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LKB1 and AMPK: central regulators of lymphocyte metabolism and function

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Summary: When T cells encounter foreign antigen and appropriate co-stimulatory signals from professional antigen-presenting cells (APCs), they initiate a coordinated program of rapid proliferation and differentiation, leading to the development of activated T cells with specific effector functions tailored toward pathogen clearance or control. One of the fundamental programs that underpin T-cell proliferation and function is the regulation of cellular metabolism. Recent efforts to identify the signal transduction pathways that regulate T-cell metabolism have led to the identification of liver kinase B1 (LKB1) and AMP-activated protein kinase (AMPK) as key regulators of T-cell metabolism. LKB1 and AMPK are part of an evolutionarily conserved signal transduction pathway that monitors cellular energy status. AMPK senses bioenergetic fluctuations in cells and works in concert with LKB1 to maintain cellular energy homeostasis by promoting catabolic pathways of ATP production and limiting processes that consume ATP. Recent data indicate that LKB1 and AMPK can influence diverse aspects of T-cell biology beyond metabolism, including T-cell development, peripheral T-cell homeostasis, and T-cell effector function. In this review, we focus on the regulation of lymphocyte metabolism by this energy-sensing pathway and discuss its influence on T-cell function.

Keywords: *T cells, LKB1, AMPK, mTOR, cellular metabolism, lymphocyte homeostasis*

Introduction

As part of their program of activation, T cells dramatically alter their metabolic activity to meet the increased metabolic demands of cell growth, proliferation, and effector function (1, 2). Glucose is a critical metabolite for proliferating T cells. It serves as the primary substrate for ATP generation, is an intermediate in glycosylation reactions, and is an essential carbon source for the synthesis of other macromolecules. Glucose is normally catabolized in cells via two connected but independent biochemical pathways: glycolysis, the non-oxidative arm of glucose catabolism which generates 2 molecules each of ATP and pyruvate per molecule of glucose, and the tricarboxylic acid cycle/oxidative phosphorylation (OXPHOS) system in mitochondria, which oxidizes glycolysis-derived pyruvate to generate 36 molecules

of ATP per glucose molecule. In contrast to resting T cells, which predominantly oxidize glucose-derived pyruvate via OXPHOS to generate ATP, activated T cells generate ATP in large part by increasing their rate of glucose uptake and glycolysis and convert the majority of glucose-derived pyruvate to lactate (2). This metabolic switch toward increased glycolytic metabolism despite available oxygen for complete glucose oxidation is known as aerobic glycolysis, or the 'Warburg effect', and is a common trait of actively proliferating cells and cancer cells (3). In contrast, quiescent T cells rely primarily on mitochondrial oxidative metabolism fueled by glucose, amino acids, lipids, and other metabolic substrates to maintain bioenergetic homeostasis. Processing glucose rapidly by engaging the Warburg effect allows proliferating T cells to generate ATP quickly and to use glucose-derived carbon and other metabolites for biosynthesis. In each phase of the immune response, T-cell metabolism must be matched to function to support T-cell survival, proliferation, and effector programs (4). As such, T cells at various stages of activation display novel metabolic signatures, and regulating energy metabolism may provide a way for T cells to reversibly switch between quiescent and highly proliferative states (5).

Work over the past several years has identified signal transduction pathways downstream of the TCR, costimulatory receptors, and cytokine receptors that couple growth signals to metabolic regulation. TCR- and CD28-dependent activation of phosphatidylinositol 3-kinase (PI3K)/Akt results in increased glucose uptake and stimulation of glycolytic activity (6, 7). Akt can promote the production of glucose-derived lipids, which are required for membrane biosynthesis, through the activation of ATP citrate lyase (2). Akt also activates the mammalian target of rapamycin (mTOR), which regulates multiple metabolic pathways including protein synthesis, autophagy, and mitochondrial biogenesis in response to growth factor signals (8). mTOR has emerged as an important regulator of T-cell metabolism, differentiation and effector function (9). mTOR activity is required for the full effector function of both $CD4^+$ (10) and $CD8^+$ (11) T cells. Inhibition of mTOR signaling blocks the differentiation of $CD4^+$ T cells toward inflammatory T-helper (Th) lineages (10, 12) while stimulating the development of Treg cells (10, 13, 14).

While PI3K/Akt/mTOR signaling promotes anabolic growth, metabolic stress and declining cellular energy is monitored by cellular energy sensors including the liver kinase B1 (LKB1)/AMP-activated protein kinase (AMPK) pathway. AMPK engages pathways of catabolic metabolism, such as fatty acid oxidation (FAO) and autophagy and inhibits anabolic processes that consume ATP including cell proliferation (15). The net result of AMPK activation is the conservation of cellular energy before the cell reaches a state of bioenergetic catastrophe that triggers cell death. Recent work has revealed that LKB1 and AMPK play important but not completely overlapping roles in T-cell biology. This review focuses on the role that LKB1 and AMPK play in energy regulation in lymphocytes and the impact that this signaling pathway has on T-cell-mediated adaptive immunity.

The LKB1-AMPK signaling: a cellular energy-sensing pathway

LKB1 and AMPK couple cellular bioenergetics to metabolic control

LKB1 and AMPK function as part of an evolutionarily conserved energy-sensing pathway that couples cellular bioenergetics to metabolic control and cell growth. LKB1 is a serine-threonine kinase (encoded by the *stk11* gene) that was first identified as the tumor suppressor responsible for Peutz-Jeghers syndrome (16, 17), an autosomal dominant disorder that leads to intestinal hamartomas, mucocutaneous lesions, and an increased risk of spontaneous epithelial carcinomas. AMPK is an ancient regulator of anabolic and catabolic metabolism found in all eukaryotes (18). AMPK was originally characterized as an enzyme activity in liver homogenates that could promote the phosphorylation and inactivation of acetyl-CoA carboxylase (ACC) (19). Subsequent biochemical isolation of a protein kinase with similar activity toward ACC (whose activity was also stimulated by AMP) lead to the identification of AMPK and its classification as an AMPK (20). The search for downstream targets of LKB1 kinase activity led to the identification of AMPK as an LKB1 substrate (21–23). In addition to AMPKα1 and α2, LKB1 phosphorylates and activates 12 other AMPK-related kinases involved in regulating diverse biological processes including cell proliferation, survival, and polarity (24) (Fig. 1).

One common function of the LKB1/AMPK pathway throughout evolution is its role in regulating cellular metabolism and energy homeostasis. Snf1 (sucrose non-fermenting-1), the yeast orthologue of mammalian AMPK (25), was independently identified as a protein kinase required for growth of yeast on alternate or non-fermentable carbon sources (26, 27). In *S. cerevisiae*, Snf1 is regulated upstream by various functional orthologues of LKB1 (Sak1, Tos3, and

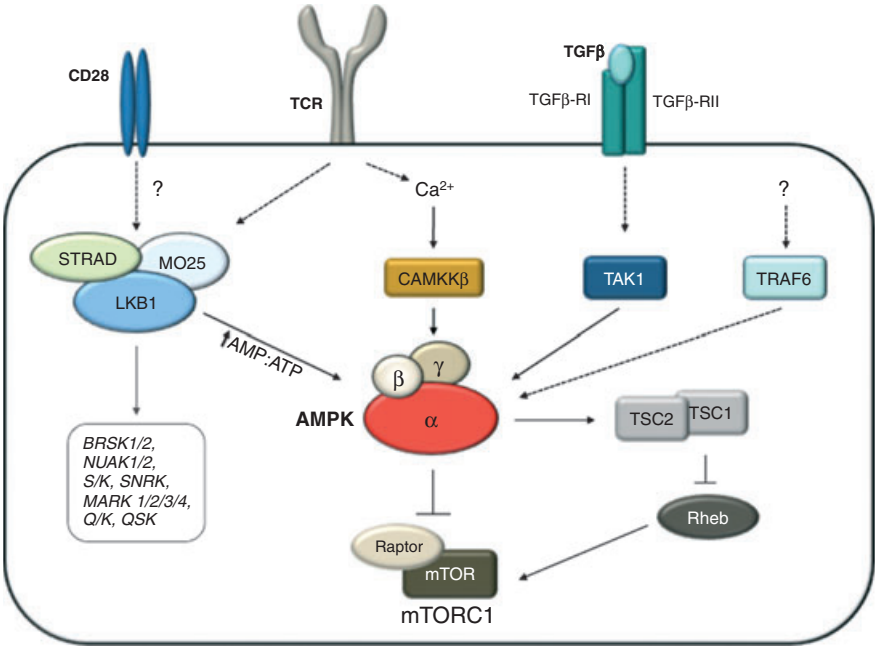

***Fig. 1.* Signaling to the AMP-activated protein kinase (AMPK) complex in T lymphocytes.** AMPK is a heterotrimeric protein kinase complex composed of α, β, and γ subunits, and is activated by multiple pathways in T cells. TCR and CD28 signal to the liver kinase B1 (LKB1) kinase complex (composed of LKB1, STE20-related adaptor, and mouse protein 25), which phosphorylates AMPK at Thr-172 of the α subunit. LKB1 also activates AMPK under low energy conditions when the AMP:ATP ratio is elevated. LKB1 also regulates 12 other AMPK-related kinases (shown in the gray box). CamKKβ and TAK1 promote AMPK phosphorylation at Thr-172 in response to TCR-mediated Ca^{2+} flux and cytokine signals such as transforming growth factor-β, respectively. TRAF6 is a positive regulator of AMPK in $CD8^{+}$ T cells, and regulates AMPK through an unknown mechanism. AMPK can suppress mTORC1 signaling through phosphorylation of the mTORC1-binding partner Raptor, which inhibits its function. AMPK promotes the activity of the TSC1-TSC2 complex by phosphorylating TSC2.

Elm1), and its activity is essential for the metabolic shift required to grow on alternate or non-fermentable carbon sources (27). Disruption of AMPK signaling in the fruit fly *Drosophila* results in developmental defects due to impaired activation of energy checkpoints (28, 29). More recently, AMPK function has been linked to cellular lifespan and aging. Several groups have demonstrated using the nematode worm *C. elegans* that disruption of AMPK signaling shortens organism lifespan (30–32). LKB1 and AMPK cooperate during the *C. elegans* Dauer stage, a developmental larval stage induced by low food abundance or reduced insulin-like signaling, to establish cell cycle quiescence. AMPK mediates *C. elegans* 'hibernation' in part by rationing lipid reserves through the regulation of adipose triglyceride lipase, ensuring that sufficient energetic resources are available to the organism to promote long-term survival (33).

Regulation of LKB1-AMPK signaling in lymphocytes

AMPK is a heterotrimeric kinase complex composed of a catalytic α subunit and β and γ regulatory subunits (*Fig.* 1). Mammals possess two genes each encoding the α (α1 and α2) and β (β1 and β2) subunits, and three genes encoding γ subunits (γ1-3) (15). The β subunit is a bridging molecule that links the α and γ subunits, although the β subunit also possesses a carbohydrate-binding domain that facilitates binding to glycogen (34). The γ regulatory subunit contains four cystathionine-β-synthase domains that interchangeably bind adenylate nucleotides (i.e. ATP or AMP). AMPK activation is regulated by the competition of ATP, ADP, and AMP for the nucleotide-binding sites of the γ subunit, and as such, its activity is sensitive to the adenylate energy charge of the cell (35, 36). It is generally believed that the α1 and α2 catalytic subunits of AMPK are functionally redundant, although they display differences in subcellular localization, with α1 primarily localized in the cytosol and α2 displaying both cytosolic and nuclear localization (37). Lymphocytes exclusively express the α1 catalytic subunit (38). AMPKα2 expression is not detected in resting or activated lymphocytes (38, 39), and deletion of *prkaa2*, the gene that encodes AMPKα2, has no effect on AMPK activity in T cells (39).

AMPK is responsive to changes in the cellular AMP:ATP ratio and is activated by stresses that stimulate ATP consumption or inhibit ATP production (40). As mentioned, AMP binding to the γ subunit of the AMPK complex

promotes increased kinase activity of the α subunit through phosphorylation of AMPKα at Thr-172 by upstream kinases and protection of Thr-172 from dephosphorylation (41, 42). TCR and calcium signals are potent activators of AMPK activity in lymphocytes (38, 39). Three upstream AMPK kinases have been identified to date: LKB1 (22), calmodulin-dependent protein kinase kinase β (CamKKβ) (43–45), and transforming growth factor-β (TGFβ)-activated kinase-1 (TAK1) (46, 47) (*Fig. 1*). Notably, all three of these kinases play roles in hematopoietic cells. Recent studies have revealed that LKB1 is essential for the maintenance of hematopoietic stem cell (HSC) homeostasis (48–50). Conditional deletion of *stk11* in HSCs promotes a cell-autonomous loss of HSC quiescence marked by increased HSC proliferation and differentiation toward multipotent progenitor cell lineages. This leads to a transient increase in multipotent progenitor cells, followed by a progressive depletion of the HSC compartment. CamKKβ has largely been associated with neuronal signaling and whole-body metabolic balance, but recent evidence indicates that CamKKβ can function as a negative regular of granulocyte differentiation *in vivo* (51). TAK1 is a critical signaling molecule for both innate and adaptive immunity. Similar to LKB1, loss of TAK1 function in the HSC compartment leads to depletion of HSC and multipotent progenitor cells due to increased apoptosis of these populations (52). TAK1 is also essential for dendritic cell survival (53). Deletion of the gene encoding for TAK1 (*Map3k7*) in the T-cell lineage leads to defects in thymocyte development, reduced peripheral T-cell activation and survival, and a marked reduction in $CD4^+Foxp3^+$ Treg populations (54). Despite the prominent role of TAK1 in T-lymphocyte survival and function, the contribution of AMPK to TAK1-mediated functions in lymphocytes remains unknown.

Like AMPKα, LKB1 exists as part of a heterotrimeric protein complex and binds two other proteins known as STE20-related adaptor and mouse protein 25 (MO25) (*Fig. 1*). LKB1 kinase activity in cells appears to be constant regardless of stimulus (55), and as such, LKB1 may exert its effects on downstream pathways based on substrate availability or the differential accessibility of targets. LKB1 kinase activity is essential for AMPK activation under conditions of bioenergetic stress, including nutrient withdrawal or disruption of mitochondrial energy production (21–23). LKB1-deficient T cells also display reduced TCR- and CD28-mediated AMPK activation (39). This finding suggests that LKB1 may be a signaling intermediary linking AMPK to antigen receptors or that TCR stimulation promotes a transient fluctuation in cellular energy (i.e. a decrease in the AMP:ATP ratio) that results in LKB1-dependent AMPK phosphorylation. AMPK is activated rapidly in response to TCR stimulation (38), and thus may function to promote rapid ATP production to 'kick start' T-cell metabolism during the early stages of activation.

Tamas and colleagues demonstrated that TCR-stimulated Ca^{2+} flux is a potent activator of AMPK through CamKKβ (38), and this may represent a physiological activator of AMPK in lymphocytes. AMPK can also be activated in cell lines by the pro-inflammatory cytokines TNF-α and IL-1 in an LKB1-independent manner (47), although it remains unclear whether TAK1 mediates these effects. TNF-receptor-associated factor 6 (TRAF6) has also been demonstrated to be a positive regulator of AMPK activity in $CD8^+$ T lymphocytes (56) (*Fig. 1*). Interestingly, TAK1 and TRAF6 have been shown to interact downstream of IL-1 signaling (57), but whether this complex functions to regulate AMPK activity in T cells is unclear. Other cytokines, such as IL-7 maintain glucose metabolism in developing and resting T cells (58), but it is unclear if these cytokines regulate basal T-cell metabolism through AMPK.

Downstream functions of LKB1-AMPK signaling

T-cell metabolism must be matched to effector function to support proper T-cell survival and function. AMPK mediates changes in cellular metabolism in response to fluctuating environmental conditions and this is a general function of AMPK across species. From a metabolic standpoint, AMPK promotes ATP conservation by inhibiting anabolic processes that consume ATP and activating pathways of catabolic metabolism to generate ATP (*Fig. 2*). The exact function of AMPK in any given tissue depends on multiple factors, including the type and duration of stimulus as well as the expression and availability of downstream substrates. AMPK can exert direct and immediate effects on metabolism through phosphorylation of downstream effectors (reviewed in 59). AMPK can also induce global changes in cellular metabolic programs through the regulation of gene transcription. A subset of AMPK targets relevant to lymphocyte function are described in *Fig. 2* and discussed below.

Direct regulation of metabolic processes by AMPK

One way that AMPK affects energy homeostasis is through the direct phosphorylation of metabolic enzymes. When AMPK is activated under conditions of energetic or metabolic stress, it limits the activity of metabolic pathways that

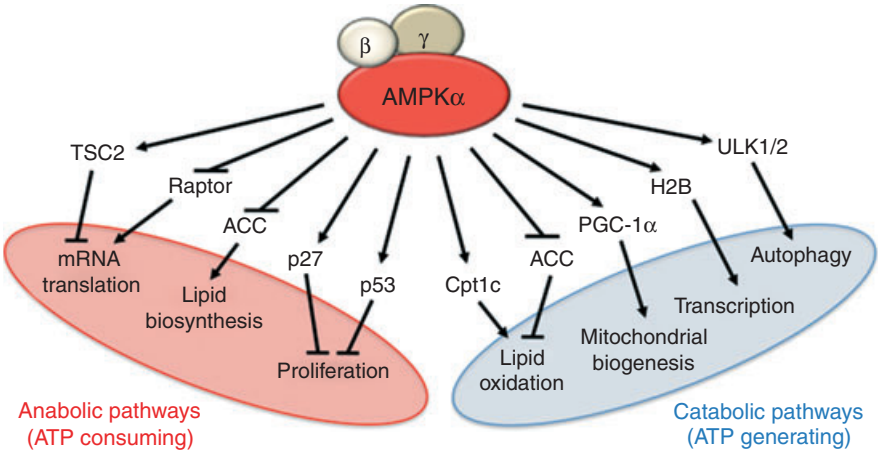

Fig. 2. AMP-activated protein kinase (AMPK) signaling maintains bioenergetic homeostasis by regulating both anabolic and catabolic pathways. AMPK promotes catabolic pathways (in blue) and antagonizes anabolic pathways (in red) to maintain cellular bioenergetic balance. AMPK reduces mRNA translation by inhibiting mTORC1 activity via multiple mechanisms. AMPK promotes TSC2-mediated inhibition of Rheb or inhibits the mTORC1 binding partner Raptor by direct phosphorylation of these targets. AMPK promotes fatty acid oxidation through the phosphorylation and inhibition of acetyl-CoA carboxylase or the transcriptional upregulation of the lipid transporter Cpt1c. AMPK initiates cell cycle arrest through the activation of p53 and p27. Phosphorylation of the transcriptional coactivator PGC-1α by AMPK enhances mitochondrial biogenesis. AMPK-dependent phosphorylation of Histone H2B at Ser-36 enhances transcription of metabolic genes by promoting transcriptional elongation of specific targets. AMPK activity promotes autophagy in part by phosphorylating the autophagy-initiating kinases ULK1 and ULK2. The downstream effectors engaged by AMPK depend on the activating stimulus, length of activation, and the expression of downstream substrates in specific tissues.

consume ATP. The classic example of this paradigm is the coordinate regulation of lipid biosynthesis and oxidation through AMPK-dependent inhibition of ACC (*Fig.* 2). ACC mediates the conversion of Acetyl-CoA to Malonyl-CoA. Accumulation of Malonyl-CoA increases the carbon pool dedicated to lipid biosynthesis, so the level of ACC activity acts as a rate-limiting step for lipid biosynthesis. At the same time, increased Malonyl-CoA levels inhibit lipid oxidation by allosterically inhibiting carnitine palmitoyl transferase 1 (CPT1), a rate-limiting enzyme for the import of fatty acyl-CoA chains into the mitochondria (60). Phosphorylation of ACC on Ser-79 by AMPK decreases ACC activity (61) and as such inhibits both lipid biosynthesis and relieves Malonyl-CoA-mediated suppression of CPT1 to promote lipid oxidation and ATP production. AMPK has also been shown to promote glycolysis under certain conditions through the phosphorylation of 6-phosphofructo-2-kinase (62).

One of the prominent metabolic pathways regulated by AMPK is autophagy, a catabolic process of 'self-digestion' that mediates the recycling of intracellular components for energy production and cell survival. AMPK promotes autophagy in part by regulating the activity of the autophagy-initiating kinases ULK1 and ULK2 (*Fig.* 2). AMPK is a binding partner for both ULK1 and ULK2 (63) and directly phosphorylates these kinases on multiple sites to promote autophagy under conditions of energy stress (64, 65). ULK1 and ULK2 play a prominent role in the autophagic degradation of mitochondria ('mitophagy') (64). ULK1-deficient animals display defects in erythrocyte development due to defects in mitophagy (66). Interestingly, AMPKα1-deficient animals display a similar defect in reticulocyte maturation (67), raising the possibility that an AMPK-ULK1 pathway regulates mitophagy during erythropoiesis. AMPK may also promote autophagy under conditions of energy stress by inhibiting mTOR complex 1 (mTORC1). Animals lacking T-cell-specific expression of ATG5, an essential mediator of autophagy in mammalian cells, display severe defects in lymphocyte homeostasis (68), suggesting that successful execution of autophagy is essential for lymphocyte function and survival. Whether LKB1 or AMPK regulate autophagy in lymphocytes via ULK1/2 remains to be determined.

Regulation of mRNA translation and protein synthesis

Translation of mRNA is one of the most energy consuming processes in proliferating cells, accounting for approximately 20% of cellular ATP consumption (69). AMPK antagonizes mRNA translation largely through negative regulation of mTORC1 signaling. Under conditions of energy stress, AMPK negatively regulates mTOR-dependent mRNA translation through activation of the TSC1-TSC2 complex (70–72) or inhibition of the mTOR binding partner Raptor (73) (*Fig.* 1). T cells lacking LKB1 or AMPKα1 display elevated

phosphorylation of the mTORC1 targets ribosomal S6 (rS6) protein and 4E-BP1 (39), indicating that LKB1 and AMPK antagonize mTORC1 downstream of TCR stimulation. In addition, AMPK can inhibit peptide chain elongation by activating the eukaryotic elongation factor (eEF2) kinase-dependent inhibition of eEF2 (74).

Another mechanism by which AMPK may regulate protein translation is through the regulation of mRNA stability. A key regulator of mRNA stability is the human antigen R (HuR) protein, an RNA-binding protein that stabilizes specific mRNA species by binding adenine/uridine-rich elements in their 3′-untranslated regions (75). AMPK is a negative regulator of HuR activity. Activation of AMPK promotes the retention of HuR in the nucleus, leading to reduced HuR levels in the cytosol and increased mRNA turnover (76). Interestingly, HuR has been implicated in the stabilization of cytokine mRNAs and specifically binds to il2 mRNA in activated T cells (77). Consistent with this, we have found that T cells lacking LKB1 display increased expression of mRNAs encoding for the pro-inflammatory cytokines IFN-γ and IL-17 (39), although it is not clear whether this is due to enhanced mRNA expression or stability.

Regulation of gene transcription by AMPK

Growth on alternate carbon sources in yeast requires transcriptional reprogramming, and this is controlled in large part by the activity of Snf1. Snf1 phosphorylates and displaces the transcriptional repressor Mig1 (78, 79), which is normally bound to glucose-repressed promoters and inhibits gene expression under glucose-replete conditions. In mammalian cells, AMPK and other AMPK-related kinases can influence metabolism by regulating gene transcription at multiple levels. First, the action of AMPK on signal transduction pathways, such as mTORC1 can influence the transcription of metabolic genes. The sterol regulatory element-binding proteins (SREBPs) are transcription factors that regulate genes involved in lipid and cholesterol biosynthesis, and their activity is positively regulated by mTOR (80). AMPK is a negative regulator of SREBP activity and may influence SREBP-mediated gene expression through both the regulation of mTORC1 activity and direct phosphorylation of SREBP (81). AMPK also affects the activity of numerous transcriptional coactivators including histone acetyltransferases (82), histone deacetylases (83, 84), sirtuins (85), and peroxisome proliferator-activated receptor-γ coactivator-1α (PGC1α) (86). PGC-1α is a key regulator of mitochondrial biogenesis (*Fig.* 2), and its activation by AMPK may regulate the switch between glycolytic and mitochondrial metabolism characteristic of $CD8^+$ memory T cells (56, 87). Finally, AMPK can promote the phosphorylation of transcription factors themselves, which either enhances or represses their function. AMPK-dependent phosphorylation of p53 (88) and Foxo3a (89) enhances their transcriptional activity, while activity of the nuclear receptor HNF4 is suppressed by AMPK phosphorylation (90).

Recent work indicates that AMPK may not act at arm's length to regulate gene transcription, but rather may play an intimate role in transcription as part of transcriptional complexes. We have recently shown that under conditions of chronic energetic stress, AMPK promotes the transcription of genes required to drive catabolic oxidative metabolism, such as the lipid oxidation-associated gene Cpt1c (91). Curiously we were able to detect AMPK as part of a stable p53 transcription factor complex that associates with chromatin (92). The physical association of signaling kinases such as AMPK with genes under their regulatory control may be more common than previously appreciated (93). It is unlikely that AMPK complexes directly with DNA as none of the α, β, or γ subunits possess DNA-binding domains. So why is AMPK localized to chromatin? The answer may lie in the ability of AMPK to directly alter chromatin dynamics through its kinase activity. Both Snf1 and AMPK can function as histone kinases (92, 94), and AMPK-dependent phosphorylation of Histone H2B at Ser-36 helps promote transcriptional elongation at actively transcribed loci (92) (*Fig.* 2). The association of AMPK with chromatin-bound transcriptional complexes may allow for the precise tethering of gene expression to minute changes in bioenergetics.

Initiation of metabolic cell cycle checkpoints by LKB1/AMPK signaling

Many cells slow their proliferation when nutrients are limiting (95). The simple explanation for this phenomenon is that reduced nutrient availability leads to decreased ATP production and that proliferation slows as a consequence. However, we and others have established that AMPK mediates a metabolic cell cycle checkpoint that is proactively triggered in mammalian cells in response to nutrient limitation (71, 73, 88). One-way AMPK achieves this through the aforementioned inhibition of mTORC1 via AMPK-dependent raptor phosphorylation (73) or activation of the cell cycle regulator p27kip1 (96) (*Fig.* 2). Bioenergetic stress also stimulates AMPK-dependent p53 phosphorylation at Ser-15,

which induces p53-dependent proliferative arrest (88) (Fig. 2).

In addition to well-defined roles in regulating apoptosis and cell cycle arrest, p53 can also exert effects on metabolism. p53 activation can promote an increased shift toward ATP production by OXPHOS. This metabolic shift is achieved in part through the p53-dependent transcriptional regulation of TP53-induced glycolysis and apoptosis regulator (TIGAR) and synthesis of cytochrome c oxidase 2 (SCO2) (97, 98). TIGAR is a fructose-2,6-bisphosphatase that negatively regulates glycolysis by degrading fructose-2,6-bisphosphate, an allosteric activator of the glycolytic enzyme 6-phosphofructo-1-kinase. SCO2 is an electron transport chain complex IV subunit required for assembly of the cytochrome C oxidase complex. Loss of p53-dependent SCO2 expression reduces the efficiency of the mitochondrial respiratory chain and promotes a metabolic switch from OXPHOS to glycolysis. Thus, AMPK-dependent activation of p53 may regulate the metabolic switch from aerobic glycolysis to OXPHOS in T cells, thus facilitating the transition of lymphocytes from an activated state to an inert or anergic state.

Regulation of cell polarity by the LKB1/AMPK pathway

One area not intrinsically linked to metabolic control, but with potential relevance to lymphocyte function is the regulation of cell polarity by LKB1/AMPK signaling. Disruption of the LKB1 and AMPKα orthologues in *D. melanogaster* (PAR4 and SNF1A, respectively) leads to defects in epithelial cell polarity marked by disruption of the apical–basal polarity of the actin cytoskeleton in these cells (28, 99, 100). In particular, PAR4 is required for the first asymmetric cell divisions during embryonic development in *Drosophila* (100). These LKB1/AMPK-dependent polarity phenotypes may be relevant to differentiating T cells, given the recent demonstration of asymmetric T-cell division and differential inheritance of fate determining molecules in lymphocytes (101, 102). The T-box transcription factor T-bet, which regulates IFN-γ production in $CD8^+$ and $CD4^+$ T cells, segregates asymmetrically in IFN-γ-producing effector T cells (103). Notably, mice with T-cell-specific deletion of LKB1 display a marked increase in circulating IFN-γ-producing $CD8^+$ and $CD4^+$ T cells (39). While any link between LKB1 and asymmetric cell division in lymphocytes is just speculation, it would be interesting to investigate whether the effect of LKB1 loss on the development of IFN-γ-producing T cells is linked to LKB1-mediated effects on cell polarity.

LKB1/AMPK signaling in T-cell biology

Tamas and colleagues (38) were the first group to demonstrate that AMPKα activity can be stimulated in T cells by antigen specific and Ca^{2+} signals. However, this study did not explore the functional consequences of TCR-mediated AMPK activation or its impact on metabolic control or lymphocyte function. The latter has been facilitated by the development of mouse models lacking AMPKα1 (39, 104) or T-cell-specific deletion of LKB1 (39, 105). Evidence from these studies suggests that the LKB1/AMPK signaling pathway works in concert to regulate common elements of T-cell metabolism. However, while AMPK is one of the key physiological effectors downstream of LKB1, LKB1 also exerts distinct effects on T-cell biology through AMPK-independent pathways.

LKB1 and AMPK regulate common metabolic pathways in lymphocytes

The demonstration by Frauwirth and Thompson (6) that CD28 costimulation could stimulate increased glycolysis in T cells provided some of the first evidence that specific signal transduction pathways regulate nutrient uptake and anabolic metabolism in response to T-cell activation. Subsequent work has linked multiple signal transduction pathways including PI3K/Akt signaling (7), Myc (106), and Notch (107) to the positive regulation of glycolysis in developing and mature T cells. In contrast, glycolysis is enhanced in both LKB1- and AMPKα-deficient T cells (39), suggesting that LKB1 and AMPK are negative regulators of TCR-induced glycolytic metabolism. Expression of the glucose transporter Glut1 and the glycolytic enzyme hexokinase-2 are elevated at both the mRNA and protein level in LKB1- and AMPKα1-deficient T cells. These findings are consistent with data in other cell types linking LKB1/AMPK signaling to transcriptional control of these key glycolytic regulators (108). LKB1 is also required for efficient surface expression of CD98/4F2hc, a subunit of an amino acid transporter that promotes the import of branched chain amino acids (105). In addition to effects on glucose metabolism, loss of LKB1 promotes increased mTORC1 activity in both developing (105) and mature (39) T cells, which is phenocopied in AMPKα1-deficient T cells (39). Notably, branched chain amino acids such as leucine are powerful agonists of mTOR activity (109), and altering their uptake may affect mTOR-dependent lymphocyte growth independent of TCR-dependent signals. Increased nutrient uptake and deregulated mTORC1 activity promotes a net increase in biosynthesis and T-cell

size. Thus, LKB1 and AMPK act in concert to limit the anabolic growth of T cells by suppressing nutrient uptake, energy production (glycolysis), and biosynthesis (mTOR).

While the metabolism of proliferating T cells is marked by high rates of aerobic glycolysis, quiescent T cells rely primarily on mitochondrial oxidative metabolism to maintain bioenergetic homeostasis. Recent work suggests that T-cell metabolic reprogramming from a glycolytic to an oxidative metabolic state may regulate the transition from effector to memory T cells. van der Windt *et al.* (87) recently demonstrated that $CD8^+$ T-memory cells display increased mitochondrial oxidative capacity relative to IL-2-driven $CD8^+$ T-effector cells. This switch to mitochondrial metabolism is regulated by IL-15 and dependent on mitochondrial-dependent lipid oxidation. The specific induction of FAO in $CD8^+$ T-effector cells triggered by cytokine withdrawal or following pathogen clearance is important for the production of $CD8^+$ T-cell memory, and appears to be dependent on TRAF6 (56) and/or the inhibition of mTOR (110). As discussed, AMPK is positioned between these two effector molecules (*Fig.* 1) and is a logical mediator of this metabolic switch. In support of this, metformin, a diabetes drug that induces LKB1-dependent AMPK activation, promotes the increased production of $CD8^+$ memory T cells *in vivo* (56). Not surprisingly, both LKB1- and AMPKα1-deficient T cells are unable to promote the metabolic switch to FAO in response to cytokine deprivation (39). Moreover, they continue to engage a pro-growth metabolic program in the absence of IL-2 marked by elevated glycolytic metabolism and sustained mTORC1 activity, which ultimately leads to T-cell death. From these data, one might predict that $CD8^+$ T-effector cells lacking AMPKα1 may be unable to form functional $CD8^+$ T-cell memory due to an inability to disengage from the Warburg effect. AMPK appears to regulate a key metabolic control point in activated T cells – the transition from glycolytic to FAO and oxidative metabolism – and supports a model by which AMPK activity influences $CD8^+$ T-cell fate through the regulation of oxidative metabolism (*Fig.* 3). It remains to be determined whether LKB1 or AMPK regulate distinct metabolic pathways in T cells.

LKB1 regulates T-cell development and proliferation independent of AMPK

While the core metabolic pathways required for T-cell growth and proliferation (i.e. glycolysis and FAO) are coordinated by the LKB1/AMPK cascade, LKB1 has distinct roles in T-cell development and peripheral function that are

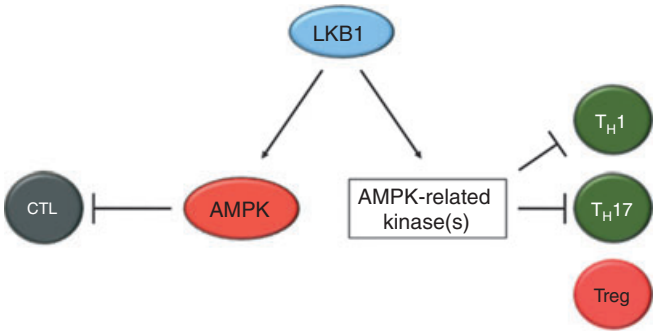

***Fig.* 3. Liver kinase B1 (LKB1) regulates T-cell function through AMP-activated protein kinase (AMPK)-dependent and AMPK-independent pathways.** LKB1 plays a critical role in T-cell development and the maintenance of proper T-cell function. LKB1 suppresses the production of IFN-γ-producing $CD8^+$ CTLs and negatively regulates $CD4^+$ T-cell differentiation toward pro-inflammatory Th1 and Th17 lineages (in green). AMPKα1 functions downstream of LKB1 to antagonize $CD8^+$ CTL function, but AMPKα1 is not required for LKB1-mediated effects on Th1 and Th17 differentiation. LKB1 may mediate its effects on $CD4^+$ T-cell differentiation through activation of one or more AMPK-related kinases. The role of LKB1 or AMPK in Treg differentiation (in red) remains unclear.

independent of AMPK function. Deletion of LKB1 in the T-cell lineage leads to severe defects in thymus cellularity, decreased numbers of $CD4^+CD8^+$ [double positive (DP)] thymocytes, and overall reduced production of $CD4^+$ and $CD8^+$ thymocytes (39, 105, 111), indicating that LKB1 is a cell-autonomous factor essential for T-cell development. These phenotypes may not necessarily be linked to metabolic regulation. The DN to DP transition is dependent on induction of glycolysis at the TCRβ checkpoint (107), but glycolysis is actually enhanced in LKB1-deficient T cells. Rather, LKB1 appears to be important for maintaining T-cell survival, as both thymocytes and T cells lacking LKB1 express lower levels of the anti-apoptotic protein Bcl-xL and display increased susceptibility to apoptosis (39, 111). Moreover, the thymic defect in T-cell-specific LKB1-null mice can be rescued by transgenic overexpression of Bcl-xL (39). Collectively the evidence suggests that LKB1 regulates overall T-cell fitness, and its loss leads to decreased thymocyte viability similar to that observed in HSCs (48–50). In contrast, AMPKα1 and AMPKα2 are dispensable for thymocyte development (39, 104). AMPKα1 and AMPKα2 are just two enzymes of the AMPK-related kinase family activated by LKB1 (24), and another AMPK-related kinase may mediate the downstream actions of LKB1 in thymocytes.

In addition to its role in thymocyte development, LKB1 is required for the proliferation of mature T cells. LKB1-deficient T cells display defective proliferation in response to TCR and costimulatory signals and are susceptible to

apoptosis in response to CD3/CD28 stimulation or cytokine withdrawal (39, 105). The effects of LKB1 on T-cell proliferation appear to be independent of cell viability as expression of Bcl-xL rescues the survival defect but not the proliferative defect of LKB1 mutant T cells (39). It has been speculated that LKB1 coordinates energy production to meet the increased energetic requirements of cell proliferation, and the loss of LKB1 invokes a metabolic checkpoint in T cells to restrict proliferation. This seems unlikely, given that LKB1 and AMPKα1 work in concert to suppress the metabolic pathways required for T-cell proliferation and that AMPKα1-null T cells proliferate normally in response to TCR stimulation (39, 104). Rather, LKB1 may act to transduce mitogenic signals downstream of TCR/CD28 engagement independent of AMPK. LKB1 has recently been shown to be phosphorylated by Lck in response to TCR stimulation, and LKB1-deficient T cells display impaired PLCγ activation and recruitment to LAT-containing signaling complexes (112). Direct involvement of LKB1 in TCR signal transduction may explain why thymocyte development and peripheral T-cell activation are defective in mice with T-cell-specific loss of LKB1, whereas these processes are unaffected in AMPKα1-deficient animals. These data argue that LKB1 functions at the center of a signaling axis in T cells that couples TCR-mediated signals to cellular bioenergetics via AMPK and proliferative programs through distinct signal transduction pathways.

LKB1 and AMPK regulate lymphocyte homeostasis

One of the striking phenotypes observed in animals with T-cell-specific deletion of LKB1 is the disruption of $CD4^+$ and $CD8^+$ T-cell homeostasis. While LKB1 mutant animals display defective thymocyte development and impaired T-cell proliferation, the T cells that do reach the periphery (either naturally or through forced expression of Bcl-xL) display an activated phenotype characterized by increased CD44 expression and elevated production of inflammatory cytokines, including IFN-γ and IL-17 (39). It remains unclear whether this phenomenon is due to increased homeostatic proliferation leading to an accumulation of $CD44^+CD62L^+$ central memory (Tcm) or $CD44^+CD62L^-$ effector memory (Tem) cells or defective apoptosis of these T-cell subsets following activation. The former is unlikely, as expression of a Bcl-xL transgene rescues peripheral T-cell numbers, but does not prevent the accumulation of Tcm- or Tem-like cells in LKB1 mutant mice. Interestingly, AMPKα1-deficient animals display homeostatic defects in $CD8^+$ but not $CD4^+$ T-cell subsets, again suggesting that AMPK mediates only a subset of LKB1 functions in T cells (*Fig. 3*).

Modulation of mTOR activity can shift the homeostatic balance of T cells, and deregulation of its activity in the absence of LKB1/AMPK signaling may account for the disruptions in $CD8^+$ T-cell homeostasis. One function of TORC1 signaling is the maintenance of ribosomal biogenesis, which is mediated by the phosphorylation and activation of p70 ribosomal S6 kinase (S6K1). S6K1-deficient mice display fewer memory cells and increased numbers of naive $CD4^+$ and $CD8^+$ T cells (113), essentially the converse of LKB1 deficiency, which results in elevated S6K activity in T cells. The TSC1-TSC2 complex, which negatively regulates mTORC1 signaling by inhibiting the function of the small GTPase Rheb (114), is positively regulated downstream of LKB1 and AMPK (*Fig. 1*). T-cell-specific deletion of TSC1 leads to disrupted T-cell homeostasis similar to that observed in LKB1 mutant mice (115, 116). TSC1 mutant mice display an overall decrease in both the $CD4^+$ and $CD8^+$ T-cell compartments, but display an accumulation of Tem-like cells that express increased levels of the activation markers CD44, CD25, and CD69 and produce inflammatory cytokines including IFN-γ (115).

Why would a signaling pathway that normally monitors cellular bioenergetics and nutrient levels function to prevent T-cell hyperproliferation and hyperactivation? TCR and CD28 engagement induces a strong mitogenic signal that directs naive T cells to grow and proliferate, and mTOR couples these mitogenic signals to anabolic growth programs. Conversely, one may consider LKB1, AMPK, and TSC1-TSC2 to be rheostats of the intracellular environment; they integrate signals from a variety of inputs, most notably cellular energy charge and nutrient availability, to maintain energy balance. Thus, LKB1/AMPK signaling may act as a brake on lymphocyte expansion when energy conditions are poor. When LKB1/AMPK/TSC signaling is defective, naive T cells are freed from these metabolic checkpoints and operate under the assumption that bioenergetics and nutrient availability are suitable to support proliferation. The differential effects of AMPKα1 on the homeostatic activation of $CD8^+$ versus $CD4^+$ T cells may argue that $CD8^+$ T cells are more sensitive to AMPK-dependent energetic checkpoints.

Can metabolic control pathways influence T-cell effector differentiation?

There is a growing body of evidence suggesting that AMPK signaling can influence inflammatory processes (117–119).

Given the impact of disrupting LKB1 or AMPKα1 on T-cell homeostasis, we hypothesized that LKB1/AMPK signaling may act as a negative regulator of peripheral $CD8^+$ and $CD4^+$ T-cell function. Deletion of LKB1 or AMPKα1 promotes a sharp increase in the proportion of IFN-γ^+CD8^+ T cells *in vivo* and enhances IFN-γ and IL-17 production by $CD8^+$ CTLs *in vitro* (39). Interestingly, naive $CD4^+$ T cells lacking LKB1 display a greater capacity to differentiate toward pro-inflammatory T-cell lineages, whereas loss of AMPKα1 has no impact on $CD4^+$ T-cell differentiation toward Th lineages (*Fig.* 3). These data suggest that LKB1 is a critical regulator of both $CD4^+$ and $CD8^+$ T-cell differentiation and antagonizes the development of pro-inflammatory T-effector cells. Deficiency of LKB1 in T cells leads to the reciprocal phenotype of mTOR-deficient T cells (9), supporting the model that these pathways act in opposition to regulate T-cell effector function. The fact that AMPK does not perform the same function as LKB1 in $CD4^+$ T cells may suggest that the circuitry connecting LKB1 with mTORC1 differs between T-cell subsets, and that an AMPK-related kinase may mediate the effects of LKB1 on Th1 and Th17 differentiation (*Fig.* 3). One possibility is that LKB1 and AMPK function in parallel rather than linear pathways in $CD4^+$ T cells. There are many routes to AMPK activation in peripheral T cells (*Fig.* 1), and it is tempting to speculate that other $CD4^+$ subsets, such as T-follicular helper (Tfh) cells and Tregs are influenced by AMPK activity independent of LKB1 activity.

Recent evidence indicates that $CD4^+$ T-cell subsets display distinct metabolic profiles specific to their differentiation state. Michalek *et al.* (120) recently demonstrated that $CD4^+$ Th cells (Th1, Th2, and Th17) display a strongly glycolytic metabolic profile, whereas immunosuppressive Tregs display reduced glycolysis and increased oxidative metabolism. These data have led to the hypothesis that the metabolism of distinct $CD4^+$ T-cell subsets may influence their fate and/or effector function. This raises an important question: can signal transduction pathways influence $CD4^+$ T-cell differentiation via changes in their metabolism? This is a difficult question to address. For example, the PI3K/Akt/mTOR pathway drives glycolytic metabolism and anabolic growth in T cells when nutrients, energy, and growth factors are in ample supply. Signaling via this pathway also antagonizes Treg differentiation. Overexpression of an active form of Akt (myr-Akt) blunts TGF-β-induced $Foxp3^+$ expression by $CD4^+$ T cells (121), a process that is partially dependent on mTORC1 signaling (122). Moreover, inhibition of mTOR signaling, either through genetic deletion or rapamycin treatment, promotes Treg differentiation while blunting Th differentiation and function (10, 13, 14). These results clearly indicate that PI3K/Akt/mTOR signaling can influence Treg differentiation. What remains unclear, however, is whether changes in glucose metabolism induced by PI3K/Akt/mTOR signaling are the driver of this process.

Given the hypothesis described above, how could LKB1/AMPK-dependent metabolic control influence the effector functions of $CD8^+$ and $CD4^+$ T cells? Increased glucose uptake, such as that observed in LKB1- or AMPKα1-deficient $CD8^+$ T cells, has been shown to enhance both T-cell activation and cytokine production (123). Thus, increased glucose metabolism associated with loss of LKB1/AMPK signaling may drive a feed-forward loop to promote the differentiation of proinflammatory Th cells through unknown mechanisms. Alternatively, regulation of metabolism by mTORC1 signaling may be involved in this process. Hyperactivation of mTORC1 contributes to the proinflammatory phenotype of $CD8^+$ T cells with reduced LKB1/AMPK signaling, as IFN-γ production in these T cells is blocked by rapamycin (39). Thus, engagement of mTOR through TCR-mediated signal transduction or specific loss of LKB1 signaling appears to favor the development of pro-inflammatory $CD4^+$ and $CD8^+$ T cells (*Fig.* 3). Interestingly, $CD4^+$ T-cell activation in the absence of mTOR leads to the development of Tregs rather than Th effector cells (10), and treatment of mice with metformin, which leads to AMPK-dependent inhibition of mTOR, promotes Treg differentiation *in vivo* (120). These data infer that energy stress may drive an LKB1/AMPK/mTOR metabolic checkpoint to promote Treg differentiation. The requirement of AMPK for T_h versus Treg function remains to be fully elucidated.

Conclusion

Cell metabolism is emerging as a key regulator of T-cell homeostasis and adaptive immunity. However, we still have much to learn about how metabolism is regulated in T cells and the influence that it has on T-cell-mediated immunity. Recent work investigating the role of LKB1/AMPK signaling in T cells has uncovered a novel function for this energy-sensing pathway in both T-cell development and peripheral T-cell function. Evidence to date suggests that LKB1 and AMPK regulate a metabolic switch in T cells that functions to antagonize anabolic pathways (glycolysis and mTORC1) and promote mitochondrial oxidative metabolism (lipid oxidation). Through the coordinated control of cellular metabolism, LKB1 and AMPK regulate a 'metabolic checkpoint' in

T cells that restricts T-cell growth in a fashion similar to that observed in cancer cells (124). However, it is clear that LKB1 and AMPK can also mediate independent functions in T cells, particularly with respect to thymocyte development and $CD4^+$ Th effector function, and may operate in distinct signaling pathways. Future work will focus on dissecting LKB1- and AMPK-specific effects in T lymphocytes, exploring the role of AMPK-related kinases in T-cell function, and investigating whether LKB1 or AMPK are involved in the regulation of inflammation or autoimmunity *in vivo*. The observation that silencing LKB1 or AMPKα1 expression in T cells promotes the development of pro-inflammatory T cells provides new insight into how T-cell-mediated inflammation is regulated. This raises an interesting question: can the LKB1-AMPK pathway be harnessed to regulate inflammatory responses? AICAR and metformin are well-known AMPK agonists that have shown efficacy in attenuating experimental autoimmune encephalomyelitis, an experimental autoimmune disease model (119, 125). Thus, manipulation of LKB1-AMPK signaling may provide a new therapeutic avenue to treat inflammation and autoimmune disease.

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