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Semin Immunol. Author manuscript; available in PMC 2017 October 03.

Published in final edited form as:

Semin Immunol. 2016 October ; 28(5): 505–513. doi:10.1016/j.smim.2016.09.003.

Nutrients and the Microenvironment to Feed a T Cell Army

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Abstract

T cells have dramatic functional and proliferative shifts in the course of maintaining immune protection from pathogens and cancer. To support these changes, T cells undergo metabolic reprogramming upon stimulation and again after antigen clearance. Depending on the extrinsic cell signals, T cells can differentiate into functionally distinct subsets that utilize and require diverse metabolic programs. Effector T cells (Teff) enhance glucose and glutamine uptake, whereas regulatory T cells (Treg) do not rely on significant rates of glycolysis. The dependence of these subsets on specific metabolic programs makes T cells reliant on these signaling pathways and nutrients. Metabolic pathways, such as those regulated by mTOR and Myc, augment T cell glycolysis and glutaminolysis programs to promote T cell activity. These pathways respond to signals and control metabolism through both transcriptional or post-transcriptional mechanisms. Epigenetic modifications also play an important role by stabilizing the transcription factors that define subset specific reprogramming. In addition, circadian rhythm cycling may also influence energy use, immune surveillance, and function of T cells. In this review, we focus on the metabolic and nutrient requirements of T cells, and how canonical pathways of growth and metabolism regulate nutrients that are essential for T cell function.

Keywords

T cell metabolism; mTOR; Glut1; glutamine; epigenetics; circadian rhythms

1.1 INTRODUCTION

Human inflammatory diseases and immunological clearance depend on efficient and appropriate T cell activation, balance, and subsequent inactivation. Deficits in these processes are a growing concern in medical care and it is estimated that 5–7% of individuals experience an autoimmune and inflammatory disorder[1]. Maintaining proper T cell activation and function is a complicated process that requires signaling pathway integration, initiation of metabolic reprogramming, and effector cell proliferation and cytokine

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CONFLICT OF INTEREST

The authors report no conflict of interest.

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production[2, 3]. Activated T cells switch from oxidative to glycolytic metabolism. This shift is somewhat counterintuitive, as glycolysis is less efficient than oxidative phosphorylation when considered as a source of ATP. Known as the Warburg Effect or aerobic glycolysis, ATP is generated primarily from glycolysis even in the presence of oxygen. This metabolic program was famously discovered in cancer cells[4], but it has been known for decades that T lymphocytes also induce aerobic glycolysis during effector responses[5]. Aerobic glycolysis can be highly efficient at promoting biosynthesis essential for effector function and rapid proliferation, but also relies on high levels of nutrient uptake, which may change with tissue location, inflammation, or even time of day.

Metabolic flexibility is critical to allow cells to rapidly adjust to changing signals and environments to support cell survival, signaling, biosynthesis, and growth. The interplay between cell extrinsic and intrinsic signals is tightly connected, and cytokines, growth factors, and receptor signaling are all integrated by well-characterized pathways, including JAK/STAT, mTOR/AMPK, and T cell receptor (TCR) signaling, among many others. These signaling pathways are controlled at both the transcriptional level, such as circadian cycling of protein expression, and post-transcriptional, as in the case of mTOR. Nutrient access also regulates signaling and availability of essential amino acids which is crucial to promote mTOR signaling[6]. The activity of T cells and their function is also modified by circadian rhythm. Circulating lymphocyte number can vary dramatically depending on the time of day, likely due to expression of homing molecules on the cell surface[7]. Studies in mice with disrupted circadian rhythm show increased incidences of obesity and metabolic syndrome, and in humans, increased cholesterol levels and obesity[8, 9]. Though the role of canonical intrinsic circadian rhythm cycling in T cells is not firmly established, altered circadian rhythms may modify circulating nutrients[10] and hormones[11] available in the environment that influence T cell responses.

1.1 Basics of T cell metabolism

The primary duty of naïve T cells is immune surveillance. T cells stay in close proximity to B cells and antigen presenting cells (APCs) in secondary lymphoid tissues and are poised to respond to presentation of specific antigen[12, 13]. Upon stimulation, T cells undergo a dramatic shift in metabolism that is marked by increased nutrient uptake and glycolysis. Mitochondrial oxidative phosphorylation (oxphos) also increases, but to a lesser extent[14]. This leads to a general shift in the metabolic flux such that activated T cells are considered predominantly glycolytic, with increased glycolysis and lactate production, and large changes in uptake of anabolic precursors such as glucose and amino acids[15–17]. Metabolic switching is likely due to increased metabolic demand for both energy, reducing equivalents, and precursors for cell components[2]. Cells that fail to meet this metabolic demand undergo programmed cell death[18]. Carbon tracing for glucose and glutamine has recently shown that a majority of carbon cell mass in rapidly proliferating cells, including T cells, is derived from amino acids, and not glucose[19]. However, a high flux of both glucose and glutamine is required for effector T cell (Teff) function[16]. After successful T cell proliferation and immunological clearance of pathogens, Teff responses are diminished and memory T cells emerge with naïve-like oxidative phosphorylation metabolism[20]. This metabolic reprogramming event is paramount to transition of effector cells to memory, as

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memory T cells require oxidative metabolism. Indeed, inhibition of glycolytic pathways enhances T cell memory while inhibition of mitochondrial respiration prevents memory cell formation[21].

1.2 T cell subsets and metabolic specification

Although the balance of inflammatory Teff and suppressive regulatory T cells (Treg) is tightly regulated during immune responses, dysregulation occurs as nutrients change in obesity, chronic inflammation, and cancer. These observations supported the potential for distinct metabolic programs for different T cell subsets. Indeed, direct measurement of the metabolism of CD4 subsets or CD8 effector or memory populations showed the use of clearly distinct metabolic programs[15, 22]. Teff activate and generally utilize aerobic glycolysis reminiscent of cancer cells. In contrast, Treg were shown to predominantly rely on mitochondrial oxidative pathways. Similarly, activated CD8 T cells utilize glycolysis while memory CD8 T cells utilize lipid oxidation. Altered nutrients in obesity, hyperglycemia, and hyperlipidemia thus can lead to shifts in immune cell subsets that promote insulin resistance and lead to an increased occurrence of autoimmune and other immune disorders[23]. While direct causes remain uncertain, histological analysis of visceral adipose tissue in obese subjects shows changes in T cell subsets including increased effector Th1 cells and macrophage infiltration but decreased Treg[24]. Hyperglycemia itself has been shown to enhance cell survival in general by increasing glycolysis and inhibiting caspases via NF-kB expression[25]. These shifts in T cell subsets may have significant implications for immunity. For example, increased Th17 cells can cross the blood brain barrier and induce inflammation in Experimental Autoimmune Encephalomyelitis (EAE) that induces neuronal cell death. Depletion of Treg exacerbates[26] while addition of induced Treg protect[27] from this excessive inflammation and tissue damage. As Treg are required for peripheral tolerance and prevention of inflammatory responses, it is not surprising that System Lupus Erythematosus (SLE) patients have low Treg numbers circulating in peripheral blood when compared to normal patients[28]. Th17 cell numbers increase in SLE flares, with concomitant rise in IL-17 production[29]. It should be noted, however, that circulating populations could be very different from those at sites of inflammation. Conversely, inhibition of Treg suppression could hamper treatment to induce anti-tumor immunity. Tumor elimination depends upon robust Teff activity and depletion of Treg during tumor growth enhances systemic tumor responses[30]. Clearly, affecting the balance of Teff and Treg clinically will require a solid understanding of the underlying disease and how T cell subsets function.

It is also important to consider that T cell subsets are not fixed and instead have a great deal of plasticity. Rather than T cell differentiation advancing from a naïve, quiescent state to stable effector or regulatory lineages, with little to no change in cell identity after differentiation, it is now clear that CD4 subsets can switch from one functional subset to another. We now know that T cells can also display characteristics of multiple subsets. For example, in vivo co-expression of Tbet and GATA3 has been shown, including co-production of IL-4 and IFNy cytokines[31, 32]. Likewise, Th17 cells can become Th1 cells, and regulatory T cells can become effector cells[33, 34] based on microenvironmental cues and epigenetic programing. The flexibility of T cells to reprogram themselves as “Th1-like”

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or “Th17-like” is becoming an accepted characteristic of T cell biology. However, the function and metabolic programs of these heterogeneous T cell populations have not been well explored, nor is it understood to what extent metabolic and nutrient shifts may impact T cell plasticity. Delineating the differences between naïve cells and dual-type or phenotypically plastic T cells may show how altered nutrient-limited conditions such as sites of inflammation or tumor microenvironment impact T cell populations and inform future therapeutics.

2. METABOLIC REGULATORS OF T CELLS

2.1 mTOR and AMPK

There are several canonical regulators of cell metabolism that are now know to play key roles in the metabolism of T cells[35–37]. The mTOR and AMPK pathways have significant and opposing roles in T cell metabolic programming and differentiation. AMPK is an AMP-sensitive signaling kinase – in times of stress, when nutrient availability is low or ATP synthesis decreased, AMPK activates cellular machinery to boost catabolic metabolism and inhibit anabolic growth[38, 39]. AMPK can also phosphorylate TSC2 and RAPTOR to inhibit mTOR signaling[40]. When biosynthetic nutrients are abundant, mTOR integrates extracellular signaling and sensing of essential amino acids. This promotes anabolic metabolism through transcriptional activity and direct action to promote glycolysis and lipogenesis[41]. The mTOR and AMPK pathways can be generalized as a rheostat, where AMPK induces catabolic metabolism and oxidative phosphorylation that slow proliferation, whereas the mTOR pathway promotes anabolic growth pathways to drive proliferation during activation (Figure 1). However, the role of mTOR in T cell metabolism is quite complicated and differential mTOR signaling can modify the balance of effector or regulatory T cell lineages. When mTOR is entirely absent, Teff cannot be generated, and T cells are pushed into the Treg fate[42]. mTOR kinase exists in two independent complexes: mTOR complex 1 or 2 (mTORC1 or mTORC2), which have distinct effects on T cell subsets. Inhibition of mTORC1 signaling with intact mTORC2 signaling[43] or overactive mTORC1 signaling[44] can lead to accumulation of non-functional Treg and onset of autoimmunity. Teff are also highly sensitive to mTORC complex regulation and loss of mTORC1 through deletion of the GTPase Rheb specifically eliminates the formation of Th1 and Th17, but not Th2 cells[45].

Selective regulation of mTORC1 or mTORC2 pathways is also important during T cell activation, as TCR-stimulation can lead to asymmetric division of mTORC1 activity in proximal mother and distal daughter cells. It was recently shown that the APC proximal cell maintains strong effector programming and mTORC1 signaling, whereas the distal cell has reduced levels of nutrient transporters and mTORC1 signaling[42, 46], potentially leading to generation of effector versus memory cell populations. In Treg, the mTOR contribution to cell proliferation is even more complicated. Inhibiting mTOR with rapamycin before TCR stimulation counterintuitively induces proliferation in normally-anergic Treg in vitro[47]. Interestingly, PTEN- or TSC2-deficient Treg that have high mTORC1 activity not only lose suppressive function, but also have decreased lineage stability and may gain inflammatory activity as ‘ex-Treg’[48, 49].

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2.2 Myc

The transcription factor Myc is also an important modulator of T cell metabolism. Myc is growth factor-induced and commonly mutated or overexpressed in cancer cells to promote glycolytic programming and glutamine uptake and catabolism[50]. Myc interacts with a variety of signaling and transcriptional networks [51] that regulate cell growth, cell death, and metabolism[52]. Indeed, there are few anabolic pathways untouched by Myc. T cell activation leads to Myc upregulation and Myc is essential for appropriate Teff activation[2]. Consistent with the role of Myc in cancer to promote aerobic glycolysis, Myc-deficient T cells fail to upregulate glycolysis and glutaminolysis following activation [53]. The asymmetric division of mTORC1 activity upon T cell activation has profound implications on Myc in proximal and distal daughter cells. Myc protein has a short half-life and mTORC1-dependent translation is critical to maintain high Myc levels. Higher levels of mTORC1 signaling in proximal cell lead to differential partitioning of Myc protein with lower Myc levels and reduced activation, mTOR signaling, and glycolytic metabolism in the distal daughter cell. This may be part of the asymmetric division of cell fates, where Myc-high cells become effectors and Myc-low daughter cells become memory T cells[46] with reduced glycolysis and mTOR signaling, as described above.

2.3 HIF1 α

Hypoxia Inducible Factor 1α (HIF1α) is an important transcriptional regulator of T cell metabolism in response to environmental stress. HIF1α can strongly promote glycolysis in conditions of hypoxia and is regulated by oxygen sensing mechanisms and mTORC1 signaling, which promotes HIF1α protein translation[54]. While mTORC1-mediated regulation provides a link to other nutrient sensing mechanisms, HIF1α was initially discovered because it is upregulated in low-oxygen environments[55]. When lymphocytes move into environments with low oxygen tension, HIF1α becomes important to promote adaptation. B and T cells appear to have a differential response to HIF1α, as HIF1αdeficient B cells develop poorly and generate autoantibodies that drive autoimmunity[56] while HIF1α-deficient T cells can still activate normally in vitro[2]. Upon T cell activation, however, HIF1α is selectively required for generation of Th17 cells[57]. This may act through interaction with the Th17 transcription factor RORγT. It may also specifically regulate the degradation of Foxp3, thus preventing Treg generation[58]. While the role of HIF1α in T cell metabolism remains poorly understood, HIF1α is known to directly target checkpoint receptor ligand interactions through regulation of PDL-1, which may have implications in the tumor microenvironment[56].

2.4 Metabolites as signaling molecules

In addition to signaling pathways that directly regulate T cell metabolism, it is also now apparent that metabolites and metabolic pathways in turn regulate signaling and differentiation events. This bidirectional connection ensures that cells only proceed to activate or differentiate if nutrient conditions are appropriate. In response to limiting glucose, it was recently shown that reduced glycolysis allows GAPDH, the enzyme responsible for catalyzing glyceraldehyde-3-phosphate to glycerate 1,3-bisphosphate in the glycolysis pathway, to translocate to the nucleus and modify IFNγ translation in T cells[14].

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While a role for GAPDH in translation has been previously shown[59], the modification of transcript for IFNy specifically correlated with induction of glycolytic metabolism to provide a new link between this metabolic enzyme and inflammation[14]. Similarly, the glycolytic metabolite phosphoenolpyruvate may directly modify calcium signaling and T cell activation, to link glucose availability and T cell receptor induced signaling[60]. It is likely that other metabolic enzymes and metabolites will have additional moonlighting jobs but have yet to be discovered. Our incomplete understanding of the metabolic regulation of T cells is slowly being filled in, opening new targets of potential treatment for diseases of autoimmunity, immune-deficiency, and cancer.

3. SUBSTRATE AVAILABILITY

3.1 Glucose uptake

Beyond the changes in signaling pathways that directly modify metabolic pathways intrinsic to T cells, access to extracellular nutrients and the regulation of nutrient uptake are also essential for T cell function. In some cases, nutrient levels are altered or may not be present, such as in tumor environment. Even if adequate nutrients are available, however, appropriate transporters must be present on the cell surface. Indeed, T cell activation leads to a sharp upregulation of nutrient transporter expression and cell surface trafficking to increase metabolic substrate availability and remove waste products. The glucose transporter Glut1 and the amino acid transporters SLC1a5 and SLC7a5 are upregulated and traffic to the cell surface in response to PI3K/Akt/mTORC1 signaling to allow T cells to uptake glucose and amino acids, including large neutral amino acids and glutamine[17, 61, 62]. Likewise, Mct1 and Mct4 transport lactate out of cells to maintain glycolysis and redox balance and are also upregulated upon activation [63]. These changes can have significant impact on T cell activation and fate. T cells rely strongly on high rates of glucose uptake[64] and elevated Glut1 expression can enhance activation and glycolysis of effector T cells, leading to a Systemic Lupus Erythematosus (SLE) like-disease[65]. Conversely, Glut1-deficient T cells have decreased glucose uptake and reduced Teff proliferation, activation, and function. Mice grafted with Glut1-deficient T cells are protected from Graft-vs-Host Disease (GvHD) and Inflammatory Bowel Disease (IBD), showing that glucose transporter alterations affect in vivo function. Treg, which rely on oxidative metabolism more so than glycolysis, were unaffected by Glut1 deficiency[61]. However, Glut1 protein expression is upregulated in hypoxic conditions and Treg may instead rely more on glycolysis in low oxygen environments[66].

3.2 Glutamine and amino acids

In addition to glucose, availability and metabolism of amino acids has significant impacts on T cell metabolism. Glutamine in particular is an important substrate for highly proliferative cells[22]. Glutamine is the most abundant amino acid in circulation[67] and is a critical fuel for anabolic metabolism. As proliferative cells remove citrate from the TCA cycle to generate fatty acids for various structures such as cell membrane, glutamine is internalized and converted to glutamate, then the TCA intermediate, α-ketoglutarate (αKG). In addition, glutamine is essential for nucleotide synthesis, generation of glutathione, and provides glutamate that is used to facilitate transport of additional amino acids[68].

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Drugs such as BPTES slow the growth of tumor cells in vitro by inhibiting the first enzymatic step in glutaminolysis, Glutaminase. Knockout of the Glutaminase enzyme inhibits tumor growth[69, 70], supporting Glutaminase an anti-cancer target. Because proliferative T cells closely resemble the metabolic state of cancer cells, glutamine metabolism also likely contributes to T cell metabolism and function. Indeed, removal of glutamine from cell culture media completely abrogates T cell activation and IL-2 production[16]. Multiple cell surface transporters have been shown to uptake glutamine[71], but a specific role for Slc1A5 (ASCT2) has been demonstrated in T cells. In a mouse model deficient of Slc1A5 deficiency, Th1 and Th17 effector T cells were reduced while Treg numbers were unaffected, showing differential effects on T cell subsets that correlate with dependence of these subsets on mTORC1. Strangely, proliferation of these effector cells was not affected, as would be expected if alterations in amino acid levels were reduced. It was further shown that ASCT2 knockout mice were less susceptible to Experiment Autoimmune Encephalomyelitis (EAE), a model of multiple sclerosis, which was attributed to reduced Th17 cells. Though Treg suppressive capacity was not directly assessed, the idea that Teff responses could be selectively modified by targeting glutamine availability is enticing[62].

In addition to providing metabolic substrates, amino acids have direct activating effects on mTORC1. Low amino acid levels inhibit mTOR association with lysosome, and thus prevent downstream signaling for cell growth and proliferation[46]. How the mTOR complexes and its related proteins identify amino acid levels remains uncertain, though SES2[72] or CASTOR proteins[73] are responsible for some amino acid sensing. While the essential amino acid leucine is likely the key substrate for mTORC1 amino acid sensing, T cells deficient in the large neutral amino acid transporter, SLC7a5, also fail to maintain mTORC1 activity and support anabolic metabolism[74]. In this case, however, it is unclear if mTORC1 responds directly these amino acids or if other metabolic deficiencies caused by lack of sufficient neutral amino acids prevent mTORC1 activation. Regardless of the precise mechanism, Slc7a5 deficient T cells failed to properly activate and maintain mTORC1 signaling and, as a consequence, failed to upregulate Glut1 and induce glycolysis necessary for effector T cell activation.

4. METABOLISM AND EPIGENETIC MODIFICATIONS IN T CELLS

Another mechanism by which nutrient status and metabolic pathways may influence T cell signaling and differentiation is through epigenetic modification of DNA and histones. The two primary epigenetic modifications, methylation and acetylation, each use metabolites as substrates. DNA methylation is controlled by DNA methyltransferases[75], and require methyl groups donated by S-adenosylmethionine derived from the one carbon metabolism pathway. This pathway integrates nutrients from glycolysis, amino acids, and choline or folate[76]. Associated with reduced gene transcription, methylation of DNA is heritable and stable [77], but responds to dietary influences. Indeed, reduced dietary methionine was found to lower DNA methylation[78]. Conversely, the dioxygenase enzymes that de-methylate DNA, such as TET2, require αKG as a substrate[79]. Thus both methylation and demethylation events are metabolically sensitive.

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Acetylation and deacetylation of histones and other proteins also play significant roles in gene transcription and cell function, affecting growth and proliferation. In rapidly dividing cells, for example, Akt-induced glycolytic flux has a direct impact on acetyl-CoA levels, which is the required coenzyme substrate for acetyl-transferase activity. Increased availability of acetyl-CoA enhanced global acetylation of histones, even when nutrients were limited[80]. Lysine acetylation can also affect enzymatic activity and protein interactions[81]. The Treg-associated transcription factor, Foxp3, for example, is stabilized by hyperacetylation, leading to increased Foxp3 expression and higher Treg numbers [82]. It was also recently shown that acetyl-CoA availability enhances CD8+ effector function, as increased acetate uptake is required for effector CD8+ T cell responses to *L. monocytogenes*. This increased acetyl-CoA allowed for the acetylation of GAPDH, enhancing glycolysis and boosting CD8+ function[83]. Because T cells rely so strongly on glycolytic reprogramming, epigenetic and protein modifications could be a significant contributor to T cell function and inflammatory responses by linking substrate availability, diet, and metabolism.

Modification of epigenetic marks can have major functional consequences. Myc expression is required for T cell activation but methylation of the enhancer regions usually bound by Myc prevents Myc association and reduces gene expression[84]. A study of CD8+ T lymphocytes from geriatric and young patients showed methylation status of T cells was significantly more variable in geriatric CD8+ T cells and this correlated with decreased immune function. Several genes important in CD8+ T cell differentiation and immune response, such as IFNy and CCL5, had decreased methylation which correlated with higher transcription levels[85]. These studies were confounded, however, by decreased naïve T cells and increased memory T cells in the older population, which could skew the methylation status data. Further, during differentiation of Th1 and Th2 human T cells, the IFNy locus itself is differentially methylated. The locus becomes hypomethylated in Th1 cells while hypermethlyated in Th2 cells[86, 87]. In naïve T cells deficient for the methyltransferase Dnmt1, activation led to reduced proliferation, concomitant loss of methylation on DNA globally, and increased IFNy production[88].

In addition to cytokines, the Foxp3 locus is tightly regulated by methylation to control Treg stability. Stable nTreg have fully demethylated DNA upstream of exon-1 of Foxp3, while iTreg and less stable Treg generally show only partial demethylation. Interestingly, it seems that full demethylation is required for stable Foxp3 expression, as iTreg can lose Foxp3 levels when TGF-β is removed from culture[89]. Stability of Foxp3 expression modified by methylation status may be a reoccurring theme, as the ‘why’ of methylation is under investigation. Moreover, from a purely clinical standpoint, methylation status of Foxp3+ cells could be used to ensure stable Treg lineages are identified correctly in graft transplantation. Additional studies on T cell-specific transcription factors shows epigenetic regulation for Tbet, GATA3, and RORγt, and these are affected both as epigenetic modifiers and as agents of chromatin remodeling[90, 91]. How substrate availability and metabolic pathways impact methylation status of lymphocyte-regulatory loci remains an important question.

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5. Tumor Infiltrating Lymphocytes and Metabolic Adaptation

Tumor cells can be highly glycolytic and poorly vascularized, causing the tumor microenvironment to have reduced glucose and amino acid levels[92], while simultaneously accumulating lactate, increased acidity, and other waste products[93]. Tumor infiltrating lymphocytes (TILs) thus face the challenge of engaging in active cell metabolism necessary for effector function when substrates are limited by nutrient availability. High lactate levels inhibited proliferation and cytokine production in human cytotoxic lymphocytes, which was attributed to disruption of a required lactate gradient, prevention of lactate export, and metabolic perturbation[94]. Additionally, in squamous tumors, highly glycolytic tumors had reduced CD8+ T cell infiltration[95].

TILs isolated from tumors exhibit characteristics of stereotypically lowered activity called exhaustion[96]. This exhaustion is due in part to chronic stimulation, but T cell activation in low nutrient conditions is also sufficient to induce an exhausted-like state of anergy[97]. Though the regulation of exhaustion remains complex, upregulation of the death receptor and ligand PD-1 and PDL-1, respectively, correlates with reduced activation and proliferation in CD4+ T cells[98] and tolerance in CD8+ T cells[99]. It is interesting that blockade of the immune checkpoint PD-1 can improve T cell function even in this metabolically challenging microenvironment[100]. Blockade of PD-1 in T cells enhances activation and proliferation[101]. Perhaps improved T cell function despite low nutrient levels is due to an increase in the ability of T cells to uptake and metabolize those nutrients. Indeed, PD-1 was shown to directly impair Akt and mTORC1 signaling and T cell glucose metabolism[102]. Blocking PD-1 interaction with its ligand, therefore, leads to increased T cell glycolysis that may improve the metabolic competitiveness of T cells in the tumor setting[103].

6. CIRCADIAN RHYTHM REGULATION OF CELL METABOLISM

It has been appreciated for some time that immune responses are influenced by time of day and circadian rhythms that are closely linked to metabolic status[104]. Entrainment of the circadian rhythm is generally provided by sunlight, sleep cycles, and feeding[10]. The daily cycles of feeding and sleeping connect energy homeostasis with metabolic substrate availability, as feeding schedules correlate with availability of glucose and amino acids in the blood[105], and loss of the normal circadian cycle can effect bodyweight and metabolic disorder[106–108]. A key function, therefore, of circadian rhythms is to coordinate metabolism with nutrient availability. The transcription factors Clock and BMAL1 constitute the original basis for circadian cycling and these form dimers to induce transcription of oscillatory inhibitory proteins PER (PER1-3), Cry (Cry1 and 2), and REV-ERB[109]. The rise and fall of these transcription factors Clock and BMAL and their downstream protein targets establish the cell-intrinsic circadian rhythm. Lymphocytes show the same 24-hour circadian cycling as most other mammalian cells[110]. Indeed, circadian cycling could affect autoimmune disease pathogenicity as well, as loss-of-function Clock mutant mice had reduced IL-23 levels and improved dermatitis, a psoriasis-like model. It was shown that Clock actually bound the promoter region of IL-23 receptor, which exhibited circadian behavior. Further, a loss-of-function mutation in the inhibitory protein Per1 exacerbated the

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dermatitis, pointing to the circadian pathway as altering autoimmune conditions[111]. Because the expression of Clock and BMAL1 is under control of retinoic acid receptor-related orphan nuclear receptors (RORs)[112], it is possible that transcriptional regulation of Clock genes are partially under control of RORγt, the regulator of Th17 development. However, the connection between T cell differentiation and Clock:BMAL1 expression remains unexplored.

T cells and cancer cells are both strongly driven by Myc which may circumvent the normal process of growth cycles limited by circadian rhythm. In cultured U2OS cells, overexpression of Myc enhanced expression of REV-ERBα and REV-ERBβ, which are responsible for a feedback inhibition loop of circadian-controlled BMAL protein expression[113], abolishing circadian cycling. This also eliminated a previously unknown cycling of glycolytic metabolism, which varied glucose consumption based on the time of day[114]. Combined with increased glutamine metabolism, Myc expression can thus push circadian rhythm-limited cells beyond their regular programming (Figure 2). Remarkably, despite cell-intrinsic circadian cycling of proteins and metabolism, activated T lymphocytes also appear unaffected by elimination of the circadian clock, at least in terms of differentiation and viral clearance. Deletion of Bmal1 in T cells did not prevent the generation of Th17 cells, though there were slight reductions in IL-2 production[115], which is important in immune regulation and activation. This points to potential other extrinsic regulators of the circadian cycles in lymphocytes. As circadian rhythms in T cells have not been fully explored, the role of circadian cycling on T cell metabolism and function remains an open question.

7. OPEN QUESTIONS

The interplay of signaling proteins, metabolic status, and transcription factor regulation is in constant flux to control T cell fate. While it is clear that metabolism must be tightly linked with cell differentiation and function, it remains an open question to what extent signaling pathways drive metabolism or vice versa. Though T cells need transcription factors to enact the metabolic programs required for function, we only poorly understand how the metabolic status of a cell in turn affects T cell specification and function. Perhaps there are more enzymes-turned-promotors lurking in the labyrinth of metabolic regulation, like GAPDH. What is becoming increasingly evident is that the surrounding environment and nutrient accessibility can strongly affect T cell function. In tumor infiltrating lymphocytes, effector function and tumor growth are in constant battle, with tumor cells out-competing T cells during tumor progression. How do we use our knowledge of metabolic vulnerabilities to tip the balance toward favoring T cell function in tumor? Perhaps metabolic inhibitors or activators can tweak the availability of substrate that reduces the specific activity of inflammatory Teff during autoimmunity, modifies tumor use of Teff nutrients, or enhances cytotoxic lymphocyte activity to prevent tumor growth. These dual and multi-function T cells may be an interesting new area for research, where a cell’s metabolic plasticity allow for survival and function in distinct nutrient environments. As we unravel the key metabolic characteristics of these different classes of T cells, it will be important to understand the role they play in immune modulation and tumor infiltration. These strategies may provide new

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and significant benefit to patients without the unfortunate side effect profiles of the old and new treatment regimens.

Acknowledgments

FUNDING

This work was supported by the National Institutes of Health (R01 DK105550 and R21 CA194829).

We would like to thank members of the Rathmell lab for intellectual support.

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Highlights

- T cells require glucose, glutamine, and associated transporters to activate
- Myc, AMPK, and mTOR are key molecular switches of T cell metabolism
- Activation of T cells induce Myc that may circumvent normal circadian rhythm
- Epigenetic marks respond to metabolic status and control T cell fate
- T cells under metabolic stress in inflammation or tumors decrease function

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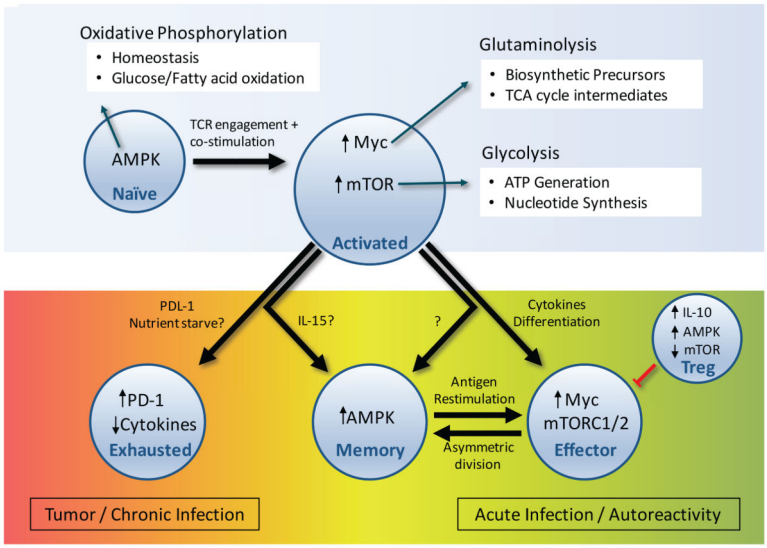

Figure 1.

Naïve T cells are predominantly oxidative and constantly monitor their environment for immune insult. Upon T cell receptor stimulation and co-stimulation by antigen presenting cells, T cells upregulate Myc expression and mTOR signaling, enhancing glycolysis and glutaminolysis (above). In tumor environment, some T cells exhibit high PD-1 expression and reduced activation. During the immune response, some cells will become memory T cells and return to naïve-like oxidative metabolism, whereas large populations of clonally selected T cells remain effector cells with high Myc and mTOR pathway signaling to respond to acute infection. In autoimmune settings, effector T cells react against self-antigens erroneously, enhancing effector responses that escape Treg suppression. Induced Treg (iTreg) are important in suppressing this peripheral autoreactivity.

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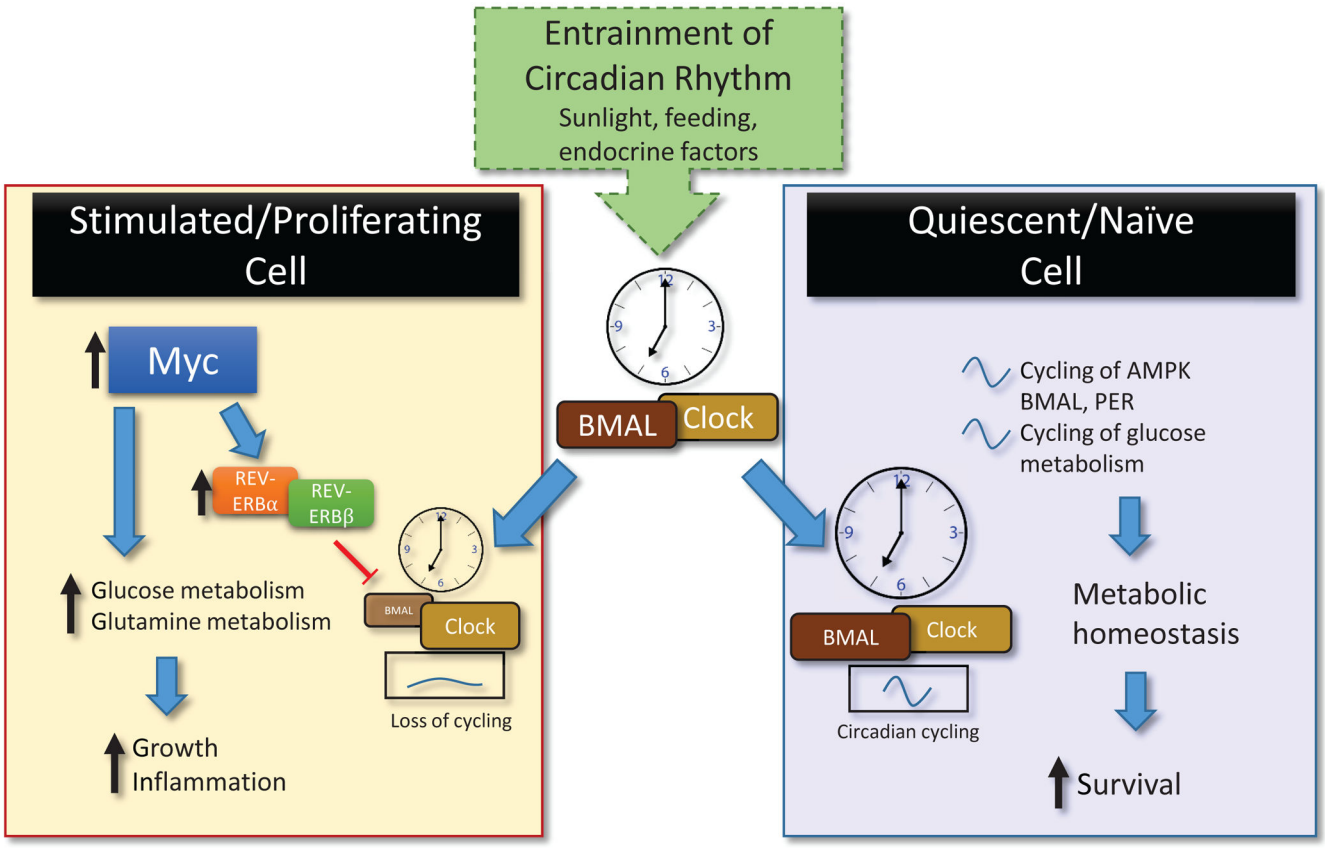

Figure 2.

The circadian clock exists in most somatic cells and is entrained by the Suprachiasmatic Nucleus in the brain via sunlight. This affects feeding behavior, sleep/wake cycles, circulating T cell counts, and more. In quiescent cells (right), cycling of metabolic sensor AMPK is coordinated with Clock:BMAL and PER1/PER2 expression, with concomitant cycling of glucose metabolism and potentially important in T cell maintenance and surveillance. Overexpression of Myc (left), increases REV-ERBα and REV-ERBβ expression to inhibit BMAL1 transcription and prevent circadian cycling. Myc expression induces glucose and glutamine metabolism and is required for T cell effector function.