in liver[83]. This raises the intriguing idea that LXR serves as a sensor of glucose. However, the downstream target of LXR, the transcription factor ChREBP, but not LXR itself, may in fact be required for the induction of glucose-regulated genes in hepatocytes[84]. The role of ChREBP or LXR in sensing glucose in T cells remains to be tested.

Although the function of ChREBP in T cells remains unclear, LXR-mediated signaling not only suppresses cell proliferation after T cell activation but also negatively affects T_H17 differentiation[85,86]. Mechanistically, the LXR-targeted gene encoding the transcription factor SREBP-1 binds to AhR and consequently inhibits AhR-driven transcription of the gene encoding interleukin 17 (IL-17)[86]. In addition, AhR forms a heterodimer with HIF-1β, which dimerizes with HIF-1α to elicit HIF-1 cellular functions (discussed above). Therefore, crosstalk among LXR, AhR and HIF-1 might occur in some cellular contexts[87]. Although it has not been confirmed in T cells, both LXR and HIF-1α are reported to be substrates of the protein deacetylase Sirt1 (discussed below), which suggests the existence of another layer of crosstalk between various metabolic checkpoints. Whereas Sirt1-mediated deacetylation enhances the function of LXR, it suppresses HIF-1 activity[88,89]. Given that AhR, HIF-1, LXR and Sirt1 are all involved in regulating the differentiation of T_H17 and T_{reg} cells, it is conceivable that the interplay among these molecules represents a layer of complexity in the response of T cells to various metabolic signals. They may either function in a competitive manner or work in concert to synergistically regulate the differentiation of T_H17 and T_{reg} cells, depending on the nature of the immunological signaling and the metabolic environment.

Protein acetylation as a metabolic checkpoint

Evidence suggests that the availability of acetyl-CoA and NAD^+ modulates protein acetylation[22]. This may represent another major metabolic checkpoint in cells. Acetyl-CoA provides the acetyl group required for protein acetylation mediated by histone acetyltransferases, whereas the conversion of NAD^+ to nicotinamide is coupled with deacetylase (Sirtuin)-mediated protein deacetylation[90,91]. Protein acetylation is one of the most common post-translational modifications and influences almost every aspect of cell physiology and pathology[92]. One form of protein acetylation is lysine acetylation, which is reversibly regulated by protein acetyltransferases such as histone acetyltransferases and deacetylases, including HDAC and Sirtuin[91]. Whereas histone acetylation functions as an essential epigenetic regulator that dictates cellular transcriptional machinery, the acetylation of non-histone proteins has been suggested to regulate various cellular processes, including metabolic pathways[93].

Acetyl-CoA is present in various cellular compartments, and its intracellular concentration largely reflects the metabolic state of the cell[94]. The mitochondrial pool of acetyl-CoA is abundant and is derived mainly from the catabolic flux of glucose, glutamine and fatty acids. However, citrate or acetate are the main precursors for the cytosolic pool of acetyl-CoA. Whereas cytosolic citrate is shuttled from mitochondria, the carbon source that generates acetate remains unclear. Extramitochondrial acetyl-CoA is not only the precursor of lipogenesis but also provides the acetyl moiety for the acetylation of cytosolic and nuclear proteins[22]. This has led to the idea that the extramitochondrial concentration of acetyl-CoA may influence protein acetylation. However, the question of whether protein acetyltransferases are sensitive to changes in acetyl-CoA concentration within a physiological range remains to be clarified.

As one of the essential redox pairs, NAD^+-NADH is tightly linked to many metabolic reactions and therefore is often suggested as both a 'readout' and a determinant of the metabolic state of a cell. Whereas the intracellular ratio of NAD^+ to NADH is estimated to be in a wide range, from 0.1 to 500, the intracellular NAD^+ concentration is in a narrow, low millimolar range[95]. Of note, protein-bound NAD^+ and

the cellular compartmentalization of NAD^+, especially the mitochondrial NAD^+ pool, may influence the estimation noted above. Nevertheless, some members of the Sirtuin family are reported to have a high K_m for NAD^+ approximately equal to the physiological intracellular concentration of NAD^+. This supports the idea that NAD^+ is rate-limiting for Sirtuin enzymatic reactions, and as a result, Sirtuin proteins may serve as metabolic sensors of intracellular NAD^+ and the redox state[96,97]. Intracellular NAD^+ concentrations are also tightly balanced through biosynthesis and degradation. Whereas in the liver and kidneys NAD^+ is synthesized mainly from tryptophan through the *de novo* pathway, T lymphocytes seem to exclusively rely on the salvage pathway and use nicotinamide or nicotinic acid (vitamin B3) as a precursor[98–100]. Of note, nicotinamide may also inhibit Sirtuin enzymatic activity as an endogenous end-product inhibitor. As a donor of ADP-ribose, the cellular NAD^+ content can be rapidly depleted by poly(ADP-ribose) polymerases (PARPs), especially PARP-1, under some conditions[101–104]. Consistent with that, PARP-1 has a very low K_m for NAD^+ (ref. 105), which puts it in a position to compete with Sirtuin proteins for the cellular NAD^+ pool[106]. After T cell activation, PARP-1 activity is increased and modulates the transcription factor NFAT[107–109]. This raises the possibility that higher PARP-1 activity in activated T cells also affects Sirtuin function by competing for cellular NAD^+.

After antigenic stimulation, the metabolic programs of T cells result in an increase in cytosolic NAD^+ and citrate, the precursor of acetyl-CoA[4,107]. These changes may serve as metabolic cues that direct T cell fate through the regulation of protein acetylation. Consistent with that, antigenic stimulation engages a dynamic change in histone acetylation in some cytokine-encoding loci in T cells, which may both promote their early-phase transcription and direct the expression patterns of lineage-specific cytokine-encoding genes during T cell differentiation[110,111]. In contrast, the transcription factor Foxp3 represents an emerging non-histone target of acetyltransferases and deacetylases in T cells. Whereas the histone acetyltransferase TIP60 forms a complex with Foxp3 and is required for Foxp3-mediated transcriptional repression[112], the histone acetyltransferase p300 promotes the acetylation of Foxp3 and enhances its protein stability[113]. Conversely, deacetylation may negatively affect Foxp3. In support of that idea, either the pharmacological inhibition of deacetylase or T cell–specific deletion of Sirt1 substantially promotes the generation and function of $Foxp^+$ T_{reg} cells *in vitro* and *in vivo*[114,115]. However, the possibility that histone acetyltransferases and Sirt1 regulate T_{reg} cell development at the epigenetic level cannot be excluded[116]. Following on the considerations noted above, deletion of PARP-1 in mice results in enhancement of the development and differentiation of $Foxp3^+$ T_{reg} cells in central and peripheral tissues[117] and induces the expression of genes encoding molecules involved in T_H1 and T_H2 differentiation[118]. Consistent with that, inhibition of PARP-1 confers protection against experimental autoimmune encephalomyelitis[119,120]. However, it remains unclear whether such protection is due to a T cell–intrinsic effect.

Sirt1 is also involved in maintaining T cell tolerance, and its expression is induced considerably in anergic T cells[121–123]. Mechanistically, the transcription factor Foxo3a works in concert with the transcription factors Egr2 and Egr3 to promote transcription of the gene encoding Sirt1 after T cell activation. Conversely, IL-2-mediated activation of the PI(3)K-Akt pathway results in the sequestration of Foxo3a in the cytoplasm and, consequently, suppression of transcription of the gene encoding Sirt1. This may partially explain how IL-2 reverses T cell anergy[123]. However, neither the metabolic signals upstream of Sirt1 nor the molecular mechanism that mediates the downstream effects of Sirt1 in these contexts are clear. Nevertheless, such findings suggest that if the availability of acetyl-CoA and NAD^+ affects the acetylation of proteins, such availability would have important consequences for T cell function. In support of that proposal, caloric restriction or fasting, whose physiological effects are manifested mainly via Sirtuin proteins and protein acetylation[124], is beneficial for T cell–dependent immune responses in physiological or pathological settings[125–130].

Other potential metabolic checkpoints

Similar to protein acetylation, almost all forms of post-translational modification, including phosphorylation, glycosylation, methylation, lipidation, nitrosylation and ROS-mediated covalent modification, are directly involved in the transfer of various metabolites as molecular moieties onto protein substrates. This raises the intriguing possibility that post-translational modification may be part of a general metabolic checkpoint. Except for nitrosylation and ROS-mediated covalent modification, many of the enzymatic activities that mediate post-translational modification generally require metabolites at much lower concentrations than their normal amount. For example, most protein kinases have a K_m for ATP of 10–20 μM (ref. 131), whereas intracellular ATP has a concentration in the low millimolar range[132]. Therefore, whether changes in the concentrations of such metabolites in the physiological range can influence post-translational modification is not clear.

However, many metabolites are not uniformly distributed in cells because of highly compartmentalized metabolic pathways[133]. The concentration of metabolites measured in whole cells or tissues represents an average cellular concentration but not necessarily a concentration that is detected by a metabolic sensor. Thus, 'preferential' partitioning of metabolites into certain subcellular domains may trigger a compartmentalized metabolic checkpoint.

One example of this is protein glycosylation, which has an essential role in the regulation of protein trafficking in the Golgi apparatus and the endoplasmic reticulum. This is tightly coupled with the glucose and glutamine catabolic pathways that provide two key metabolic elements required for glycosylation: the sugar moieties (nucleotide-sugar donors) and ATP (energy). Consistent with that, the availability of nucleotide sugars and ATP in endoplasmic reticulum directly regulates the glycosylation of cell-surface receptors and metabolic flux[134,135]. Similarly, mitochondria, which represent the cellular powerhouse and a major intracellular signaling hub, may elicit various forms of compartmentalized metabolic checkpoints through the regulation of ATP and ROS production and the shuttling of various metabolites between mitochondria and the cytosol.

A growing body of evidence suggests that enzymes in the metabolic pathways also can function in signaling. These enzymes probably transduce metabolic signals to the downstream signaling pathway. One such candidate is PKM2, which has high expression in embryonic tissues, tumors and activated T cells[4,136,137]. PKM2 exists in a dimeric form and a tetrameric form; these forms determine not only its enzymatic activity but also its subcellular localization. The active tetramer has a high affinity for its substrate, phosphoenolpyruvate (PEP), and localizes mainly to the cytosol, whereas dimeric PKM2 'preferentially' localizes in the nucleus and has a low affinity for PEP. In the nucleus, dimeric PKM2 may directly interact with the transcription factors β-catenin and HIF-1 and promote their transactivation. Despite its relatively low affinity for PEP, dimeric PKM2 is able to use PEP as a phosphate donor and catalyzes the *in vitro* phosphorylation of some protein targets, including the transcription factor STAT3 (refs. 138–140).

Notably, the concentration of PEP required for protein phosphorylation is probably in the range of the physiological concentration of PEP[139]. The tetramer/dimer ratio and the enzymatic activity of PKM2 are controlled by cellular ATP, ROS, fructose-1,6-P and serine and also by direct interaction with signaling proteins[141–144]. Collectively, these findings suggest that the signaling function of PKM2 is intrinsically linked to its enzymatic function, and such functional interconnection may help to directly couple metabolic signals to the downstream signal-transduction pathways. The function of PKM2 as a metabolic sensor in T cells remains unexplored at present.

Conclusions and perspectives

Among the unanswered questions about metabolic checkpoints is how and when T cells terminate the signaling events initiated by the metabolic signals. We consider several possible scenarios that are not mutually exclusive. T cells may be triggered to migrate from a nutrient- and/or oxygen-deficient environment to a nutrient- and/or oxygen-sufficient environment. However, whether such migration is under the control of metabolic signals remains to be tested. A second scenario involves metabolic reprogramming. Many metabolic sensors and transducers, including TORC1, AMPK and Sirt1, can directly or indirectly 'rewire' the metabolic pathways to relieve the metabolic signals upstream of them. Similarly, either blocking the cellular processes that consume metabolites or enhancing the cellular processes that recycle metabolites may serve as an alternative means of regulating metabolic signals via feedback. One example of this is the control of autophagy, which after being engaged can generate ATP that decreases AMPK activity and increases TORC1 activity. Another is the GCN2-medated suppression of protein translation, a major energy- and amino acid–consuming process, which allows the cell to replenish the amino-acid pool and render GCN2 inactive. Finally, the scenarios described above may simply represent different forms of feedback regulatory loops, which would further reinforce the metabolic checkpoint response and T cell fate 'decisions'.

The function of metabolic checkpoints in the immune system is an emerging area of investigation. The metabolic sensors and transducers we have proposed obviously have signaling functions that are independent of their roles in mediating metabolic checkpoints. This may represent a general feature of the crosstalk between metabolic checkpoints and other signaling pathways. Crosstalk between different metabolic checkpoints and other stress-mediated checkpoints may represent additional, emerging signaling nodes. For fuller understanding of the underlying complexity of metabolic checkpoints, new techniques and methodologies are warranted. These include but are not limited to the *in situ* quantitative measurement of intracellular small molecules, live-cell imaging of intracellular metabolites and the physical interaction between metabolites and proteins through the use of fluorescent biosensors, and cell biology approaches for manipulating the concentrations of intracellular metabolites within physiological ranges.

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1. Schumacher, T.N., Gerlach, C. & van Heijst, J.W. Mapping the life histories of T cells. *Nat. Rev. Immunol.* **10**, 621–631 (2010).
2. Green, D.R. Overview: apoptotic signaling pathways in the immune system. *Immunol. Rev.* **193**, 5–9 (2003).
3. Wang, R. & Green, D.R. The immune diet: meeting the metabolic demands of lymphocyte activation. *F1000 Biology Reports* **4**, 5–9 (2012).
4. Wang, R. *et al.* The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. *Immunity* **35**, 871–882 (2011).
5. Gerriets, V.A. & Rathmell, J.C. Metabolic pathways in T cell fate and function. *Trends Immunol.* **33**, 168–173 (2012).
6. Frauwirth, K.A. *et al.* The CD28 signaling pathway regulates glucose metabolism. *Immunity* **16**, 769–777 (2002).
7. Michalek, R.D. *et al.* Estrogen-related receptor-α is a metabolic regulator of effector T-cell activation and differentiation. *Proc. Natl. Acad. Sci. USA* **108**, 18348–18353 (2011).
8. Okkenhaug, K. *et al.* Impaired B and T cell antigen receptor signaling in p110δ PI 3-kinase mutant mice. *Science* **297**, 1031–1034 (2002).
9. Fruman, D.A. *et al.* Impaired B cell development and proliferation in absence of phosphoinositide 3-kinase p85α. *Science* **283**, 393–397 (1999).
10. Macintyre, A.N. *et al.* Protein kinase B controls transcriptional programs that direct cytotoxic T cell fate but is dispensable for T cell metabolism. *Immunity* **34**, 224–236 (2011).
11. O'Shea, J.J. & Paul, W.E. Mechanisms underlying lineage commitment and plasticity of helper CD4$^+$ T cells. *Science* **327**, 1098–1102 (2010).
12. Zhou, L., Chong, M.M. & Littman, D.R. Plasticity of CD4$^+$ T cell lineage differentiation. *Immunity* **30**, 646–655 (2009).
13. Harty, J.T. & Badovinac, V.P. Shaping and reshaping CD8$^+$ T-cell memory. *Nat. Rev. Immunol.* **8**, 107–119 (2008).
14. Shi, L.Z. *et al.* HIF1alpha-dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. *J. Exp. Med.* **208**, 1367–1376 (2011).
15. Michalek, R.D. *et al.* Cutting edge: Distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4$^+$ T cell subsets. *J. Immunol.* **186**, 3299–3303 (2011).
16. van der Windt, G.J. *et al.* Mitochondrial respiratory capacity is a critical regulator of CD8$^+$ T cell memory development. *Immunity* **36**, 68–78 (2012).
17. Hartwell, L.H. & Weinert, T.A. Checkpoints: controls that ensure the order of cell cycle events. *Science* **246**, 629–634 (1989).
18. Elledge, S.J. Cell cycle checkpoints: preventing an identity crisis. *Science* **274**, 1664–1672 (1996).
19. Jacob, F., Perrin, D., Sanchez, C. & Monod, J. Operon: a group of genes with the expression coordinated by an operator. *C. R. Hebd. Seances Acad. Sci.* **250**, 1727–1729 (1960).
20. Marijuán, P.C., Navarro, J. & del Moral, R. On prokaryotic intelligence: strategies for sensing the environment. *Biosystems* **99**, 94–103 (2010).
21. Krell, T. *et al.* Bacterial sensor kinases: diversity in the recognition of environmental signals. *Annu. Rev. Microbiol.* **64**, 539–559 (2010).
22. Wellen, K.E. & Thompson, C.B. A two-way street: reciprocal regulation of metabolism and signalling. *Nat. Rev. Mol. Cell Biol.* **13**, 270–276 (2012).
23. Kaelin, W.G. Jr. The von Hippel-Lindau tumour suppressor protein: O2 sensing and cancer. *Nat. Rev. Cancer* **8**, 865–873 (2008).
24. Keith, B., Johnson, R.S. & Simon, M.C. HIF1α and HIF2α: sibling rivalry in hypoxic tumour growth and progression. *Nat. Rev. Cancer* **12**, 9–22 (2012).
25. Semenza, G.L. Hypoxia-inducible factors in physiology and medicine. *Cell* **148**, 399–408 (2012).
26. Epstein, A.C. *et al. C. elegans* EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. *Cell* **107**, 43–54 (2001).
27. Hirsilä, M., Koivunen, P., Gunzler, V., Kivirikko, K.I. & Myllyharju, J. Characterization of the human prolyl 4-hydroxylases that modify the hypoxia-inducible factor. *J. Biol. Chem.* **278**, 30772–30780 (2003).
28. Gerald, D. *et al.* JunD reduces tumor angiogenesis by protecting cells from oxidative stress. *Cell* **118**, 781–794 (2004).
29. Selak, M.A. *et al.* Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-α prolyl hydroxylase. *Cancer Cell* **7**, 77–85 (2005).
30. Sitkovsky, M. & Lukashev, D. Regulation of immune cells by local-tissue oxygen tension: HIF1α and adenosine receptors. *Nat. Rev. Immunol.* **5**, 712–721 (2005).
31. Nizet, V. & Johnson, R.S. Interdependence of hypoxic and innate immune responses. *Nat. Rev. Immunol.* **9**, 609–617 (2009).
32. Dang, E.V. *et al.* Control of T_H17/T_{reg} balance by hypoxia-inducible factor 1. *Cell* **146**, 772–784 (2011).
33. Kojima, H., Sitkovsky, M.V. & Cascalho, M. HIF-1α deficiency perturbs T and B cell functions. *Curr. Pharm. Des.* **9**, 1827–1832 (2003).
34. Lukashev, D. *et al.* Cutting edge: hypoxia-inducible factor 1alpha and its activation-inducible short isoform I.1 negatively regulate functions of CD4$^+$ and CD8$^+$ T lymphocytes. *J. Immunol.* **177**, 4962–4965 (2006).
35. Chi, H. Regulation and function of mTOR signalling in T cell fate decisions. *Nat. Rev. Immunol.* **12**, 325–338 (2012).
36. Laplante, M. & Sabatini, D.M. mTOR signaling in growth control and disease. *Cell* **149**, 274–293 (2012).
37. Mihaylova, M.M. & Shaw, R.J. The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. *Nat. Cell Biol.* **13**, 1016–1023 (2011).

38. Hardie, D.G., Ross, F.A. & Hawley, S.A. AMPK: a nutrient and energy sensor that maintains energy homeostasis. *Nat. Rev. Mol. Cell Biol.* **13**, 251–262 (2012).
39. Han, J.M. *et al.* Leucyl-tRNA synthetase is an intracellular leucine sensor for the mTORC1-signaling pathway. *Cell* **149**, 410–424 (2012).
40. Dennis, P.B. *et al.* Mammalian TOR: a homeostatic ATP sensor. *Science* **294**, 1102–1105 (2001).
41. Yu, Y. *et al.* Phosphoproteomic analysis identifies Grb10 as an mTORC1 substrate that negatively regulates insulin signaling. *Science* **332**, 1322–1326 (2011).
42. Hsu, P.P. *et al.* The mTOR-regulated phosphoproteome reveals a mechanism of mTORC1-mediated inhibition of growth factor signaling. *Science* **332**, 1317–1322 (2011).
43. Inoki, K., Zhu, T. & Guan, K.L. TSC2 mediates cellular energy response to control cell growth and survival. *Cell* **115**, 577–590 (2003).
44. Gwinn, D.M. *et al.* AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol. Cell* **30**, 214–226 (2008).
45. Tamás, P. *et al.* Regulation of the energy sensor AMP-activated protein kinase by antigen receptor and Ca^{2+} in T lymphocytes. *J. Exp. Med.* **203**, 1665–1670 (2006).
46. Mayer, A., Denanglaire, S., Viollet, B., Leo, O. & Andris, F. AMP-activated protein kinase regulates lymphocyte responses to metabolic stress but is largely dispensable for immune cell development and function. *Eur. J. Immunol.* **38**, 948–956 (2008).
47. MacIver, N.J. *et al.* The liver kinase B1 is a central regulator of T cell development, activation, and metabolism. *J. Immunol.* **187**, 4187–4198 (2011).
48. Powell, J.D., Lerner, C.G. & Schwartz, R.H. Inhibition of cell cycle progression by rapamycin induces T cell clonal anergy even in the presence of costimulation. *J. Immunol.* **162**, 2775–2784 (1999).
49. Zheng, Y. *et al.* A role for mammalian target of rapamycin in regulating T cell activation versus anergy. *J. Immunol.* **178**, 2163–2170 (2007).
50. Yang, K., Neale, G., Green, D.R., He, W. & Chi, H. The tumor suppressor Tsc1 enforces quiescence of naive T cells to promote immune homeostasis and function. *Nat. Immunol.* **12**, 888–897 (2011).
51. Youle, R.J. & Narendra, D.P. Mechanisms of mitophagy. *Nat. Rev. Mol. Cell Biol.* **12**, 9–14 (2011).
52. Kim, J., Kundu, M., Viollet, B. & Guan, K.L. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat. Cell Biol.* **13**, 132–141 (2011).
53. Shang, L. *et al.* Nutrient starvation elicits an acute autophagic response mediated by Ulk1 dephosphorylation and its subsequent dissociation from AMPK. *Proc. Natl. Acad. Sci. USA* **108**, 4788–4793 (2011).
54. Egan, D.F. *et al.* Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. *Science* **331**, 456–461 (2011).
55. Kamada, Y. *et al.* Tor-mediated induction of autophagy via an Apg1 protein kinase complex. *J. Cell Biol.* **150**, 1507–1513 (2000).
56. Kamada, Y. *et al.* Tor directly controls the Atg1 kinase complex to regulate autophagy. *Mol. Cell. Biol.* **30**, 1049–1058 (2010).
57. Li, C. *et al.* Autophagy is induced in $CD4^+$ T cells and important for the growth factor-withdrawal cell death. *J. Immunol.* **177**, 5163–5168 (2006).
58. Pua, H.H., Dzhagalov, I., Chuck, M., Mizushima, N. & He, Y.W. A critical role for the autophagy gene Atg5 in T cell survival and proliferation. *J. Exp. Med.* **204**, 25–31 (2007).
59. Pua, H.H., Guo, J., Komatsu, M. & He, Y.W. Autophagy is essential for mitochondrial clearance in mature T lymphocytes. *J. Immunol.* **182**, 4046–4055 (2009).
60. Jia, W., Pua, H.H., Li, Q.J. & He, Y.W. Autophagy regulates endoplasmic reticulum homeostasis and calcium mobilization in T lymphocytes. *J. Immunol.* **186**, 1564–1574 (2011).
61. Procaccini, C. *et al.* An oscillatory switch in mTOR kinase activity sets regulatory T cell responsiveness. *Immunity* **33**, 929–941 (2010).
62. Delgoffe, G.M. *et al.* The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment. *Immunity* **30**, 832–844 (2009).
63. Delgoffe, G.M. *et al.* The kinase mTOR regulates the differentiation of helper T cells through the selective activation of signaling by mTORC1 and mTORC2. *Nat. Immunol.* **12**, 295–303 (2011).
64. Lee, K. *et al.* Mammalian target of rapamycin protein complex 2 regulates differentiation of Th1 and Th2 cell subsets via distinct signaling pathways. *Immunity* **32**, 743–753 (2010).
65. Pearce, E.L. *et al.* Enhancing CD8 T-cell memory by modulating fatty acid metabolism. *Nature* **460**, 103–107 (2009).
66. Harding, H.P. *et al.* Regulated translation initiation controls stress-induced gene expression in mammalian cells. *Mol. Cell* **6**, 1099–1108 (2000).
67. Wek, R.C., Jiang, H.Y. & Anthony, T.G. Coping with stress: eIF2 kinases and translational control. *Biochem. Soc. Trans.* **34**, 7–11 (2006).
68. Huang, L., Baban, B., Johnson, B.A. III & Mellor, A.L. Dendritic cells, indoleamine 2,3 dioxygenase and acquired immune privilege. *Int. Rev. Immunol.* **29**, 133–155 (2010).
69. Bunpo, P. *et al.* The eIF2 kinase GCN2 is essential for the murine immune system to adapt to amino acid deprivation by asparaginase. *J. Nutr.* **140**, 2020–2027 (2010).
70. Nicholson, L.B., Raveney, B.J. & Munder, M. Monocyte dependent regulation of autoimmune inflammation. *Curr. Mol. Med.* **9**, 23–29 (2009).
71. Sundrud, M.S. *et al.* Halofuginone inhibits TH17 cell differentiation by activating the amino acid starvation response. *Science* **324**, 1334–1338 (2009).
72. Munn, D.H. *et al.* GCN2 kinase in T cells mediates proliferative arrest and anergy induction in response to indoleamine 2,3-dioxygenase. *Immunity* **22**, 633–642 (2005).
73. Iyer, S.S. *et al.* Protein energy malnutrition impairs homeostatic proliferation of memory CD8 T cells. *J. Immunol.* **188**, 77–84 (2012).
74. Quintana, F.J. *et al.* Control of T_{reg} and T_H17 cell differentiation by the aryl hydrocarbon receptor. *Nature* **453**, 65–71 (2008).
75. Veldhoen, M., Hirota, K., Christensen, J., O'Garra, A. & Stockinger, B. Natural agonists for aryl hydrocarbon receptor in culture medium are essential for optimal differentiation of Th17 T cells. *J. Exp. Med.* **206**, 43–49 (2009).
76. Veldhoen, M. *et al.* The aryl hydrocarbon receptor links T_H17-cell-mediated autoimmunity to environmental toxins. *Nature* **453**, 106–109 (2008).
77. Mezrich, J.D. *et al.* An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells. *J. Immunol.* **185**, 3190–3198 (2010).
78. Opitz, C.A. *et al.* An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor. *Nature* **478**, 197–203 (2011).
79. Bensinger, S.J. & Tontonoz, P. Integration of metabolism and inflammation by lipid-activated nuclear receptors. *Nature* **454**, 470–477 (2008).
80. Repa, J.J. & Mangelsdorf, D.J. The role of orphan nuclear receptors in the regulation of cholesterol homeostasis. *Annu. Rev. Cell Dev. Biol.* **16**, 459–481 (2000).
81. Janowski, B.A., Willy, P.J., Devi, T.R., Falck, J.R. & Mangelsdorf, D.J. An oxysterol signalling pathway mediated by the nuclear receptor LXRα. *Nature* **383**, 728–731 (1996).
82. Forman, B.M., Ruan, B., Chen, J., Schroepfer, G.J. Jr. & Evans, R.M. The orphan nuclear receptor LXRα is positively and negatively regulated by distinct products of mevalonate metabolism. *Proc. Natl. Acad. Sci. USA* **94**, 10588–10593 (1997).
83. Mitro, N. *et al.* The nuclear receptor LXR is a glucose sensor. *Nature* **445**, 219–223 (2007).
84. Denechaud, P.D. *et al.* ChREBP, but not LXRs, is required for the induction of glucose-regulated genes in mouse liver. *J. Clin. Invest.* **118**, 956–964 (2008).
85. Bensinger, S.J. *et al.* LXR signaling couples sterol metabolism to proliferation in the acquired immune response. *Cell* **134**, 97–111 (2008).
86. Cui, G. *et al.* Liver X receptor (LXR) mediates negative regulation of mouse and human Th17 differentiation. *J. Clin. Invest.* **121**, 658–670 (2011).
87. Schults, M.A. *et al.* Diminished carcinogen detoxification is a novel mechanism for hypoxia-inducible factor 1-mediated genetic instability. *J. Biol. Chem.* **285**, 14558–14564 (2010).
88. Lim, J.H. *et al.* Sirtuin 1 modulates cellular responses to hypoxia by deacetylating hypoxia-inducible factor 1alpha. *Mol. Cell* **38**, 864–878 (2010).
89. Li, X. *et al.* SIRT1 deacetylates and positively regulates the nuclear receptor LXR. *Mol. Cell* **28**, 91–106 (2007).
90. Cantó, C. & Auwerx, J. NAD^+ as a signaling molecule modulating metabolism. *Cold Spring Harb. Symp. Quant. Biol.* **76**, 291–298 (2011).
91. Imai, S. & Guarente, L. Ten years of NAD-dependent SIR2 family deacetylases: implications for metabolic diseases. *Trends Pharmacol. Sci.* **31**, 212–220 (2010).
92. Yang, X.J. & Seto, E. Lysine acetylation: codified crosstalk with other posttranslational modifications. *Mol. Cell* **31**, 449–461 (2008).
93. Guan, K.L. & Xiong, Y. Regulation of intermediary metabolism by protein acetylation. *Trends Biochem. Sci.* **36**, 108–116 (2011).
94. Cai, L. & Tu, B.P. On acetyl-CoA as a gauge of cellular metabolic state. *Cold Spring Harb. Symp. Quant. Biol.* **76**, 195–202 (2011).
95. Lin, S.J. & Guarente, L. Nicotinamide adenine dinucleotide, a metabolic regulator of transcription, longevity and disease. *Curr. Opin. Cell Biol.* **15**, 241–246 (2003).
96. Smith, B.C., Hallows, W.C. & Denu, J.M. A continuous microplate assay for sirtuins and nicotinamide-producing enzymes. *Anal. Biochem.* **394**, 101–109 (2009).
97. Imai, S., Armstrong, C.M., Kaeberlein, M. & Guarente, L. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature* **403**, 795–800 (2000).
98. Rongvaux, A., Andris, F., Van Gool, F. & Leo, O. Reconstructing eukaryotic NAD metabolism. *BioEssays* **25**, 683–690 (2003).
99. Rongvaux, A. *et al.* Nicotinamide phosphoribosyl transferase/pre-B cell colony-enhancing factor/visfatin is required for lymphocyte development and cellular resistance to genotoxic stress. *J. Immunol.* **181**, 4685–4695 (2008).
100. Houtkooper, R.H., Canto, C., Wanders, R.J. & Auwerx, J. The secret life of NAD+: an old metabolite controlling new metabolic signaling pathways. *Endocr. Rev.* **31**, 194–223 (2010).
101. Goodwin, P.M., Lewis, P.J., Davies, M.I., Skidmore, C.J. & Shall, S. The effect of gamma radiation and neocarzinostatin on NAD and ATP levels in mouse leukaemia cells. *Biochim. Biophys. Acta* **543**, 576–582 (1978).
102. Krishnakumar, R. & Kraus, W.L. The PARP side of the nucleus: molecular actions, physiological outcomes, and clinical targets. *Mol. Cell* **39**, 8–24 (2010).
103. Schreiber, V., Dantzer, F., Ame, J.C. & de Murcia, G. Poly(ADP-ribose): novel functions for an old molecule. *Nat. Rev. Mol. Cell Biol.* **7**, 517–528 (2006).
104. Skidmore, C.J. *et al.* The involvement of poly(ADP-ribose) polymerase in the degradation of NAD caused by γ-radiation and N-methyl-N-nitrosourea. *Eur. J. Biochem* **101**, 135–142 (1979).
105. Mendoza-Alvarez, H. & Alvarez-Gonzalez, R. Poly(ADP-ribose) polymerase is a catalytic dimer and the automodification reaction is intermolecular. *J. Biol. Chem.* **268**, 22575–22580 (1993).
106. Bai, P. *et al.* PARP-1 inhibition increases mitochondrial metabolism through SIRT1 activation. *Cell Metab.* **13**, 461–468 (2011).

107. Berger, S.J., Manory, I., Sudar, D.C., Krothapalli, D. & Berger, N.A. Pyridine nucleotide analog interference with metabolic processes in mitogen-stimulated human T lymphocytes. *Exp. Cell Res.* **173**, 379–387 (1987).
108. Valdor, R. *et al.* Regulation of NFAT by poly(ADP-ribose) polymerase activity in T cells. *Mol. Immunol.* **45**, 1863–1871 (2008).
109. Olabisi, O.A. *et al.* Regulation of transcription factor NFAT by ADP-ribosylation. *Mol. Cell. Biol.* **28**, 2860–2871 (2008).
110. Fields, P.E., Kim, S.T. & Flavell, R.A. Cutting edge: changes in histone acetylation at the IL-4 and IFN-γ loci accompany Th1/Th2 differentiation. *J. Immunol.* **169**, 647–650 (2002).
111. Avni, O. *et al.* T_H cell differentiation is accompanied by dynamic changes in histone acetylation of cytokine genes. *Nat. Immunol.* **3**, 643–651 (2002).
112. Li, B. *et al.* FOXP3 interactions with histone acetyltransferase and class II histone deacetylases are required for repression. *Proc. Natl. Acad. Sci. USA* **104**, 4571–4576 (2007).
113. van Loosdregt, J. *et al.* Regulation of Treg functionality by acetylation-mediated Foxp3 protein stabilization. *Blood* **115**, 965–974 (2010).
114. Tao, R. *et al.* Deacetylase inhibition promotes the generation and function of regulatory T cells. *Nat. Med.* **13**, 1299–1307 (2007).
115. Beier, U.H. *et al.* Sirtuin-1 targeting promotes $Foxp3^+$ T-regulatory cell function and prolongs allograft survival. *Mol. Cell. Biol.* **31**, 1022–1029 (2011).
116. Floess, S. *et al.* Epigenetic control of the foxp3 locus in regulatory T cells. *PLoS Biol.* **5**, e38 (2007).
117. Nasta, F., Laudisi, F., Sambucci, M., Rosado, M.M. & Pioli, C. Increased $Foxp3^+$ regulatory T cells in poly(ADP-Ribose) polymerase-1 deficiency. *J. Immunol.* **184**, 3470–3477 (2010).
118. Oumouna, M. *et al.* Poly(ADP-ribose) polymerase-1 inhibition prevents eosinophil recruitment by modulating Th2 cytokines in a murine model of allergic airway inflammation: a potential specific effect on IL-5. *J. Immunol.* **177**, 6489–6496 (2006).
119. Chiarugi, A. Inhibitors of poly(ADP-ribose) polymerase-1 suppress transcriptional activation in lymphocytes and ameliorate autoimmune encephalomyelitis in rats. *Br. J. Pharmacol.* **137**, 761–770 (2002).
120. Farez, M.F. *et al.* Toll-like receptor 2 and poly(ADP-ribose) polymerase 1 promote central nervous system neuroinflammation in progressive EAE. *Nat. Immunol.* **10**, 958–964 (2009).
121. Zhang, J. *et al.* The type III histone deacetylase Sirt1 is essential for maintenance of T cell tolerance in mice. *J. Clin. Invest.* **119**, 3048–3058 (2009).
122. Sequeira, J. *et al.* sirt1-null mice develop an autoimmune-like condition. *Exp. Cell Res.* **314**, 3069–3074 (2008).
123. Gao, B., Kong, Q., Kemp, K., Zhao, Y.S. & Fang, D. Analysis of sirtuin 1 expression reveals a molecular explanation of IL-2-mediated reversal of T-cell tolerance. *Proc. Natl. Acad. Sci. USA* **109**, 899–904 (2012).
124. Bordone, L. & Guarente, L. Calorie restriction, SIRT1 and metabolism: understanding longevity. *Nat. Rev. Mol. Cell Biol.* **6**, 298–305 (2005).
125. Sun, D. *et al.* Regulation of immune function by calorie restriction and cyclophosphamide treatment in lupus-prone NZB/NZW F1 mice. *Cell. Immunol.* **228**, 54–65 (2004).
126. Piccio, L., Stark, J.L. & Cross, A.H. Chronic calorie restriction attenuates experimental autoimmune encephalomyelitis. *J. Leukoc. Biol.* **84**, 940–948 (2008).
127. Petro, T.M. Regulatory role of resveratrol on Th17 in autoimmune disease. *Int. Immunopharmacol.* **11**, 310–318 (2011).
128. Pae, M., Meydani, S.N. & Wu, D. The role of nutrition in enhancing immunity in aging. *Aging Dis.* **3**, 91–129 (2012).
129. Liu, Y., Yu, Y., Matarese, G. & La Cava, A. Cutting edge: fasting-induced hypoleptinemia expands functional regulatory T cells in systemic lupus erythematosus. *J. Immunol.* **188**, 2070–2073 (2012).
130. Ahmed, T. *et al.* Calorie restriction enhances T-cell-mediated immune response in adult overweight men and women. *J. Gerontol. A Biol. Sci. Med. Sci.* **64**, 1107–1113 (2009).
131. Edelman, A.M., Blumenthal, D.K. & Krebs, E.G. Protein serine/threonine kinases. *Annu. Rev. Biochem.* **56**, 567–613 (1987).
132. Gribble, F.M. *et al.* A novel method for measurement of submembrane ATP concentration. *J. Biol. Chem.* **275**, 30046–30049 (2000).
133. Moses, V. & Lonberg-Holm, K.K. The study of metabolic compartmentalization. *J. Theor. Biol.* **10**, 336–351 (1966).
134. Fang, M. *et al.* The ER UDPase ENTPD5 promotes protein N-glycosylation, the Warburg effect, and proliferation in the PTEN pathway. *Cell* **143**, 711–724 (2010).
135. Wellen, K.E. *et al.* The hexosamine biosynthetic pathway couples growth factor-induced glutamine uptake to glucose metabolism. *Genes Dev.* **24**, 2784–2799 (2010).
136. Mazurek, S. Pyruvate kinase type M2: a key regulator of the metabolic budget system in tumor cells. *Int. J. Biochem. Cell Biol.* **43**, 969–980 (2011).
137. Shaw, R.J. & Cantley, L.C. Decoding key nodes in the metabolism of cancer cells: sugar & spice and all things nice. *F1000 Biol. Rep.* **4**, 2 (2012).
138. Ignacak, J. & Stachurska, M.B. The dual activity of pyruvate kinase type M2 from chromatin extracts of neoplastic cells. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **134**, 425–433 (2003).
139. Gao, X., Wang, H., Yang, J.J., Liu, X. & Liu, Z.R. Pyruvate kinase M2 regulates gene transcription by acting as a protein kinase. *Mol. Cell* **45**, 598–609 (2012).
140. Semenova, G. & Chernoff, J. PKM2 enters the morpheein academy. *Mol. Cell* **45**, 583–584 (2012).
141. Mazurek, S., Grimm, H., Boschek, C.B., Vaupel, P. & Eigenbrodt, E. Pyruvate kinase type M2: a crossroad in the tumor metabolome. *Br. J. Nutr.* **87** (suppl. 1), S23–S29 (2002).
142. Christofk, H.R., Vander Heiden, M.G., Wu, N., Asara, J.M. & Cantley, L.C. Pyruvate kinase M2 is a phosphotyrosine-binding protein. *Nature* **452**, 181–186 (2008).
143. Anastasiou, D. *et al.* Inhibition of pyruvate kinase M2 by reactive oxygen species contributes to cellular antioxidant responses. *Science* **334**, 1278–1283 (2011).
144. Grüning, N.M. *et al.* Pyruvate kinase triggers a metabolic feedback loop that controls redox metabolism in respiring cells. *Cell Metab.* **14**, 415–427 (2011).