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Michael D. Buck
Washington University School of Medicine in St. Louis

David O'Sullivan
Washington University School of Medicine in St. Louis

Erika L. Pearce
Washington University School of Medicine in St. Louis

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T cell metabolism drives immunity

Michael D. Buck, David O’Sullivan, and Erika L. Pearce

Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO 63110

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.

“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) and 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+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+CD25+ double-positive (DP) T cells (Pui et al., 1999).

Successful expression of TCRβ with pTα 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
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 phosphorylino-
sitol 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 de-
pendent on activation of the kinase Akt by PI3K (Swaison et al., 2005; Juntila 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 the T system that defers their development (D’Cruz et al., 2010; Rodriguez-Borrado 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–181a/b1 have altered cel-
lar metabolism caused by a significant increase in PTEN ex-
pression (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 trans-
porter 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 sur-
vival of developing and quiescent naive T cells by increasing
expression of the ant apoptotic 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-7R
lack NKT cells (Henao-Mejia et al., 2013). Glucose can enter the mitochondria as py-
crivate, where it is converted to acetyl-CoA and joins the TCA cycle. 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 bio-
mass 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 ca-
tabolism via the tricarboxylic acid (TCA) cycle and OXPHOS
(Fig. 1; Vander Heiden et al., 2009; MacIver et al., 2013). Al-
though aerobic glycolysis is less efficient than OXPHOS at
yielding an abundance of ATP per molecule of glucose, aero-
bic glycolysis can generate metabolic intermediates import-
ant 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 nu-
cleotide and amino acid synthesis (Wang et al., 2011; Pearce
et al., 2013). Glucose can also enter the mitochondria as py-
ruvate, where it is converted to acetyl-CoA and joins the TCA
cycle by condensing with oxaloacetate to form citrate. Break-
down 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).
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 (Laplante and Sabatini, 2012). CD28 ligation enhances PI3K activity, which recruits 3-phosphoinositide-dependent protein kinase-1 (PDPK1) and Akt. PDPK1, 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 (Laplante 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 (ERRα), and hypoxia inducible factor-1α (HIF-1α) 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 (Sheinss 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
glycolytic transcriptional program initiated by c-Myc to support T cell population expansion (Chou et al., 2014; Karmaus and Chi, 2014). HIF-1α, 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-1α–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-1α in settings of inflammation or injury (Tannahill et al., 2013; Chouchani et al., 2014). Innate immune receptor activation increases intracellular succinate from 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.
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-1α activity are important for the metabolic reprogramming of naïve T cells after activation, it is interesting to speculate that the metabolite succinate may also support the transition from a naïve 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-1α activity downstream of mTOR. Mice deficient in HIF-1α 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; Hyunh 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-1α (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 naïve T cells and an increased number of CD44high 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 (Chan 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 (Thorén 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 nonapoptotic 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 (Santarlasci 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 (Santarlasci 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; Castillo 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 (Angeli 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; Angeli 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 (Youssif 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; Maclver 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 (Kaech 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 palmitoyltransferase 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-1α 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 also appears 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 acetylation (HAT) and histone deacetylation (HDAC) activity (Imai and Guarente, 2010; Cantó and Auwerx, 2011; Wellen and Thompson, 2012). Protein acetylation is a reversible PTM that influences metabolic pathways support lymphocyte survival by similar mechanisms or have evolved to serve other purposes. 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). 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; Murcida 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-ENK 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 (Feurer 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 (PDH1) expression, which promotes aerobic glycolysis through inhibition of pyruvate dehydrogenase (PDH). Inhibition of PDH1 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 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.

We thank Edward Pearce and members of the Pearce laboratories for their insight and critical reading of the manuscript. This work was supported by grants from the National Institutes of Health (R01-CA181125 and R01-AI081985 to E.L. Pearce), The Burroughs Wellcome Fund (Investigator in the Pathogenesis of Infectious Disease Award to E.L. Pearce), and the National Science Foundation Graduate Research Fellowship (DGE-1143954 to M.D. Buck).

The authors declare no competing financial interests.

Submitted: 22 May 2015
Accepted: 16 July 2015

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Published August 10, 2015

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