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REVIEW

Cellular Metabolism on T-Cell Development and Function

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Cell metabolism is closely related to the host immunity in many respects. We herein briefly summarized the recent progress on the roles of cellular metabolism in T-cell development, homeostasis, differentiation and functions. Relatively quiescent naïve T cells only require energy for survival and migration, and they mainly metabolize glucose to carbon dioxide through oxidative phosphorylation. However, activated T cells engage in robust cell proliferation, produce of a range of effector molecules and migrate through peripheral tissues, so they utilizes glycolysis to convert glucose to lactate (termed aerobic glycolysis) to meet the significantly increased metabolic demands. Importantly, the differentiation of T-cell subsets and memory T cells (Tm) was also significantly shaped by distinct cellular metabolic pathways including glucose, amino acids (AA), fatty acids (FA), and others. Understanding the regulatory metabolic networks on immunity may offer new insights into the immune-related disorders and open novel potential therapies to prevent and treat immune diseases.

Keywords: aerobic glycolysis, $CD4^+$ T cells, glucose, immunity, metabolism, mTOR

Abbreviations 2-DG: 2-deoxyglucose; AA: amino acid; AAR: AA starvation response; AMPK: AMP-activated protein kinase; CaMKKII: calcium/calmodulin-dependent protein kinase kinase II; CPT1α: carnitine palmitoyltransferase 1α; DN: CD4/CD8 double-negative; DP: CD4/CD8 double-positive; EAE: experimental autoimmune encephalomyelitis; ERRα: estrogen-related receptor-α; FA: fatty acid; GVHD: graft-versus-host disease; Gluts: glucose transporters; HIF1: hypoxia-inducible factor 1; HKII: hexokinase II; HSCs: haematopoietic stem cells; LNAAs: large neutral amino acids; Lkb1: liver kinase B1; mTORC1: mammalian target of rapamycin complex 1; p70S6K: phosphorylate p70S6 kinase; PPAR: peroxisome proliferator-activated receptor; PI3K: phosphatidylinositol 3-kinase; TCA cycle: tricarboxylic acid cycle; Teff: effector T cells; Treg: regulatory $CD4^+CD25^+$ T cells; TRAF6: Tumor necrosis factor receptor-associated factor 6; Tm: memory T cells; TSC: tuberous sclerosis complex; SREBP2: Sterol Regulatory Element-Binding Protein 2; SP: CD4 or CD8 single positive.

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INTRODUCTION

With the essential requirements for the ability to access appropriate nutrients and energy to support cellular differentiation and functions, cells including immune cells have to alter their metabolic requirements, metabolisms and states to match their demands. Accumulated studies have shown that the regulation of nutrient uptake and utilization is of critical importance for the immune cell homeostasis, differentiation and immunity. General speaking, resting leukocytes use primarily an aerobic oxidative metabolism, whereas stimulations lead to a shift to glycolysis or even aerobic glycolysis as the primary metabolic program [1–3]. However, different leukocyte subsets show metabolic distinctions and specific cellular signal alterations in both transcriptional and posttranscriptional levels. Furthermore, metabolism productions may even generate signals to promote innate immunity and inflammation [4]. It is also noticed that commensal bacteria may influence host immunity via nutrient- and metabolite-dependent mechanisms [5]. In the present review, we will focus on our current understanding of the cellular metabolisms and their related intracellular signal pathways in T-cell development, differentiation, homeostasis, activation and memory processes.

Cellular Metabolism and Intrathymic T-Cell Development

As early as in the 80s of last century, It has been shown that the increase in cellular Ca^{2+} uptake induced by the thyroid hormone T3 is causally related to its subsequent effect on cellular cAMP concentration and the glucose analog 2-deoxyglucose (2-DG) uptake in rat thymocytes [6–7]. 2-DG uptake and thymidine incorporation of rat thymocytes declined with ageing [8]. Early events in thymocyte activation with Concanavalin A (ConA) and interleukin-2 (IL-2) were enhanced phosphatidylinositol turnover and the induction of ornithine decarboxylase accompanied by an increase in glucose uptake [9]. Meanwhile, a change from partial aerobic glucose degradation to CO_2 (26%) to almost complete anaerobic conversion of glucose to lactate (85%) was observed in these stimulated thymocytes [9]. The altered metabolic state present in metabolic syndrome rats showed signs of modulation of glucose internalization by the glucose transporters (Gluts) including Glut1, Glut 3, and Glut 4 [10]. Hypercholesterolemia accompanying tumor growth acts as an impact factor increasing thymocyte sensitivity to apoptosis [11]. Based on the Glut1 surface expression, a unique subset of CD4/CD8 double-positive (DP) thymocytes expressing high levels of Glut1 was identified [12]. This population of immature $Glut1^{+}$DP cells is rapidly cycling and can be further distinguished by specific expression of the transferrin receptor and the CXCR4 chemokine receptor, as compared with the $Glut1^{-}$ DP cell subset [12]. Thus, these DP cells constitute a population with distinct metabolic and chemotactic properties. Recently, more and more signal pathways were identified to regulate T-cell development through modulating cellular metabolisms (Figure 1)

The phosphatidylinositol 3-kinase (PI3K)-Akt-mammalian target of rapamycin complex 1 (mTORC1) pathway is one of the important signals to control cell metabolism in addition to its other diverse biological activity [13]. Mice lacking PDK1 or both PI3Kδ and γ isoforms during early thymopoiesis had a profound developmental block at the CD4/CD8 double negative (DN) stage (DN3/DN4) of thymocytes [14–18]. Loss of both Akt1 and Akt2 caused a reduced thymocyte cellularity and altered thymocyte subsets including CD4 and CD8 single positive (SP) cells with a significant development blocking behind DN3 development [19]. Akt1- and Akt2-deficient DN3 cells had significantly reduced glucose uptake compared with wild-type controls [19]. In the absence of PI3K-PDK1-Akt signaling, DN4 thymocytes failed to up-regulate the expression of Glut1, CD98 (component of the L-amino acid (AA) transporter for the uptake of AA) and CD71 (transferrin receptor, key transporter for the uptake of iron)

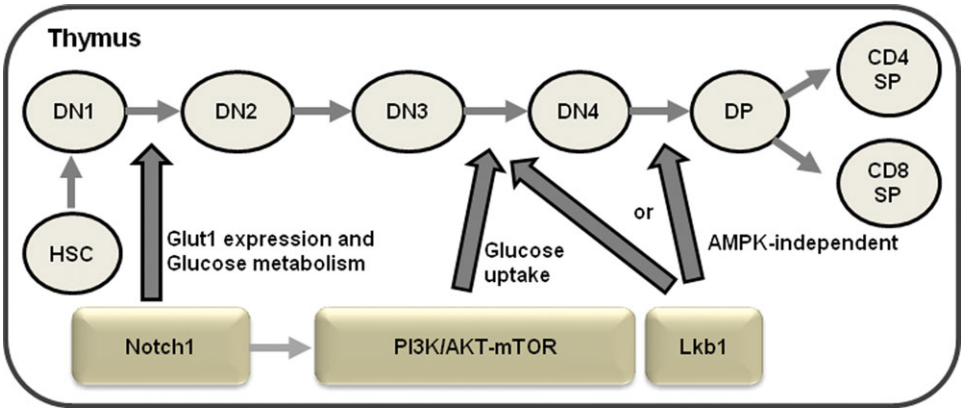

FIGURE 1. The effects of cellular metabolism-related genes on different developing stages of thymocytes. The roles of Notch, PI3K-Akt-mTOR and Lkb1 on developing thymocytes were briefly summarized. DN: CD4/CD8 double negative thymocytes, DP: CD4/CD8 double positive thymocytes, SP: CD4 or CD8 single positive thymocytes, HSCs: haematopoietic stem cells.

[19, 20]. These studies indicate that PI3K-Akt signaling is crucial in allowing these thymocyte subsets to match their cellular metabolism with metabolic demands, which is crucial for early T lymphocyte development in the thymus.

The AMP-activated protein kinase (AMPK) is an important regulatory molecule for cellular energy balance and considered as a master switch of glucose and lipid metabolisms in various organs, especially in skeletal muscle and liver. AMPK, which is activated by an increased ratio of AMP to ATP and requires phosphorylation by kinases like the tumor suppressor liver kinase B1 (Lkb1, also called Stk11) and calcium/calmodulin-dependent protein kinase kinase II (CaMKKII), is a well-known energy regulator that maximizes energy generation by promoting catabolic pathways [21, 22]. It is reported that Lkb1 and its substrate AMPK coordinate cellular metabolism with cell growth of haematopoietic stem cells (HSCs). Deletion of the Lkb1 gene in mice caused increased HSC division, rapid HSC deletion, pancytopenia and decreased thymocyte number [23]. Lkb1-deficient HSCs had reduced mitochondrial membrane potential and ATP levels. Mice with a T cell-specific Lkb1-deletion by Lck-cre system displayed an increased proportion of DN thymocytes with accumulation in the DN3 ($CD25^{high}CD44^{low}$) stage and decreased numbers of DP and SP thymocytes [24–26]. The well-defined physiological target of Lkb1 is the serine/threonine kinase AMPK. However, AMPKα1-deficient mice displayed no significant defects in thymocyte development [24, 27], indicating that Lkb1 might regulate T-cell development through an AMPK-independent pathway.

HSCs deficient for two catalytic α-subunits of AMPK showed similar changes in mitochondrial function [23]. AMPK inactivates mTORC1 through activation of the tuberous sclerosis complex (TSC), which inhibits mTORC1 and phosphorylating Raptor [28–30]. AMPK can promote the function of FoxO family transcription factors, which subsequently regulates energy metabolism, cell cycle, apoptosis and oxidative stress [31]. T-cell-specific TSC1-deficient mice using Lck-cre system had normal thymopoiesis as indicated by the observation that the total thymic cell number, and the percentage and cell number of CD4 or CD8 SP cells in the thymus of TSC1KO mice were identical as wild-type littermates in the early age, though mTORC1 activity was increased in TSC1-deficient thymocytes [32–34]. Thus, mTORC1 may not be critically involved in the thymocyte development and AMPK may regulate thymocyte development in an mTOR-independent pathway.

Notch signaling pathway plays a key role in regulating cellular metabolism and has an essential role in early T-cell development. Mice with an inducible knockout of Notch1 had a severe deficiency of thymocyte development, with developmental arrest of the most immature $CD25^{-}CD44^{+}$ DN thymocytes [35]. Upon arrival from the bone marrow, DN thymocytes initiate V(D)J rearrangement to generate antigen receptors. If a T-cell receptor (TCR) β chain is successfully rearranged, DN cells undergo β-selection and transition through the DN3 and DN4 phases of thymocyte development. This particular selection event then leads to significant cell proliferation. Interestingly, the glucose transporter Glut1 is specifically induced at this stage and may indicate an increase in glycolysis [12, 36]. Glut1 is then significantly down-regulated as thymocytes mature to more quiescent DP or CD4 and CD8 SP cells. Notch signaling is critical in β-selection of DN thymocytes via regulating Glut1 expression and glycolytic rate [37]. Absence of Notch signals caused DN thymocyte apoptosis due to lower Glut1 expression and decreased glycolytic kinetics [37]. The mechanism by which Notch signaling promotes thymocyte glucose metabolism is not fully clarified, but Notch leads to activation of PI3K-Akt signaling pathway, which is well established to drive glucose metabolism and aerobic glycolysis in a variety of systems. Inhibition of PI3K or Akt in DN thymocytes suppressed glucose metabolism, whereas over-expression of constitutively active Akt1 (Myr-Akt) restored glucose metabolism in Notch-deprived thymocytes and reversed the blockade of the early pre-T-cell development caused by disruption of Notch signaling [37]. Thus, Notch may regulate glucose metabolism via a PI3K-Akt pathway.

Recently, it is reported that miRNA181 modulated the phosphatase PTEN expression to control PI3K signaling, which was a primary stimulation for anabolic metabolism in immune cells. MiRNA181-deficient mice showed severe defects in T and NKT lymphoid development and T-cell homeostasis associated with impaired PI3K signaling [38]. These results uncover that miRNA181 is essential for NKT cell development and establish this family of miRNAs as central regulators of PI3K signaling and global metabolic fitness during immune cell development and homeostasis.

Cellular Metabolism and Naïve T-Cell Homeostasis

In the immune system, resting naive T cells are not really resting but rather continually migrating through the secondary lymphoid tissues on immune surveillance. This process is ATP expensive and requires basal replacement biosynthesis. Resting T cells rely predominantly on the high-energy-yielding processes of fatty acid (FA) β-oxidation and pyruvate and glutamine oxidation via the tricarboxylic acid cycle (TCA cycle) (Figure 2). It is known that peripheral resting T cells require cell-extrinsic signals offered by TCR and/or cytokine receptors like IL-7R to maintain this basal energy–generating metabolism to avoid cell death by neglect and to maintain peripheral T-cell homeostasis [39]. Mature resting T cells do not have fixed metabolic characteristics, but rather are under dynamic regulation. Environmental signals can control nutrient utilization in resting T cells, thus determining their trophic state, ability to initiate cell proliferation, and resistance to apoptosis. In Bcl-X(L) transgenic animals, cell size and metabolic activity of naive T cells were regulated through the TCR and correlated with TCR-dependent Glut1 expression [2]. Culture of naive T cells with IL-7 can partially maintain cell size, glucose uptake, and glycolysis. These changes are linked to the pro-survival effects of IL-7, as glucose deprivation inhibits IL-7-mediated cell survival despite Bcl-2 induction [39]. The IL-7R regulates glucose uptake largely through the PI3K-Akt-mTOR pathway, which can promote cell surface trafficking of Glut1 [39–41]. This regulation of glycolysis by the IL-7R is critical for the basal T-cell metabolism in vivo, as conditional deletion of IL-7R in mature T cells in vivo leads to cellular atrophy and an inability to maintain glycolysis [42]. In addition, recent studies

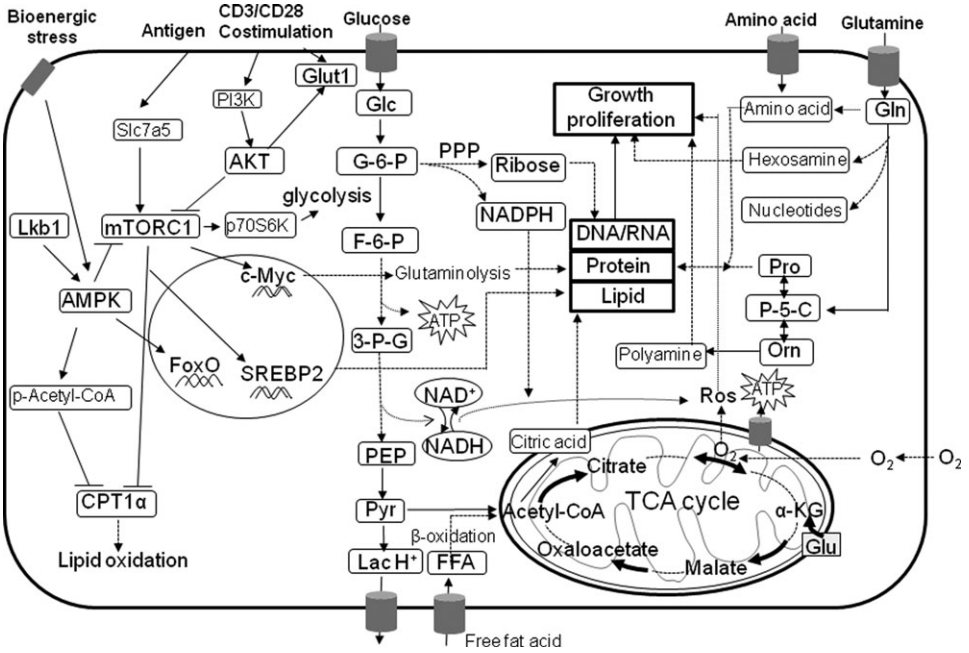

FIGURE 2. Simplified pathways for cellular metabolisms. The metabolism pathways of glucose, amino acid, fat acid and glutamine were briefly summarized. The pathways activated by TCR and co-molecular activations are involved in cellular metabolism in T cells were shown. 3-P-G: 3-Phosphoglycerate; A-KG: α-Ketoglutarate; AKT: Protein kinase B; AMPK: AMP activated protein kinase; ATP: Adenosine triphosphate; Lkb1: Liver kinase B1; CPT1α: Carnitine palmitoyltransferase 1α; F-6-P: Fructose 6-phosphate; Glc: Glucose; G-6-P: Glucose 6-phosphate; Glut1: Glucose transporter 1; LacH: Lactate-H; mTOR1: Mammalian target of rapamycin complex 1; Orn: Ornithine; Slc7a5: Solute carrier family 7 member 5; P-5-C: Pyrroline-5-carboxylate; PEP: Phosphoenolpyruvate; PI3K: Phosphatidylinositide 3-kinase; Pro: Proline dehydrogenase; Pyr: Pyruvic acid; ROS: Reactive oxygen species; SREBP2: Sterol Regulatory Element-Binding Protein 2; TCA cycle: Tricarboxylic acid cycle.

showed that IL-7-induced growth of naive $CD8^+$ T cells is dependent on AAs and that AA transporters are one of the target molecules of IL-7 signaling [43], indicating broad metabolism-regulating roles for IL-7 in resting T cells. IL-15 is another important cytokine for naïve T-cell homeostasis. Fast muscles in IL-15-transgenic mice exhibited high expression of intracellular mediators of oxidative metabolism including sirtuin 1, peroxisome proliferator-activated receptor (PPAR)-δ, PPAR-γ coactivator-1α, and PPAR-γ coactivator-1β [44]. These findings support a role for IL-15 in induction of oxidative metabolism in muscles. However, whether IL-15 plays the same roles in the regulation of oxidative metabolism in naïve T cells requests to be investigated.

Cellular Metabolism and T-Cell Activation

During activation and immune response, T cells must rapidly proliferate and exert effector function with a dramatically increased metabolic requirement to support biosynthesis of intracellular constituents including lipid membranes, nucleic acids and proteins [3], [45, 46]. T cells meet this demand and maintain sufficient intermediate metabolites for cell growth by simultaneously increasing glucose and glutamine metabolism while decreasing lipid oxidation [1] (Figure 2). It is reported that glycolysis and lactate production of human peripheral T cells are greatly increased by the mitogens phytohaemagglutinin or phorbol-12-myristate-13-acetate and ionomycin

[47, 48]. Immunization led to a rapid increase in Glut1 expression, indicating an increase in glucose uptake and metabolism during acute T-cell stimulation in vivo [49]. Increased glucose uptake in T cells with over-expression of Glut1 led to increased cytokine production and proliferation and, ultimately, to lymphoproliferative diseases [49, 50]. Conversely, inadequate nutrients or metabolic inhibition decreased T-cell proliferation and activation or lead to T-cell anergy or cell death [50–52]. The glycolytic inhibitor 2-DG can protect animals from experimental autoimmune encephalomyelitis (EAE) [53]. On the other hand, T cells responded to antigen by up-regulating expression of many AA transporters, but a single system L (leucine-preferring system) transporter, Slc7a5, mediated uptake of large neutral AAs (LNAAs) in activated T cells. Slc7a5-deficient T cells were unable to metabolically reprogram in response to antigen and did not undergo clonal expansion or effector differentiation. The metabolic catastrophe caused by loss of Slc7a5 reflected the requirement for sustained uptake of the LNAA leucine for activation of the serine-threonine kinase complex mTORC1 and for expression of the transcription factor c-Myc [54]. Thus, the metabolic changes strongly contribute to and are essentially required for the efficient and appropriate T cell activation. The metabolic program of activated T cells is regulated on both the transcriptional and post-transcriptional levels.

Enhanced glycolysis and metabolic reprogramming upon T-cell activation are dependent on co-stimulation signals [55]. In particular, CD28 plays a number of key roles to promote the glucose metabolism and aerobic glycolysis essential for cell growth and proliferation through the PI3K-Akt-mTOR pathway. Neither activation by cross-linking the TCR/CD3 complex nor ligation of CD28 alone resulted in a significant change in Glut1 expression. In contrast, stimulation with anti-CD3/CD28 led to a synergistic induction of Glut1 expression and glycolysis in T cells [55]. Both Akt and mTORC1 can promote aerobic glycolysis to support effector T (Teff) cell growth and function.

Although Akt and mTORC1 can influence gene transcription through a number of mechanisms, these kinases also promote glucose metabolism via posttranslational effects. It has been shown that Akt could promote trafficking of the glucose transporter Glut1 to the cell surface and prevents Glut1 internalization upon activation [2, 56]. Akt can directly phosphorylate glycolytic enzymes to promote increased glycolytic flux. Akt can directly phosphorylate hexokinase II (HKII) to promote HKII localization to the mitochondria and increased enzymatic activity [57]. On the other hand, Akt also enhances cell surface trafficking of AA transport proteins [58]. Activation of mTORC1 by Akt also promotes post-translational events to stimulate aerobic glycolysis and coordinate pathways to support T-cell growth [59]. One of the major functions of the mTORC1 complex is to phosphorylate p70S6 kinase (p70S6K), which regulates glycolysis [60]. Indeed, the immunosuppressant rapamycin treatment prevents the increased glycolysis upon T cell activation and blocks T-cell growth and proliferation, leading instead to a state of anergy [61]. SREBP2 is also activated by mTORC1 to promote lipid synthesis [62]. Akt/mTORC1 decreases the expression of CPT1a, a rate-limiting factor in lipid uptake into mitochondria for oxidation, to reduce lipid oxidation and conserves lipids for growth rather than for ATP generation [63]. In some cells, mTORC1 couples phosphatidylinositol-3 kinase (PI3K) and Akt to the control of glucose uptake and glycolysis. However, a recent report showed that mTORC1 activity in $CD8^+$ T cells was independent on PI3K and Akt but is critical to sustain glucose uptake and glycolysis in $CD8^+$ T cells [64]. PI3K- and Akt-independent pathways mediated by mTORC1 regulate the expression of hypoxia-inducible factor 1 (HIF1) transcription factor complex to sustain glucose metabolism and glycolysis in $CD8^+$ T cells via multiple pathways [64]. These results reveal a mechanism linking nutrient and oxygen sensing to transcriptional regulation of $CD8^+$ T-cell differentiation.

Whereas mTORC1 promotes anabolic processes to stimulate cell growth, AMPK is a well-known energy regulator that maximizes energy generation by promoting catabolic pathways [21]. The AMPK complex is activated by an increased ratio of AMP to ATP and requires phosphorylation. Several kinases including Lkb1 and CaMKKII can activate AMPK [22]. Lkb1 is essential for AMPK activation under conditions of bioenergetic stress [65]. T cells lacking Lkb1 displayed defects in cellular proliferation and survival upon activation and in response to metabolic stress, with increased rates of glycolysis and decreased ability to up-regulate lipid oxidation under stress conditions [24]. Despite poor proliferation, Lkb1-deficient T cells showed increased T-cell activation/memory phenotype $CD44^{high}CD62L^{low}$ and inflammatory cytokine production at basal and under TCR stimulation [24]. AMPKα1-deficient T cells displayed reduced viability compared to control cells in response to metabolic stress by 2-DG treatment and an increase in the basal glycolytic rate and Glut1 and HKII expression of resting T cells [24]. However, AMPKα1-deficient T cells did not show cell proliferation and survival defects, which were observed in Lkb1 deficient-T cells [24], suggesting that Lkb1 may regulate T-cell proliferation and survival through other pathways in an AMPK-independent manner. The molecular and biochemical mechanisms for the regulatory roles of AMPK in early activation of T cells are not well known. Activated AMPK has a wide variety of metabolic substrates, including acetyl-CoA carboxylase. Acetyl-CoA carboxylase phosphorylation by AMPK inhibits the synthesis of malonyl-CoA [21], which is a precursor in lipid synthesis and an inhibitor of CPT1a, and subsequently suppresses lipid synthesis and promotes lipid oxidation. AMPK can also phosphorylate TSC2 at a site that prevents mTORC1 activation [61]. Thus AMPKα1-deficient T cells had high basal levels of mTORC1 activation and glycolysis [24]. In addition, the transcription factor c-Myc is crucial for the metabolic switch in glucose metabolism that accompanies the activation of naïve T cells [66]. Deletion of c-Myc in naïve T cells prevented TCR-induced glucose uptake and glycolysis, and activated c-Myc-deficient T cells failed to grow or proliferate [66–68]. On the other hand, the transcription factor IRF4 was induced in a manner dependent on affinity for TCR and acted as a dose-dependent regulator of the metabolic function during T cell activation [69]. IRF4 critically regulated the expression of key molecules required for the aerobic glycolysis of Teff cells and was essential for the clonal expansion and maintenance of immune function of antigen-specific $CD8^+$ T cells [69]. Thus, IRF4 is an indispensable molecule for expansion of high-affinity clones during immune responses, which links metabolic function with the clonal selection and differentiation of Teff cells.

Once activated, T cells differentiate into various different Teff cell subsets depending on the local microenvironments and cytokine availability. Many of these Teff cell subsets maintain an elevated glycolytic rate in response to cytokine signaling and display different cell metabolic phenotypes. In response to IL-2 signaling, CTL maintain high levels of glucose uptake and lactate production indicative of elevated glycolysis [70]. It is reported that anergic T cells are also metabolically anergic as evidenced by the failure of the up-regulation of the essential machinery to support increased metabolism upon full stimulation [51]. On the other hand, blocking leucine, glucose and energy metabolism by *N*-acetyl-leucine amide, 2-DG and 5-aminoimidazole-4-carboxamide ribonucleoside respectively during T-cell activation leads to anergy in Th1-differentiated cells [51], indicating the role of cell metabolism in inducing T-cell anergy state.

In addition, it is shown that mitochondria-produced reactive oxygen species in T cells is required for activation of nuclear factor of activated T cells and subsequent IL-2 expression [71], indicating that mitochondrial metabolism is a critical component of T-cell activation at least in the respect of IL-2 production. Interestingly, it is reported that the regulation of glutamine use is an important component of

T-cell activation [72]. The increased glutamine metabolism upon T-cell activation is dependent on ERK/MAPK pathways and is definitely required for T-cell proliferation [72]. Recently, it is demonstrated that alloreactive Teff cells use FA as a fuel source to support their in vivo activation in a graft-versus-host disease (GVHD) model [73]. During GVHD process, alloreactive T cells increased FA transport, elevated levels of FA oxidation enzymes, up-regulated transcriptional coactivators to drive oxidative metabolism, and increased their rates of FA oxidation [73]. Pharmacological blockade of FA oxidation decreased the survival of alloreactive T cells but did not impact the survival of T cells during normal immune reconstitution [73]. These studies indicate that signal pathways controlling FA metabolism might serve as potential therapeutic targets to treat GVHD.

On the other hand, $CD4^+CD25^+$ Treg (regulatory $CD4^+CD25^+$ T cells) cells employ various metabolic strategies to mediate their immunosuppression in addition to using immunosuppressive cytokines and cytolysis pathways. Depletion of any one of 5 different essential AAs by the expression of the relevant enzymes on DCs leads to an inhibition of T-cell activation and proliferation, combined with a synergistic induction of $CD4^+CD25^+$ Treg cells by TGF-β [74]. The proliferative response of activated Teff cells requires glutathione (GSH), an abundant intracellular antioxidant. The synthesis of GSH is limited by the availability of cystine and Teff cells are inefficient at transporting cystine, the predominant form of this AA in the extracellular milieu, thus creating a metabolic dependence of Teff cells on DCs [75]. Treg cells may also suppress Teff cells by altering GSH metabolism and inducing oxidative stress. It is reported that the interaction of cytotoxic T-lymphocyte antigen 4 (CTLA-4) on Treg cells with CD80/CD86 on DCs triggers a signaling response in DCs that inhibits GSH synthesis [74]. Treg cells also compete with Teff cells for cysteine uptake. These two processes decrease the cysex pool to inhibit T-cell activation and proliferation. Furthermore, $CD4^+CD25^+$ Treg cells can induce indoleamine 2,3-dioxygenase in DCs, which catalyzes the oxidative catabolism of tryptophan [76]. These studies support the important implications for metabolic regulation of neighboring cells such as APCs and T cells and also indicate the essential requirement of Teff cells on the surrounding nitration environments.

T-cell activation induces notable changes in their migratory patterns, which are important for efficient immune response and are mediated by the regulated expression of chemokine receptors and adhesion molecules. Teff cells mainly migrate to nonlymphoid tissues and inflammatory sites, while they have less capacity to home to peripheral LNs than do naive and memory T cells (Tm) [77,78]. For example, activated T cells rapidly down-regulate CCR7 and CD62L but up-regulate expression of tissue-homing receptors like the integrins VLA-4 and cutaneous lymphocyte-associated antigen [79]. In addition to the proteolytic cleavage, CD62L expression is also controlled on the transcriptional level by cytokine-driven Teff cell differentiation [80]. Recently, it is reported that PI3K and mTOR pathways mediate TCR/cytokine-induced down-regulation of CD62L and CCR7, two crucial molecules that regulate lymphocyte recirculation [78]. Inhibition of PI3K significantly prevented both the proteolytic cleavage pathways and transcriptional mechanisms that down-modulate CD62L expression in activated T cells [78]. PI3K and mTOR, which are signaling molecules usually associated with the control of T-cell metabolism, were also essential for the down-regulation of CCR7 expression on Teff cells [78]. Thus, mTOR as a nutrient sensor has the ability to control CD62L and CCR7 expression on T cells, indicating that molecular mechanisms have evolved to synchronize T-cell activation/trafficking and cellular nutrient/energy availability.

Furthermore, intracellular calcium (Ca^{2+}) flux can also provide the functional links between TCR ligation, mitochondrial OXPHOS and cell proliferation [45]. Uptake of Ca^{2+} by mitochondria can stimulate Ca^{2+}-dependent dehydrogenases of the TCA

cycle, promoting mitochondrial NADH and ATP production by OXPHOS during early T-cell activation. Lack of the apoptosis regulators Bax and Bak in T cells, which displays defects in intracellular Ca^{2+} homeostasis, exhibit reduced Ca^{2+}-dependent mitochondrial ROS production and T-cell proliferation after TCR stimulation [81]. Thus, intracellular Ca^{2+} could regulate T-cell function via mitochondrial pathways.

Cellular Metabolism and T-Cell Differentiation

Th1, Th2 and Th17 cells expressed high levels of the glucose transporter Glut1 on cell surface and were highly glycolytic, whereas $CD4^+CD25^+$ Treg cells expressed low levels of Glut1 and had a mixed metabolisms involving glycolysis, lipid oxidation, and OXPHOS [49] (Table 1). Inhibition of glucose metabolism either by withdrawal of glucose in the medium or by the addition of the hexokinase inhibitor 2-DG was capable of selectively inhibiting the cytokine production of Teff cells including Th1, Th2 and Th17 in vitro [49, 53]. Importantly, this was true in vivo too, as treatment with 2-DG protected animals from EAE [53]. Conversely, aged Glut1-transgenic mice had selectively increased Teff cells which were readily able to produce IL-2, IL-4 and IL-17 cytokines in related to the cellular glucose metabolism [49]. Deficiency in the transcription factor HIF1α resulted in greatly reduced glycolytic activity in purified naive T cells under Th17-polarizing conditions, and subsequently decreased Th17 differentiation through decreased IL-23R and increased Foxp3 expression [53, 82]. Thus, increased glucose uptake alone is sufficient in vivo to selectively enhance Teff cell function. Decreased Glut1 expression by AMPK stimulation could increase $CD4^+CD25^+$ Treg cell generation dependently on lipid oxidation in an asthma model [49]. On the other hand, it is reported that HIF-1, a key metabolic sensor, regulates the balance between $CD4^+CD25^+$ Treg and Th17 cell differentiation [82]. HIF-1 enhances Th17 development through direct transcriptional activation of RORγt in a Stat3/p300-dependent manner. Concurrently, HIF-1 attenuates $CD4^+CD25^+$ Treg cell development by binding Foxp3 by proteasomal degradation pathway [82]. Importantly, mice with HIF-1α-deficient T cells are resistant to induction of Th17-dependent EAE, and blocking glycolysis during Th17 cell differentiation reduced the development of Th17 cells and favored the differentiation of $CD4^+CD25^+$ Treg cells [82]. Furthermore, naive $CD4^+$ T cells expressed low levels of orphan nuclear receptor estrogen-related receptor-α (ERRα) protein that increased upon activation. ERRα deficiency reduced activated T-cell number and cytokine production. ERRα broadly affected cellular metabolic gene expression and glucose metabolism critical for Teff cells [83]. Particularly, the up-regulation of Glut1 protein, glucose uptake and mitochondrial

TABLE 1. Cellular metabolic switch in T cell subset differentiation.

	Th1	Th2	Th17	Treg	Tm
Specific transcriptional Factor	T-bet	Gata3	RORγt	Foxp3	Eomesodermin
Produced cytokines	IFN-γ IL-2	IL-4 IL-13	IL-17A IL-17F	TGF-β IL-10	IFN-γ
Glycolysis	+	+	+	−	−
Lipid oxidation	−	−	−	+	+
Metabolism-related molecules	Glut1(+) PPARα(−)	Glut1(+) PPARγ(−) autophage	HIF1α(+) PPARγ(−) SREBP(−)	HIF1α(−) PPARα(+) PPARγ(+)	CPT1α(+)

(+) or (−) indicate positive or negative effects.
Glut1: glucose transporter 1; HIF1α: hypoxia inducible factor 1 α; PPARα/γ: peroxisome proliferator-activated receptor α/γ; CPT1α: carnitine palmitoyltransferase 1α.

processes were suppressed in activated ERRα-deficient T cells [83]. Further studies showed that this defect appeared as a result of inadequate glucose metabolism [83]. Additionally, $CD4^+CD25^+$ Treg cell development likely requires lipid oxidation, and lipid addition selectively restored $CD4^+CD25^+$ Treg cell generation after acute ERRα inhibition, whereas Teff cell differentiation mainly uses glucose metabolism [83]. In addition, recent results indicated that extracellular salt and short-chain FA could affect Th17 and induced $CD4^+CD25^+$ Treg cell homeostasis, respectively [84, 85]. These results support the possibility that the metabolic microenvironments can influence T-cell polarization.

It is reported that small molecule halofuginone could selectively inhibit mouse and human Th17 differentiation by activating a cytoprotective signaling pathway, the AA starvation response (AAR) [86]. Halofuginone also induces the AAR in vivo and efficiently protects mice from Th17-associated EAE [86]. These results indicate that the AAR pathway is a potent and selective regulator of inflammatory $CD4^+$ T-cell differentiation. On the other hand, disruption of mTORC1 activity leads to a profound loss of $CD4^+CD25^+$ Treg cell immunosuppressive activity in vivo and the development of a fatal inflammatory disorder [87]. Mechanistically, raptor/mTORC1 signaling in $CD4^+CD25^+$ Treg cells promotes cholesterol and lipid metabolism for coordinating $CD4^+CD25^+$ Treg cell proliferation and up-regulation of the suppressive molecules CTLA4 and ICOS to establish $CD4^+CD25^+$ Treg cell immunosuppressive function [87]. These results demonstrate that mTORC1 connects immunological signals from TCR and IL-2 to cellular lipogenic pathways and functional fitness in $CD4^+CD25^+$ Treg cells, and also highlight a central role of metabolic programming of $CD4^+CD25^+$ Treg cell immunosuppressive ability.

These findings strongly highlight the importance of metabolic cues in T-cell fate determination and suggest that metabolic modulation could ameliorate certain T-cell-based immune pathologies. These data demonstrate that $CD4^+$ T-cell subsets require distinct metabolic programs that can be manipulated in vivo to control $CD4^+CD25^+$ Treg and Teff cell development in inflammatory diseases and to ameliorate certain T-cell-based immune pathologies.

T cells lacking Lkb1 displayed enhanced differentiation toward Th1 and Th17 $CD4^+$ T-cell lineages [24]. A significant increase in the level of IFN-γ/IL-2 double-positive $CD8^+$ T cells was observed in mice with a T-cell-specific deficiency of Lkb1 [24]. However, AMPKα1 deficiency increased IFN-γ production in $CD8^+$ T cells but failed to cause a significant alteration in Th1 and Th17 $CD4^+$ T-cell differentiation [24]. The different impacts of Lkb1 and AMPK on $CD4^+$ and $CD8^+$ T-cell lineage differentiation should cause our attenuations to the distinct metabolic regulation on T-cell subsets.

Cellular Metabolism and Tm Cells

Unlike naïve T cells, Tm cells undergo intermittent cell division, which occurs about once every 2–3 weeks for typical resting T cells and is balanced by an equivalent degree of cell death. Tm cells may be independent of contact with self-p/MHC molecules but crucially depend on contact with a combination of IL-15 and IL-7 for homeostatic proliferation and survival [88]. These cells express high level of CD127 molecules regulated by FoxO1 and high CD122 regulated by T-bet and eomesodermin [89], thus allowing them to readily respond to IL-7 and IL-15 for their survival and intermittent homeostatic proliferation [90]. Tm cell response depends on lipid oxidation. Tm cells express high levels of the mitochondrial lipid transporter carnitine palmitoyltransferase 1α (CPT1α), and inhibition or RNA interference of this protein significantly diminished mitochondrial function and reduced Tm cell survival [91]. Conversely, retroviral CPT1α expression enhanced $CD8^+$ Tm cell generation in an adoptive transfer model.

Though the homeostatic control of Tm cell subsets is quite similar [88], one difference for the homeostatic control of Tm cell subsets is that dependency on IL-15 is less marked for $CD4^+$ Tm cells than $CD8^+$ Tm cells, probably because expression of CD122 is much lower on $CD4^+$ Tm cells. Furthermore, continuous TCR signaling through contact with p/MHC ligands is needed for the maintenance of the $CD8^+$ Tm cells [92], maintenance of central $CD4^+$ Tm cells does not require a TCR-self-p/MHC interaction, though it is still controversial.

Studies using mTORC1 inhibitor rapamycin treatment or RNA interference to inhibit expression of mTOR, raptor or FK506-Binding protein 12 in antigen-specific $CD8^+$ T cells showed that mTOR negatively regulated $CD8^+$ Tm cell differentiation in an intrinsic manner through the mTORC1 pathway [93]. Metabolically, rapamycin treatment reduced mTORC1 activity and increased AMPK phosphorylation that correlated with an increased ability of $CD8^+$ T cells to perform lipid oxidation in the absence of significantly impaired glycolytic metabolism in the activated $CD8^+$ T cells [93].

On the other hand, Tumor necrosis factor receptor-associated factor 6 (TRAF6), an adaptor protein in the TNFR and IL-1R/TLR superfamily, regulates $CD8^+$ Tm cell development by modulating FA metabolism. Mice with a T-cell-specific deletion of TRAF6 had a profound defect in the generation of Tm cells after primary immunization, though they mount robust $CD8^+$ Teff cell responses [94]. Meanwhile, activated $CD8^+$ T cells lacking TRAF6 display defective AMPK activation and mitochondrial FA oxidation in response to growth factor withdrawal [94]. Thus, TRAF6 plays a key role for $CD8^+$ T cells to switch from glycolytic to oxidative metabolism, and subsequently regulates $CD8^+$ Tm cell response through AMPK activity and inducing lipid oxidation.

One recent study tried to address whether changes in glucose metabolism ultimately influence the ability of activated T cells to become long-lived Tm cells. Enforcing glycolytic metabolism by over-expressing the glycolytic enzyme phosphoglycerate mutase-1 severely impaired the ability of $CD8^+$ T cells to form long-term memory [95]. Conversely, activation of $CD8^+$ T cells in the presence of an inhibitor of glycolysis, 2-DG, enhanced the generation of $CD8^+$ Tm cells [95], indicating that modification of glucose metabolism could significantly influence the formation of long-lived $CD8^+$ Tm cells.

Tm cells display effector function in an innate-like kinetics. Recent studies showed that rapid IFN-γ production of effector $CD8^+$ Tm cells was closely linked to increased glycolytic flux after activation [96]. Effector $CD8^+$ Tm cells exhibited more glyceraldehyde-3-phosphate dehydrogenase activity at early time points than did naive T cells activated [96]. Mechanism studies showed that this immediate-early glycolysis required the serine-threonine kinase Akt and the metabolic-checkpoint kinase mTORC2 but was insensitive to rapamycin [96]. Thus, Akt-dependent glycolytic potential might facility the efficient IFN-γ recall response of $CD8^+$ Tm cells.

Conclusions and Perspectives

It has recently become clear that the cellular metabolism pathways play a critical role in shaping T-cell development, homeostasis, activation/differentiation and memory processes. During immune response, T cells will migrate from lymphoid organs to sites of cancer or infection, where oxygen, nutrients and growth factors may become limited. Thus, T cells should metabolically adapt to these changing conditions in order to survive and perform their functions. The success of anti-metabolites as immunosuppressive treatments demonstrates that broad metabolic checkpoint molecules could be used as potential targeting molecules to treat immunological diseases. To this purpose, it is essential and important for us to identify the metabolic phenotype and fuel usage of each lymphocyte subset and better understand how these pathways modulate immune cell fate decision, homeostasis and functional

processes. We believe that understanding the regulatory metabolic networks in immune cells will offer significant insights into the immune-related disorders and open novel potential therapy possibilities to prevent and treat immune diseases.

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Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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