

T cell metabolism drives immunity

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Lymphocytes must adapt to a wide array of environmental stressors as part of their normal development, during which they undergo a dramatic metabolic remodeling process. Research in this area has yielded surprising findings on the roles of diverse metabolic pathways and metabolites, which have been found to regulate lymphocyte signaling and influence differentiation, function and fate. In this review, we integrate the latest findings in the field to provide an up-to-date resource on lymphocyte metabolism.

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Abbreviations used: ACC1, acetyl-CoA carboxylase 1; AMPK, adenosine monophosphate-activated protein kinase; ASS1, argininosuccinate 1; CPT1a, carnitine palmitoyl-transferase 1a; DN, double negative; DP, double positive; ERR α , estrogen-related receptor α ; ETC, electron transport chain; FAS, fatty acid synthesis; HAT, histone acetyltransferase; HDAC, histone deacetylase; IDO, indoleamine-2,3-dioxygenase; PDK1, pyruvate dehydrogenase kinase; PDPK1, 3-phosphoinositide-dependent protein kinase-1; PI3K, phosphatidylinositol 3-kinase; PKA, protein kinase A; PTM, post-translational modification; ROS, reactive oxygen species; TCA, tricarboxylic acid; TPPII, tripeptidyl peptidase II; TSC1, tuberous sclerosis complex 1.

"Part of the secret of success in life is to eat what you like and let the food fight it out inside."

— Mark Twain

Simply stated, we are what we eat. Our genetics, coupled with environmental influences, dictate how we metabolize the nutrients that we consume and how this shapes our growth, function, and overall health. The same principles hold true at the cellular level. Just as a track runner quickly engages their muscles to propel themselves from rest to sprint in response to a starting gun, pathogen-derived or inflammatory signals drive T cells out of quiescence, resulting in rapid modulation of gene expression and the acquisition of new functions. These changes range from increased production of cytokines and cytolytic molecules to the ability to undergo cell division and migration. Intimately integrated into this program of activation is the regulation of cellular metabolism.

The engagement of specific metabolic pathways profoundly affects cell differentiation and function. Metabolic reprogramming is controlled by key receptor signaling events and growth factor cytokines, as well as availability of nutrients. In addition, metabolic products provide substrates that can alter the functional fate of a cell through posttranslational modifications (PTMs) or epigenetic remodeling. Several recent articles have covered these and other emerging topics in T cell metabolism (Chapman and Chi, 2014; Bird, 2015; Lochner et al., 2015; O'Sullivan and Pearce, 2015; Palmer et al., 2015; Ramsay and Cantrell, 2015; Ron-Harel et al., 2015). In this

review, we provide a general but comprehensive overview of lymphocyte metabolism integrated with current research. Our focus will be on data and concepts derived primarily from T cell studies, with tie-ins from other fields when relevant.

T cell development and quiescence

Although the bulk of T cell metabolism research centers on mature T cells, even at their inception and throughout their development, T cells cycle through states of metabolic quiescence and activation. Hematopoietic stem cell progenitors that are double negative (DN) for CD4 and CD8 co-receptors migrate from the bone marrow and seed the thymus where they rearrange their antigen receptor gene loci to produce a functional TCR. Signals from the receptor Notch1 maintain cell survival and promote T cell lineage commitment (Pui et al., 1999; Radtke et al., 1999; Maillard et al., 2006). Induced deletion of Notch1 during neonatal development results in arrest at the most immature DN1 (CD44⁺CD25⁻) stage (Radtke et al., 1999), whereas enforced expression of constitutively active Notch1 in bone marrow cells blocks B cell differentiation and instead causes the ectopic development of CD4⁺CD8⁺ double-positive (DP) T cells (Pui et al., 1999).

Successful expression of TCR β with pT α and CD3 forms the pre-TCR, which signals with Notch1 to drive cells out of quiescence as they enter β selection (Saint-Ruf et al., 1994; Ciofani et al., 2004; Ciofani and Zúñiga-Pflücker, 2005). RAG recombinase expression declines and expression of the transferrin receptor CD71 and other nutrient transporters are induced as the

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cells proliferate (Ciofani and Zúñiga-Pflücker, 2005; Kelly et al., 2007). Signaling from the pre-TCR, Notch1, and the chemokine receptor CXCR4 converge to activate phosphatidylinositol 3-kinase (PI3K); this stimulates the switch to anabolic metabolism (metabolic pathways that create biomass from smaller molecules; Ciofani and Zúñiga-Pflücker, 2005; Janas et al., 2010). Increased expression of the glucose transporter Glut1 is required during this stage, and its expression is dependent on activation of the kinase Akt by PI3K (Swainson et al., 2005; Juntilla et al., 2007; Wieman et al., 2007). PI3K–Akt signaling also activates the mechanistic target of rapamycin (mTOR), and signals from this kinase augment the glycolytic metabolism used to support cell growth and proliferation (MacIver et al., 2013).

Disruptions in PI3K signaling also affect the transition of DP thymocytes to single-positive CD4 and CD8 T cells and the development of NKT cells, which require sustained signaling to join *trav*α to distal *Ja* gene segments that define their invariant TCR (D'Cruz et al., 2010; Rodríguez-Borlado et al., 2003). PTEN (phosphatase and tensin homologue) is the principal negative regulator of the PI3K pathway. Thymocytes from mice that lack the microRNA cluster miR-181a1b1 have altered cellular metabolism caused by a significant increase in PTEN expression (Henao-Mejia et al., 2013). Glucose uptake, measured by acquisition of the fluorescent glucose analogue 2-NBDG, and glycolytic rate are reduced in these cells, and nutrient transporter expression is diminished. As a result of dysregulated PI3K signals, these mice have deficiencies in DP cells and completely lack NKT cells (Henao-Mejia et al., 2013).

The cytokine IL-7 has a pivotal role in ensuring the survival of developing and quiescent naive T cells by increasing expression of the antiapoptotic factor Bcl-2 (B cell lymphoma 2; Akashi et al., 1997; Maraskovsky et al., 1997; Tan et al., 2001; Yu et al., 2003). Mice deficient in IL-7 or the IL-7Rα chain have defects in T cell development (Peschon et al., 1994; von Freeden-Jeffry et al., 1995). IL-7 signals through the JAK3–STAT5 pathway but can also activate PI3K (Pallard et al., 1999; Wofford et al., 2008). A recent study suggests that in addition to maintaining the survival of developing lymphocytes, IL-7 signaling promotes the growth and proliferation of DN4 cells by increasing levels of trophic receptors, such as CD71 and the amino acid transporter CD98 (Pearson et al., 2012; Boudil et al., 2015), activities that were previously attributed mainly to Notch1 signaling. However, Notch1 can induce IL-7Rα expression and therefore its effects could be downstream of IL-7 signals (González-García et al., 2009; Magri et al., 2009).

Mature naive T cells exit from the thymus into the periphery. As quiescent cells, they primarily oxidize glucose-derived pyruvate in their mitochondria via oxidative phosphorylation (OXPHOS), or they use fatty acid oxidation (FAO) to generate ATP (Fig. 1; Fox et al., 2005; Wang et al., 2011; van der Windt and Pearce, 2012; Pearce and Pearce, 2013; Pearce et al., 2013). A balance between tonic TCR signals and IL-7 is needed to sustain naive T cells. Homeostatic proliferation of naive T cells is supported by TCR ligation with self-peptides presented on MHC molecules in the periphery (Ernst et al., 1999; Goldrath

and Bevan, 1999; Muranski et al., 2000). However, unrestrained Akt activation, or deletion of negative regulators of TCR stimulation, leads to loss of quiescence (Yang et al., 2011). T cells defective in tuberous sclerosis complex 1 (TSC1), a negative regulator of mTOR signaling, prematurely exit from quiescence and have increased rates of apoptosis and hyperactive responses to TCR stimulation (Yang et al., 2011). In addition, TCR-mediated PI3K–Akt activation down-regulates IL-7Rα (Cekic et al., 2013), but, as discussed in the previous paragraph, IL-7 signaling is essential to prevent apoptosis and ensure survival of the naive T cell pool (Rathmell et al., 2001; Surh and Sprent, 2008). A recent study showed that the metabolite adenosine, which is a byproduct of metabolic activity, suppresses TCR signaling in a dose dependent manner (Cekic et al., 2013). The G-protein-coupled adenosine receptor subtype A_{2A}R is predominantly expressed in T cells. Binding with adenosine activates cAMP-dependent protein kinase A (PKA), which suppresses TCR-mediated activation of the PI3K pathway and prevents IL-7Rα down-regulation (Cekic et al., 2013).

Activation and effector T cell differentiation

Metabolic reprogramming during T cell activation. Once in the periphery, a mature naive T cell is like a bomb, lying dormant in the lymphoid organs and circulation until it is triggered to activate and explode in a proliferative chain reaction. T cell activation stimulated by TCR ligation and binding with costimulatory molecules induces metabolic remodeling of the naive T cell to a program of anabolic growth and biomass accumulation; this is marked by the engagement of aerobic glycolysis, a process in which glucose is converted into lactate even though sufficient oxygen is present to support glucose catabolism via the tricarboxylic acid (TCA) cycle and OXPHOS (Fig. 1; Vander Heiden et al., 2009; MacIver et al., 2013). Although aerobic glycolysis is less efficient than OXPHOS at yielding an abundance of ATP per molecule of glucose, aerobic glycolysis can generate metabolic intermediates important for cell growth and proliferation, and provides a way to maintain redox balance (NAD⁺/NADH) in the cell (Fig. 2; Vander Heiden et al., 2009; Anastasiou et al., 2011; Macintyre and Rathmell, 2013). For example, glucose-6-phosphate and 3-phosphoglycerate (3PG) produced during glycolysis can be metabolized in the pentose phosphate and serine biosynthesis pathways, respectively, donating important precursors for nucleotide and amino acid synthesis (Wang et al., 2011; Pearce et al., 2013). Glucose can also enter the mitochondria as pyruvate, where it is converted to acetyl-CoA and joins the TCA cycle by condensing with oxaloacetate to form citrate. Breakdown of substrates in the TCA cycle not only provides reducing equivalents for OXPHOS, but also precursors for biosynthesis. Glucose-derived citrate can be exported into the cytosol to generate acetyl-CoA by ATP citrate lyase (ACL) for use in lipid synthesis (Bauer et al., 2005; Hatzivassiliou et al., 2005; DeBerardinis et al., 2008). Similarly, oxaloacetate can be used to produce aspartate, an additional precursor for generating nucleotides (Fig. 2; DeBerardinis et al., 2007).

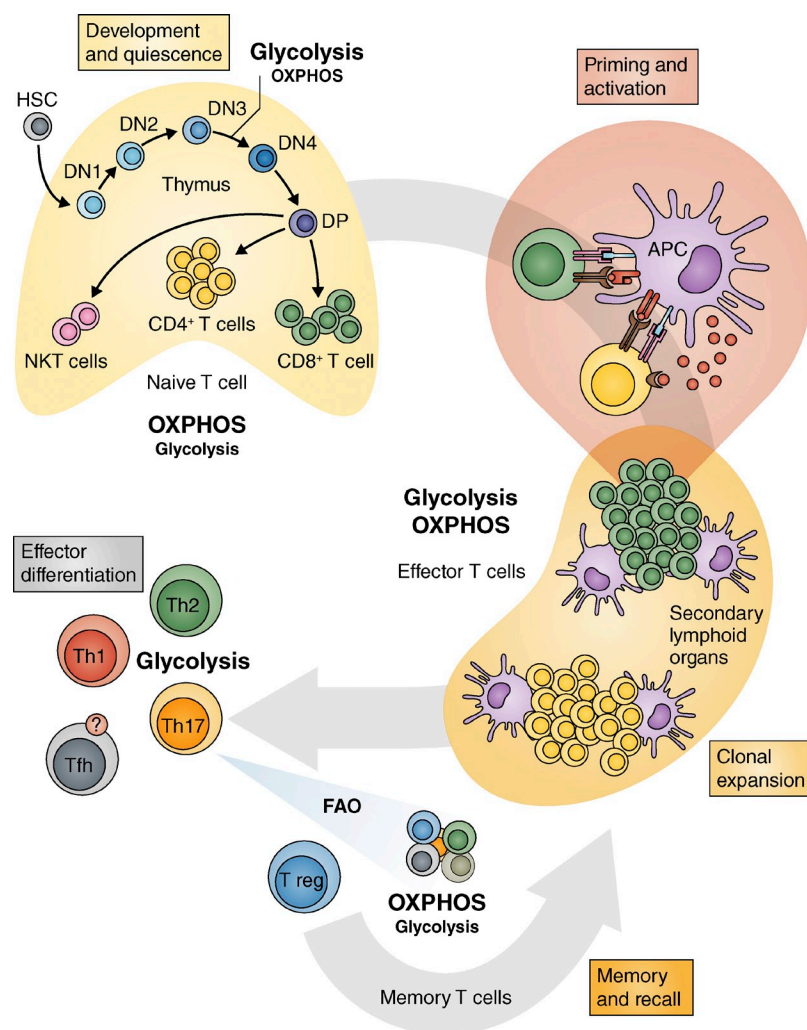


Figure 1. Metabolism drives the life cycle of T cells.

T cells engage specific metabolic pathways during development that underpin their differentiation and function. Naive T cells mature and exit from the thymus primarily relying on OXPHOS for their metabolic needs, although they augment with glycolytic metabolism during times of proliferation that follow TCR gene rearrangements. In secondary lymphoid organs, TCR ligation, costimulation, and growth factor cytokine signals induce clonal expansion and metabolic reprogramming of an antigen-specific T cell. This conversion to an activated effector T cell is marked by the engagement of aerobic glycolysis and increased OXPHOS activity. Glycolytic metabolism differentiates CD4 Th1, Th2, and Th17 effector cells (and possibly Tfh cells) from T reg cells. Promoting FAO and catabolic metabolism enhances T reg and memory T cell development (blue arrow). Memory T cells are a quiescent population of cells that primarily use OXPHOS, but both OXPHOS and glycolysis increase rapidly after antigen rechallenge and facilitate their recall responses.

Several transcription factors and signaling pathways coordinately support and regulate this change in T cell metabolic programs after activation. Growth factor cytokines such as IL-2 and ligation of costimulatory molecules promote the switch to glycolysis through the enhancement of nutrient transporter expression and activation of the key metabolic regulator mTOR (Fig. 1; Frauwirth et al., 2002; Jones and Thompson, 2007; Wieman et al., 2007; Kolev et al., 2015). mTOR exists as two complexes, mTORC1 and mTORC2, and integrates extrinsic and intrinsic signals related to nutrient levels, energy status, and stress to induce changes in cellular metabolism, growth, and proliferation (Laplanche and Sabatini, 2012). CD28 ligation enhances PI3K activity, which recruits 3-phosphoinositide-dependent protein kinase-1 (PDK1) and Akt. PDK1, together with mTORC2, phosphorylates Akt, which in turn activates mTORC1. Both Akt and mTOR promote aerobic glycolysis and support effector T cell differentiation, growth, and function (Delgoffe et al., 2011; Pollizzi et al., 2015). Akt regulates nutrient transporter expression and can phosphorylate the glycolytic enzyme hexokinase II, promoting its localization to the mitochondria and augmenting its enzymatic

activity (Miyamoto et al., 2008; John et al., 2011). mTORC1 activation increases protein translation via phosphorylation of 4E-BP1 and p70S6 kinase (Laplanche and Sabatini, 2012) and promotes lipid synthesis by activating SREBP2 (sterol regulatory element-binding protein 2; Porstmann et al., 2008).

The up-regulation of transcription factors c-Myc, estrogen-related receptor α (ERR α), and hypoxia inducible factor-1 α (HIF-1 α) coordinately drives the expression of genes involved in intermediary metabolism that fuel the rapid proliferation of effector T cells during clonal expansion (Michalek et al., 2011b; Wang et al., 2011; Doedens et al., 2013). First discovered as an oncogene important for cell growth and proliferation (Sheiness et al., 1978; Cole, 1986), c-Myc has been shown to be a critical regulator of metabolic reprogramming after T cell activation (Wang et al., 2011). c-Myc drives the expression of enzymes that promote aerobic glycolysis and glutaminolysis and coordinates these metabolic pathways with lipid, amino acid, and nucleic acid synthesis. However, c-Myc expression is not continually sustained after T cell activation (Nie et al., 2012; Best et al., 2013). A recent study suggests that c-Myc induces the transcription factor AP4, which maintains the

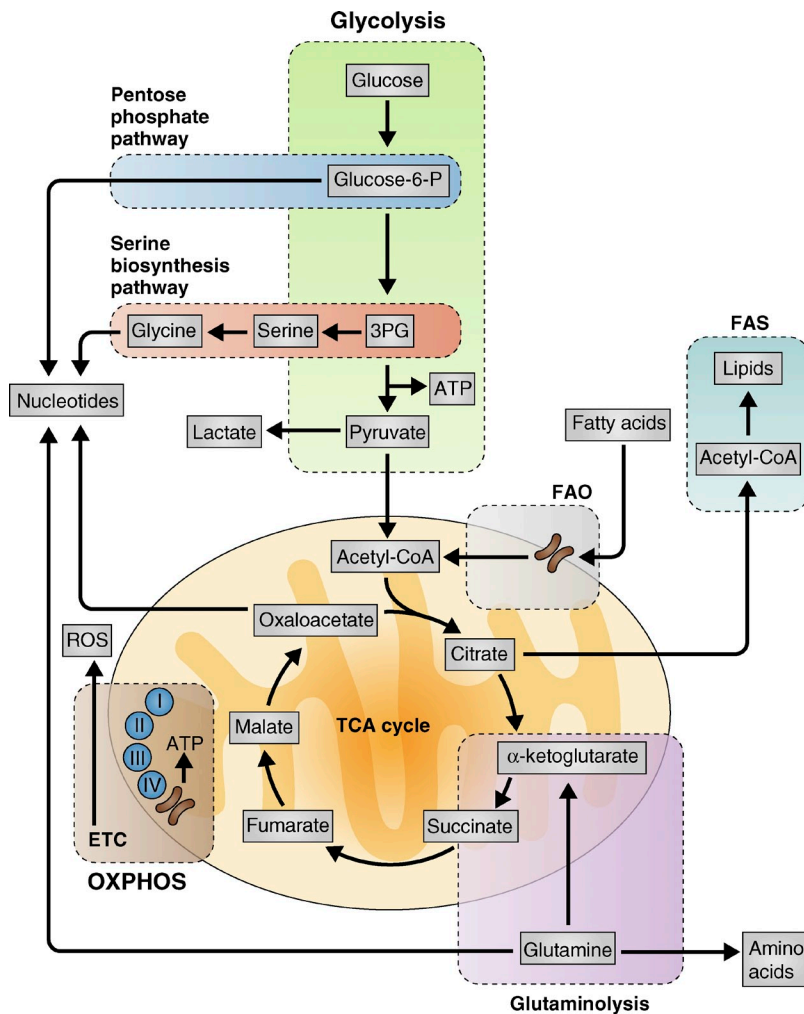


Figure 2. Metabolic pathways that support T cells. ATP is the molecular currency of energy in the cell. It can be derived from glucose through two integrated pathways. The first of these, glycolysis (green), involves the enzymatic breakdown of glucose to pyruvate in the cytoplasm. The TCA cycle (orange) encompasses the second pathway, where pyruvate is converted to acetyl-CoA in the mitochondria and shuttled through several enzymatic reactions to generate reducing equivalents to fuel OXPHOS (brown). Other substrates can also be metabolized in the TCA cycle, such as glutamine via glutaminolysis (purple) or fatty acids via β -oxidation (FAO; gray). These connected biochemical pathways can also provide metabolic precursors for biosynthesis. Intermediates from glucose catabolism during glycolysis can shuttle through the pentose phosphate (dark blue) and serine biosynthesis pathways (red) to fuel nucleotide and amino acid production. Oxaloacetate from the TCA cycle can similarly be used to generate aspartate for use in nucleotide synthesis. Precursors for amino acid and nucleotide biosynthesis can be obtained from glutamine. Citrate from the TCA cycle can be exported from the mitochondria and converted to acetyl-CoA for FAS (light blue). ROS generated from the ETC during OXPHOS can also act as secondary signaling molecules.

glycolytic transcriptional program initiated by c-Myc to support T cell population expansion (Chou et al., 2014; Karmaus and Chi, 2014). HIF-1 α , a transcription factor that responds to oxygen levels, also increases glucose uptake and catabolism through glycolysis (Kim et al., 2006; Finlay et al., 2012). Deletion of its negative regulator, von Hippel-Lindau (VHL), enhances HIF-1 α -mediated CD8 T cell glycolysis and effector responses to persistent viral infection (Doedens et al., 2013).

ROS signaling. Although much of the attention on metabolic reprogramming in activated T cells has focused on the engagement of aerobic glycolysis, recent research has revealed the importance of mitochondrial-driven activities in this process. In addition to energy production, the electron transport chain (ETC) is a major source of reactive oxygen species (ROS; Turrens, 2003), which are important for T cell responses (Fig. 2; Chaudhri et al., 1988; Devadas et al., 2002; Jones et al., 2007). T cells deficient for Rieske iron sulfur protein (RISP), a subunit of mitochondrial complex III, have impaired activation and antigen-specific T cell expansion *in vitro* and *in vivo* due to defects in mitochondrial-derived ROS signaling (Sena et al., 2013). More recently, using a forward genetic screen, another

group identified mice with enhanced CD8 T cell responses to viral and tumor challenge (Okoye et al., 2015). The source of the heightened immunity gained after germline mutagenesis was the increased expression of an orphan protein, identified as lymphocyte expansion molecule (LEM). Interestingly, augmented OXPHOS and mitochondrial ROS levels were detected in CD8 T cells isolated from LEM-deficient mice after infection, whereas heterozygous LEM-deficient CD8 T cells had reduced OXPHOS and mitochondrial ROS levels. LEM helps stabilize a protein involved in inserting ETC complex proteins in the mitochondrial membrane, which may account for the increased ROS and enhanced proliferation evident in CD8 T cells from these mice (Okoye et al., 2015).

Although ROS is produced as a general byproduct of mitochondrial metabolism, new studies have specifically linked the metabolite succinate to both the generation of ROS and activation of HIF-1 α in settings of inflammation or injury (Tannahill et al., 2013; Chouchani et al., 2014). Innate immune receptor activation increases intracellular succinate from glutamine via glutamine-dependent anaplerosis and the γ -aminobutyric acid shunt pathway, and this leads to HIF-1 α stabilization and activation (Tannahill et al., 2013). During

ischemia reperfusion injury, which happens when blood supply to a tissue is disrupted and then restored, succinate accumulates from reverse activity of the enzyme succinate dehydrogenase (SDH) and is rapidly oxidized upon reperfusion. This leads to overreduction of the electron carrier coenzyme Q, causing reverse electron transport through mitochondrial complex I and, subsequently, excessive ROS production (Chouchani et al., 2014; O'Neill, 2014). Given that mitochondrial ROS and HIF-1 α activity are important for the metabolic reprogramming of naive T cells after activation, it is interesting to speculate that the metabolite succinate may also support the transition from a naive to an activated effector T cell.

Metabolic programming of T helper cell differentiation.

Activation of T cells is intimately tied to the engagement of specific metabolic pathways, so it is no surprise that distinct metabolic programs also support the differentiation of CD4 T helper (Th) cells into their separate lineages. Initial studies found that suppression of mTOR with rapamycin promoted the generation of FoxP3⁺ regulatory T (T reg) cells even in the presence of Th17-polarizing cytokines *in vitro* (Kopf et al., 2007), and genetic deletion of mTOR in T cells augmented production of T reg cells upon activation, but not Th1, Th2, or Th17 cells (Delgoffe et al., 2009). These results are consistent with the metabolic profiles of these cells: Th1, Th2, and Th17 cells strongly engage glycolysis via mTOR signaling, whereas T reg cells depend more on the oxidation of lipids (Fig. 1; Michalek et al., 2011a). Th17 cells in particular have been found to heavily rely on glycolysis for their development and maintenance, stimulated by HIF-1 α activity downstream of mTOR. Mice deficient in HIF-1 α have increased generation of T reg cells, and blocking glycolysis with 2-Deoxy-D-glucose (2-DG) inhibits Th17 cell differentiation (Dang et al., 2011; Shi et al., 2011). T reg cell homeostasis and survival depends on the delicate balance between mTORC1 activation from PI3K-Akt and regulation from PTEN (Zeng et al., 2013; Huynh et al., 2015; Shrestha et al., 2015). Signaling through mTORC1 versus mTORC2 also selectively differentiates CD4 T cells into the Th1 and Th2 lineages, respectively (Lee et al., 2010; Delgoffe et al., 2011), although activation of mTORC1 and its component Raptor is still required for T cell exit from quiescence to begin the transition into Th2 cells (Yang et al., 2013). Less is known about T follicular helper (Tfh) cell metabolism compared with other T cell subsets, but their lineage-defining transcription factor Bcl6 has been shown to suppress glycolysis potentiated by c-Myc and HIF-1 α (Johnston et al., 2009; Nurieva et al., 2009; Oestreich et al., 2014).

Substrate utilization in activated T cells

Glucose is a key metabolic substrate for T cells. Upon T cell activation, Glut1 traffics to the cell surface from intracellular vesicles (Rathmell et al., 2000; Frauwirth et al., 2002; Wieman et al., 2007). Overexpression of Glut1 in mice results in larger naive T cells and an increased number of CD44^{hi} T cells, suggesting that glucose acquisition mediates early steps in T cell activation, such as promoting the expression of activation

markers and increasing cell size (Jacobs et al., 2008). Consistent with these observations, T cell specific deletion of Glut1 impairs CD4 T cell activation, clonal expansion, and survival (Macintyre et al., 2014). When deprived of glucose, CD8 T cells display defects in functional capacity with reduced IFN- γ , granzyme, and perforin production (Cham and Gajewski, 2005; Cham et al., 2008; Jacobs et al., 2008). More recently, it was shown that T cells can become activated and proliferate when glucose catabolism through aerobic glycolysis is limited, as they can rely on OXPHOS (Chang et al., 2013; Sena et al., 2013). However, in this case, effector function is compromised, with impaired cytokine production caused by posttranscriptional regulation by the glycolytic enzyme GAPDH. When disengaged from glycolysis, GAPDH can function as a RNA-binding protein (RBP) and prevent the translation of cytokine messenger RNAs containing AU-rich elements in their 3'-UTRs (Chang et al., 2013). Therefore, in addition to providing precursors for biomass, augmenting aerobic glycolysis in activated T cells allows for the acquisition of full effector function.

Amino acids. Although glucose is a critical substrate for T cells, glutamine is also essential during T cell activation (Frauwirth et al., 2002; Carr et al., 2010; Wang et al., 2011). T cells increase the expression of glutamine transporters, and their deletion impairs the transition to an effector T cell (Carr et al., 2010; Sinclair et al., 2013). Clear differences in concentrations of other amino acids also exist in quiescent compared with activated T cells, corresponding to their distinct metabolic requirements (Pearson et al., 2012; Ananieva et al., 2014). New research has begun to uncover the vast array of additional amino acid transporters and catabolizing enzymes that regulate amino acid levels, revealing previously unappreciated roles for amino acids in T cell metabolism and function.

Deficiency in the neutral amino acid transporter Slc7a5 (LAT1), which transports leucine, prevents the metabolic reprogramming, clonal expansion, and/or effector function of both CD4 and CD8 T cells (Hayashi et al., 2013; Sinclair et al., 2013). These cells had impaired mTORC1 activation and were unable to induce key metabolic processes, such as enhancing glutamine and glucose uptake (Sinclair et al., 2013). This deficiency, however, did not impair the ability of CD4 T cells to differentiate into T reg cells. Leucine can activate mTOR via leucyl-tRNA synthetase, and thus it is not surprising that reduced leucine uptake impairs mTOR activation (Han et al., 2012). However, the effects of Slc7a5 deficiency were more severe than those induced by mTOR inhibition using rapamycin (Sinclair et al., 2013), suggesting either that rapamycin may not have completely blocked mTOR activation, or that leucine deficiency has effects over and above limiting mTOR activation (Thoreen et al., 2009; Powell, 2013). Additionally, although no overt decrease in global protein expression occurred in Slc7a5-deficient cells, protein expression of the key metabolic transcription factor, c-Myc, was diminished, despite its increased mRNA expression upon activation (Sinclair et al., 2013). This raises the intriguing question of whether leucine deficiency

results in posttranslational regulation of c-Myc expression. Alternatively, this effect could simply result from a limitation in the supply of amino acids in Slc7a5-deficient cells, which is not sufficient to keep up with the demands of synthesizing proteins such as c-Myc, which have a short half-life (Sinclair et al., 2013).

Results from another study suggest that modulation of intracellular leucine concentrations can be used to regulate metabolic reprogramming. It was found that the expression of the cytosolic branched chain aminotransferase (BCATc), which can reduce intracellular leucine concentrations through a transamination reaction, limited mTORC1 activation (Ananieva et al., 2014). BCATc expression was up-regulated upon CD4 T cell activation, and T cells that lacked BCATc had increased intracellular leucine, which correlated with enhanced activation of mTORC1 and glycolytic phenotype. Increased BCATc expression has been observed in anergic T cells, which have impaired metabolic function (Zheng et al., 2009; Ananieva et al., 2014). These data could suggest that leucine depletion by BCATc contributes to T cell anergy through suppression of mTOR activity.

The alanine serine and cysteine transporter system (ASCT2/Slc1a5), which also transports glutamine, is another solute carrier whose expression increases after T cell activation (Levring et al., 2012). It was recently found that loss of ASCT2 decreased glutamine import and impaired OXPHOS and glucose metabolism in activated CD4 T cells (Nakaya et al., 2014). Surprisingly, the loss of ASCT2 did not inhibit proliferation or IL-2 production. However, ASCT2-deficient cells cultured in vitro had a decreased ability to differentiate into Th1 and Th17 cells, but not Th2 or T reg cells. Interestingly, glutamine transport into cells can substantially enhance leucine transport via Slc7a5, as increased intracellular glutamine levels result in glutamine export and concomitant import of leucine by this transporter (Nicklin et al., 2009). Supporting this additional role for glutamine in T cell activation, addition of leucine to T cells lacking ASCT2 helps rescue their polarization defects (Nakaya et al., 2014).

Depletion of extracellular arginine has been found to impair T cell proliferation and aerobic glycolysis, but not mitochondrial OXPHOS (Fletcher et al., 2015). However, provided that extracellular concentrations of citrulline are sufficient, T cells can partially compensate by synthesizing arginine de novo via an argininosuccinate 1 (ASS1)-dependent process (Qualls et al., 2012; Fletcher et al., 2015; Tarasenko et al., 2015). One study suggested that ASS1 activity may contribute to T cell function in ways beyond simple synthesis of arginine, as deletion of ASS1 can negatively impact in vitro Th1 and Th17 cell polarization, even in the presence of extracellular arginine (Tarasenko et al., 2015). Arginine metabolism also has a role in macrophage polarization and dictating metabolic phenotype (Galván-Pena and O'Neill, 2014; Rath et al., 2014), but whether arginine metabolism also has such roles in T cells remains to be determined.

A recent study suggests that intracellular recycling of amino acids also contributes to T cell amino acid homeostasis.

Deficiency in cytosolic protease tripeptidyl peptidase II (TPPII), which digests proteins for the recycling of amino acids, lead to increased sensitivity to perturbations in intracellular amino acids concentrations, impaired IFN- γ production, and a susceptibility to viral infections (Lu et al., 2014). Lack of TPPII activity in both human and murine T cells resulted in impaired glycolysis caused by enhanced degradation of the key glycolytic enzyme hexokinase II, an effect that likely contributed to their impaired cytokine production (Lu et al., 2014). Another study also found that TPPII deficiency caused susceptibility to viral infections, although under these particular experimental conditions, defects in cytokine production were not observed and the T cell dysfunction was attributed to premature immunosenescence (Stepensky et al., 2015). Although it remains to be tested, this premature cell senescence could be linked to reduced glycolytic flux. In a model of oncogenic stress-induced senescence, inhibition of hexokinase II or glucose uptake induces senescence in human epithelial cells (Gitenay et al., 2014).

Many products of amino acid catabolism also have important nonanaplerotic roles that can alter cell signaling and function. The metabolic byproduct of tryptophan catabolism, kynurenine, can ligate the aryl hydrocarbon receptor and enhance polarization of CD4 T cells to a T reg phenotype (Mezrich et al., 2010; Opitz et al., 2011). Another example of this is catabolism of phenylalanine by IL-4-induced gene 1 protein (IL4I1). When highly expressed by tumors or APCs, IL4I1 can inhibit T cell proliferation (Boulland et al., 2007; Lasoudris et al., 2011). This effect appears to be caused by the production of H₂O₂, a product of phenylalanine catabolism. IL4I1 is also expressed in Th17 and T reg cells (Santarasci et al., 2012; Scarlata et al., 2015). The specific purpose for IL4I1 expression in these T cell subsets remains ambiguous, although its expression in Th17 cells was speculated to have a self-regulatory role, where its induction led to diminished proliferation (Santarasci et al., 2012). However given that low concentrations of H₂O₂ can act as a signaling molecule (Veal et al., 2007), IL4I1 might also play a role in cell signaling pathways independent of mechanisms that inhibit proliferation.

Fluctuations in environmental amino acid concentrations, as well as metabolic products from amino acid catabolism, can dramatically alter T cell activity and polarization. A well-documented example of this is indoleamine-2,3-dioxygenase (IDO)-mediated tryptophan catabolism. IDO, which is often expressed at high levels by APCs or tumor cells, can deplete tryptophan within a tissue microenvironment, and this in turn can lead to inhibition of effector T cell proliferation and induction of anergy (Munn et al., 2002; Uyttenhove et al., 2003). Depletion of tryptophan causes activation of the integrated stress response inducer general control nonderepressible 2 (GCN2) kinase, which results in the inhibition of translation initiation and metabolic remodeling (Munn et al., 2005; Guo and Cavener, 2007; Castilho et al., 2014).

Studies into the interactions of APCs with T cells have highlighted multiple pathways through which APCs modulate extracellular concentrations of amino acids, or their catabolic

products, to regulate T cell responses. In a tumor, TGF- β -producing DCs can enhance expression of transporters for histidine, leucine, valine, and tryptophan, depleting these amino acids from the extracellular microenvironment and directly impairing T cell proliferation (Angelini et al., 2002). T reg cells can also enhance expression of particular amino acid catabolizing enzymes, including arginase 1, histidine decarboxylase, threonine dehydrogenase, and IL4I1, in skin grafts and bone marrow-derived DCs (Cobbold et al., 2009). Limitations in these amino acids, singularly or in combination, enhanced T reg cell polarization when T cells were activated in vitro (Cobbold et al., 2009). Although depletion of amino acids from the microenvironment appears to be a way in which APCs can negatively regulate T cell activity, the opposite also occurs, whereby APCs can support T cell activation through supplementing a microenvironment. For example, DCs and monocytes can release cysteine, which is thought to support T cell activation and function (Sido et al., 2000; Angelini et al., 2002). Cysteine supply is a limiting factor in T cell proliferation and is used extensively for protein and glutathione synthesis, as well as providing beneficial catabolic products, such as taurine, which may support T cell function through regulating osmolality (Kaesler et al., 2012; Sikalidis, 2015).

Lipid metabolism. Lipids or fatty acids encompass another critical substrate group for T cells. They are a vital component of cell membranes, provide a high yielding energy source, and can also supply substrates for cell signaling and PTMs (Lochner et al., 2015; Thurnher and Gruenbacher, 2015). After T cell activation, the demand for lipids rapidly increases. Within 24 h, in vitro-activated T cells augment fatty acid synthesis (FAS), while concomitantly decreasing FAO, thus enhancing the accumulation of fatty acid metabolites needed for membrane synthesis (Wang et al., 2011). c-Myc and mTOR have important roles in coordinating these metabolic changes (Wang et al., 2011; Yang et al., 2013), and SREBP transcription factors are critical for reprogramming lipid metabolism (Kidani et al., 2013). SREBPs induce expression of genes involved in FAS and mevalonate pathways, which supply de novo synthesized fatty acids and cholesterol, respectively (Thurnher and Gruenbacher, 2015). CD4 T cells deficient in Raptor, and thus mTORC1 signaling, have impaired de novo FAS, most likely caused by reduced expression of SREBP1 and SREBP2 protein (Yang et al., 2013).

Loss of SREBP function in CD8 T cells results in a failure to induce metabolic pathways needed for clonal expansion during a viral infection (Kidani et al., 2013). Exogenous cholesterol rescues the defects in SREBP-deficient T cells, suggesting that at least in this context, a lack of cholesterol is the main limiting factor. This requirement for cholesterol synthesis is consistent with results showing that perturbing sterol homeostasis in activated T cells—by activating the liver X receptor (LXR), which targets genes that are involved in cholesterol cellular export—impairs T cell proliferation. The inhibitory effect of LXR activation can be overcome through the addition of mevalonate, a cholesterol precursor (Bensinger

et al., 2008). Inhibition of 3-hydroxy-3-methylglutaryl-coenzymeA (HMG-CoA) reductase, an enzyme in the mevalonate pathway, results in a Th2 cell bias in the experimental autoimmune encephalomyelitis (EAE) disease model, due to impaired biosynthesis of isoprenoids and a subsequent reduction in prenylation of Ras and RhoA GTPases (Youssef et al., 2002). These data suggest that in addition to cholesterol homeostasis, other products of the mevalonate pathway can also influence T cell differentiation. The impact of commonly used drugs that lower cholesterol by inhibiting HMG-CoA reductase can affect both prenylation and cholesterol synthesis, and thus it is plausible these drugs have multiple effects on activated T cells.

The synthesis of fatty acids is also important for effector T cell function. Although activated T cells readily acquire and use extracellular fatty acids, it appears that there may also be cell-intrinsic requirements for de novo-synthesized fatty acids (Berod et al., 2014; Lee et al., 2014; O'Sullivan and Pearce, 2014; O'Sullivan et al., 2014). Inhibition of acetyl-CoA carboxylase 1 (ACC1), an enzyme in FAS, was shown to limit Th17 cell differentiation and promote the development of T reg cells. This effect translated into improved disease outcomes in EAE (Berod et al., 2014). Inhibition of ACC1 impaired phospholipid synthesis in Th17 cells while also impairing glycolytic flux, both through aerobic glycolysis and the TCA cycle. In contrast, T reg cells were able to sustain their requirements for fatty acids through acquisition from extracellular sources (Berod et al., 2014). ACC1 deficiency also impairs Th1 and Th2 development, suggesting that CD4 effector T cells have a common requirement for FAS (Berod et al., 2014). In contrast, T cell-specific deletion of ACC1 does not impair CD8 effector T cell development after infection, although effector T cell expansion is diminished due to increased cell death, indicating that FAS is required for the persistence of CD8 effector T cells (Lee et al., 2014). Collectively, these findings suggest that there are varying requirements for de novo-synthesized fatty acids between different T cell subsets. Interestingly, defects after ACC1 inhibition in either Th17 cells or CD8 effector T cells can be rescued through the addition of excess free fatty acids to the media (Berod et al., 2014; Lee et al., 2014), indicating that these cells can compensate for the lack of FAS if the extracellular fatty acid supply is plentiful. Addition of extracellular fatty acids can also enhance T cell proliferation (Gorjão et al., 2007). It is plausible that the demand for fatty acids is so substantial in these highly proliferative populations that de novo FAS can be supplemented through extracellular uptake. This concept would be consistent with a recent study suggesting that lipid released from adipose tissue may enhance T cell proliferation in vivo (Kim et al., 2015b). Responses to TNF-mediated signaling in the hypothalamus induced B and T cell proliferation in the spleen, an effect mediated by an induction of lipolysis through sympathetic nervous system signaling to adipose tissue and a resultant increase in circulating leptin and free fatty acids (Kim et al., 2015b).

Although in general the balance of FAS to FAO within effector T cell populations is weighted heavily toward FAS, effector T cells can use FAO (Byersdorfer et al., 2013; O'Sullivan

et al., 2014). Given that the demand for energy is high in these cells, it is likely that they need some metabolic flexibility in their fuel sources, an idea that is consistent with recent work highlighting the importance of adenosine monophosphate-activated protein kinase (AMPK) in effector T cell function (Blagih et al., 2015). The extent to which FAO occurs in effector T cells is likely to be highly context dependent, in part due to the heterogeneity of this population of cells during an immune response. Studies using animal models of graft versus host disease (GvHD) have found that alloreactive T cells increase fatty acid uptake and enhance FAO compared with other effector T cells (Gatza et al., 2011; Byersdorfer et al., 2013; Glick et al., 2014). Suppressing Akt during activation can induce a metabolic profile suggestive of FAO utilization (Crompton et al., 2015), and culturing CD8 effector T cells in low glucose enhances FAO (O'Sullivan et al., 2014). A recent report also found that inhibition of T cell signaling through ligation of PD-1 induces changes in the metabolic profile of activated T cells, including decreased aerobic glycolysis and enhanced FAO (Patsoukis et al., 2015). Collectively, these data suggest that the utilization of FAO in effector T cells may be influenced by several factors, such as activation state, exposure to antigen, inflammatory signals, and microenvironmental nutrient availability.

Memory T cell metabolism

Effector T cell populations contract after pathogen clearance and undergo apoptosis, leaving behind a small population of long-lived memory T cells that can respond vigorously upon antigen rechallenge (Williams and Bevan, 2007). Although both naive and memory T cells acquire effector functions upon activation, memory T cells have an accelerated response to antigen, proliferate faster, and produce more cytokines than their naive counterparts. Work from our laboratory and others has shown that changes in metabolism also drive memory T cell development (Fig. 1; Araki et al., 2009; Pearce et al., 2009; Rao et al., 2010).

AMPK and mTOR. Increases in intracellular AMP-to-ATP concentrations activate the energy stress sensor AMPK, a signal that also promotes FAO (Jones and Thompson, 2007). AMPK is important for the development of memory T cells, and administration of the metabolic stressor and AMPK activator metformin enhances the generation of memory T cells after infection (Pearce et al., 2009; Rolf et al., 2013). In addition, AMPK allows for effector T cells to metabolically adapt during nutrient stress and modulates T cell effector function through suppression of mTOR (Tamás et al., 2006; MacIver et al., 2011; Blagih et al., 2015). Inhibiting mTOR with rapamycin boosts memory T cell development in vivo (Araki et al., 2009; Pearce et al., 2009; Rao et al., 2010). Loss of the mTORC1-negative regulator TSC1 compromises formation of memory T cell precursors that are present during the primary effector response (Kaeck et al., 2003; Shrestha et al., 2014). Similarly, suppressing mTORC2 fosters memory T cell generation (Pollizzi et al.,

2015). Inhibition of mTOR and activation of AMPK also strongly stimulate the catabolic process of autophagy. Autophagy has been shown to support T cell viability and bioenergetics after activation (Pua et al., 2007; Hubbard et al., 2010). Consistent with the idea that activation of catabolic pathways promotes the development of memory T cells, a recent study found that deletion of the autophagy molecules Atg5 or Atg7 compromised the formation of CD8 memory T cells after viral infection (Xu et al., 2014).

FAO and mitochondria. Work from our laboratory has shown that CD8 memory T cells are dependent on FAO for their development, long-term persistence, and ability to robustly respond to antigen stimulation (Fig. 1; Pearce et al., 2009; van der Windt et al., 2012, 2013). Enhancing FAO in memory T cells through increased expression of carnitine palmitoyl-transferase 1a (CPT1a)—a critical mitochondrial transporter of long-chain fatty acids and rate limiting step to β -oxidation—increases CD8 memory T cell numbers after infection (van der Windt et al., 2012). During an immune response, common γ chain cytokines like IL-15 and IL-7 have an essential role in supporting catabolic metabolism by promoting mitochondrial biogenesis, CPT1a expression, and FAO (van der Windt et al., 2012). As a result, memory T cells have increased mitochondrial mass and greater spare respiratory capacity (SRC) compared with naive and effector T cells, which endows them with a bioenergetic advantage for survival and recall after antigen rechallenge (van der Windt et al., 2012, 2013; Gubser et al., 2013). Interestingly, a study investigating heterologous prime-boost vaccination found that memory T cell differentiation is hastened and enhanced after secondary or tertiary immunization, including acquisition of substantially greater mitochondrial mass and SRC (Fraser et al., 2013). In a sense, memory T cells are metabolically primed and ready to respond to secondary infections. This idea has recently been extended to innate immune cells under the novel concept of trained immunity, whereby metabolic priming confers superior immunity to a secondary pathogenic insult (Cheng et al., 2014). Previous engagement of aerobic glycolysis in monocytes driven by mTOR and HIF-1 α was found to induce epigenetic modifications that endowed them with enhanced function against other infections (Cheng et al., 2014).

The specific role of FAO in promoting memory T cell development and survival remains to be elucidated, but it appears that metabolic reprogramming associated with FAO enhances mitochondria-associated processes. Induction of FAO in memory T cells enhances SRC, which is the reserve capacity of mitochondria to produce energy over and above normal energy outputs (van der Windt et al., 2012). This parameter is probably important for the longevity of memory T cells, especially in times of stress or nutrient restriction, conditions that may present themselves when infection is resolved and growth factor signals are scarce. Surprisingly, endothelial cells, unlike most other cell types, use carbon derived from FAO for nucleotide synthesis and proliferation (Schoors et al., 2015), providing another way in which FAO supports cell function.

Another unexpected discovery was that memory T cells preferentially use *de novo* FAS to fuel FAO (O'Sullivan et al., 2014). Specifically, CD8 memory T cells use glucose to produce triacylglycerides (TAGs) that are subsequently hydrolyzed by lysosomal acid lipase (LAL) to support mitochondrial FAO (O'Sullivan et al., 2014). It was also recently shown that glucose metabolism is critical for CD4 memory T cell survival, and this is controlled by Notch signaling (Maekawa et al., 2015). The requirement for FAS in CD8 memory T cells is supported by a recent study showing that glycerol import into the cell via IL-7-induced aquaporin-mediated transport is required for memory T cell longevity (Cui et al., 2015). Glycerol is the molecular backbone for TAGs. Aquaporin 9 (AQP9)-deficient T cells had reduced glycerol import and TAG synthesis and impaired memory T cell survival after viral infection (Cui et al., 2015).

The reasons why CD8 memory T cells synthesize and then catabolize fatty acids in an apparently futile cycle rather than simply acquire extracellular fatty acids are not understood. However, this synthesis/catabolism cycle has also been shown to occur in muscle and adipose tissues (Dulloo et al., 2004; Yu et al., 2002). If viewed on a purely energetic level, this process appears counter-productive, as there would be no net gain in ATP. It is possible that building and burning fatty acids allows memory T cells to sustain their glycolytic and lipogenic machinery while maintaining mitochondrial health during times of quiescence, allowing for the rapid recall ability that is characteristic of memory T cells after antigen recognition and activation (Gubser et al., 2013; van der Windt et al., 2013). It could also potentially provide a mechanism for balancing redox state or metabolic intermediates. An energetically futile cycle has been described in yeast grown in glucose rich media, whereby trehalose cycling provides a buffer system to maintain intracellular phosphate levels and balance glycolytic intermediates (van Heerden et al., 2014).

Emerging topics and concluding remarks

It is an exciting time for the field of immunometabolism. Although the body of literature surrounding this topic is increasing at an exponential rate, much remains to be explored. We are just beginning to understand the many connections between metabolism and gene regulation in T cells (Lu and Thompson, 2012; Wang and Green, 2012; Kaelin and McKnight, 2013; Öst and Pospisilik, 2015). Acetyl-CoA and NAD⁺ generated from oxidative metabolism are used for histone acetyltransferase (HAT) and histone deacetylase (HDAC) activity (Imai and Guarente, 2010; Cantó and Auwerx, 2011; Wellen and Thompson, 2012). Protein acetylation is a reversible PTM that influences epigenetic changes mediated by HATs and HDACs and also controls the actions of transcription factors and molecular chaperones (Glozak et al., 2005). T cell metabolic reprogramming during activation increases cytosolic NAD⁺ and citrate, the precursor of acetyl-CoA, which may direct cell-fate decisions through protein acetylation (Berger et al., 1987). In agreement with this idea, lineage-specific cytokine-encoding genes that affect T cell differentiation undergo dynamic changes in

histone acetylation after antigen-driven stimulation (Avni et al., 2002; Fields et al., 2002). The actions of the transcription factor FoxP3 in directing T reg cell development are controlled through opposing activities of the HATs p300 and TIP60, and the HDAC Sirtuin1 (Sirt1; Li et al., 2007; Tao et al., 2007; van Loosdregt et al., 2010; Beier et al., 2011). Acetylation also affects the activity of circadian clock proteins, and reciprocally, the circadian acetylome has been found to regulate the epigenome and mitochondrial metabolic pathways (Koike et al., 2012; Lu and Thompson, 2012; Masri et al., 2013). Organisms rely on the cell autonomous transcription-translation oscillator loop managed by solar time to accommodate physiological changes brought about by the daily pattern of rest, activity, and feeding (Curtis et al., 2014). The circadian clock also in part regulates Th17 cell development (Yu et al., 2013). Circadian rhythm controls nutrient acquisition and metabolic flux, and it will be interesting to see how the body's internal clock may connect to lymphocyte metabolism, regulation, and function (Rey and Reddy, 2013).

Intermediates from glucose catabolism can be converted into substrates that are needed to support cell growth and proliferation. Recent studies of one-carbon metabolism in cancer research involving the serine and glycine biosynthetic pathways may have implications for T cell metabolism, given the many common features shared between activated T cells and proliferating cancer cells. Although the role of one-carbon metabolism in generating units for nucleic acid synthesis from folate has long been appreciated, it was more recently recognized that this pathway is an important source of NADPH to maintain redox balance and methyl groups for methylation (Locasale, 2013; Fan et al., 2014). For example, depletion of methylenetetrahydrofolate dehydrogenase (MTHFD) in cancer cells results in a decreased cellular NADPH/NADP⁺ ratio and increased oxidized glutathione, enhancing sensitivity to oxidative stress (Fan et al., 2014). Serine and glycine metabolism also have a vital role in cell survival under harsh environmental conditions of nutrient scarcity and hypoxia (Kim et al., 2015a). Tumors foster these conditions, but tumor cells are able to survive and function under these stressors. A recent study found that increased expression of mitochondrial serine hydroxymethyltransferase and glycine decarboxylase confer a survival advantage for glioma cells by allowing them to lower their oxygen consumption and metabolize toxic molecules in the tumor microenvironment (Kim et al., 2015a). T cells also migrate and travel to sites of infection or tumors and must adapt to these hypoxic or nutrient-depleted environments (Pearce et al., 2013). It remains to be explored whether these metabolic pathways support lymphocyte survival by similar mechanisms or have evolved to serve other purposes.

Another area of interest is how substrate availability affects T cell differentiation and their functional fate. Studies investigating the relationship between the gut microbiome and lymphocytes have found that metabolites produced by commensal bacteria have important implications for maintaining immune cell gastrointestinal homeostasis and defense against pathogens. Short chain fatty acids such as butyrate, acetate, and propionate

produced by bacteria induce differentiation of colonic T reg cells (Furusawa et al., 2013; Smith et al., 2013) and also Th17 cells under certain conditions (Park et al., 2015). The vitamin A metabolite retinoic acid can synergize with TGF- β to stimulate T reg conversion (Coombes et al., 2007; Denning et al., 2007; Mucida et al., 2007; Sun et al., 2007; Elias et al., 2008). However, vitamin A metabolite deficiency also abrogates Th1 and Th17 cell immunity (Hall et al., 2011) and, more recently, was found to diminish type 3 innate lymphoid cells (ILC3s), but expand ILC2 cells (Spencer et al., 2014). Lymphocytes can also regulate whole body metabolism by affecting the tissues in which they reside. ILC2s can promote beiging of white adipose tissue and control caloric expenditure through secretion of methionine-enkephalin peptides (Brestoff et al., 2015). Loss of insulin sensitivity as a result of inflammation of adipose tissue in obesity and type 2 diabetes results in part from deficiencies in adipose tissue-specific T reg cell populations controlled by the transcriptional regulator PPAR- γ (Feuerer et al., 2009; Cipolletta et al., 2012). These are just a few examples of what remains to be explored in the interplay between lymphocytes, their environment, and metabolism.

In the development of novel therapeutics for the treatment of human disease, targeting T cell metabolism provides a unique opportunity to manipulate T cell function (O'Sullivan and Pearce, 2015). For example, it has been demonstrated that compared with Th1 and T reg cells, Th17 cells have elevated pyruvate dehydrogenase kinase 1 (PDK1) expression, which promotes aerobic glycolysis through inhibition of pyruvate dehydrogenase (PDH). Inhibition of PDK1 using dichloroacetate (DCA) selectively impairs Th17 proliferation and survival and reduces T cell-mediated inflammation in models of inflammatory bowel disease and EAE (Gerriets et al., 2015). Targeting trophic transporters on T cells may also provide a way in which to manipulate T cell function through altering their nutrient uptake; for example, JPH203, which inhibits amino acid transporter Slc7a5, could be used to inhibit inflammatory T cells without impairing T reg cell function (Hayashi et al., 2013; Sinclair et al., 2013). In the context of cancer, the development of adoptive cellular immunotherapies using in vitro-expanded tumor infiltrating lymphocytes (TILs) could benefit from tailoring culture conditions to optimize TIL metabolism before transfer into the patient (Restifo et al., 2012). Exaggerated glycolysis and cell size resulting from in vitro expansion conditions, such as high glucose, can be detrimental to TIL survival and persistence in vivo, and strategies to limit glycolysis directly or suppress Akt activation in TILs have already shown promising results in this context (Sukumar et al., 2013; Crompton et al., 2015). Inhibiting glycolysis and oxidative metabolism with 2-DG and metformin may also hold therapeutic potential in other disease settings as highlighted by a recent study using models of system lupus erythematosus (Yin et al., 2015).

It is apparent that many diverse processes integrate with lymphocyte signaling, gene regulation, and function to shape T cell metabolism. Understanding the metabolic regulation that dictates T cell fate and how nutrient availability and

microenvironmental factors influence T cell function will provide further insight into immune cell biology and could lead to new approaches to treating human diseases.

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