REVIEW

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Immunometabolism of T cells and NK cells: metabolic control of effector and regulatory function

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Abstract

Metabolic flux can dictate cell fate, including immune cell effector and regulatory function. The metabolic regulation of cell function is well characterized with respect to effector, memory, and regulatory T cells. This knowledge may allow for manipulation of T cell metabolic pathways that set the stage for more effective T cell therapy. Natural Killer (NK) and T-lymphocytes have complementary roles in the defense against pathogens. However, studies of NK cell metabolism are only beginning to emerge and there is comparatively little knowledge on the metabolic regulation of NK-cell activation and effector function. Given their common lymphoid lineage, effector functions and cellular memory potential our current knowledge on T cell metabolism could inform investigation of metabolic reprogramming in NK cells. In this review, we compare the current knowledge of metabolic regulation in T cell and NK cell development, activation, effector and memory function. Commonalties in glucose transport, hypoxia-inducible factors and mTOR highlight metabolic control points in both cells types. Contrasting the glycolytic and oxidative nodes of metabolic regulation in T cells versus NK cells may provide insight into the contribution of specific immune responses to disease and promote the development of immunotherapeutic approaches targeting both innate and adaptive immune responses.

Keywords Cell metabolism · Oxidative · Glycolysis · Aerobic · Glucose · Fat · Lymphocyte

Introduction

NK cells and T cells participate in innate and adaptive immunity

Natural Killer (NK) and T lymphocytes are components in the body's defense against infections, autoimmunity, and cancer. As part of innate immunity, NK cells respond to a broad range of danger signals [1]. NK cell function is influenced by chemical messengers (including cytokines) and through signal integration from activating and inhibitory cell-surface receptors [1, 2]. Upon activation, NK cells can induce cell death directly in target cells by receptor-mediated interactions and/or by the release of cytotoxic granules [1]. NK cells further augment the innate response and initiate adaptive immunity through direct interactions and by releasing pro-inflammatory cytokines such as interferon gamma (IFN-γ) [1, 2]. Similar to T cells, NK cells can develop memory, leading to enhanced secondary responses upon re-stimulation [3]. As a critical component of adaptive immunity, T cells mount antigen-specific responses through T cell receptor (TCR) recognition combined with co-stimulation by other inflammatory signals [4]. While the activation process differs, NK cells and T cells share similarities with respect to effector functions. Both cell types can robustly proliferate when stimulated, exert cytotoxicity against target cells, and produce IFN-γ [5]. The purpose of this review is to highlight the current knowledge of similarities and differences in T cell versus NK cells regarding the role of cellular metabolism linked to effector and regulatory function. These

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two cell populations share characteristics relevant to immunotherapy and diseases such as autoimmunity.

There is a reciprocal relationship between NK and T cells, which should be considered when immunotherapies aim to augment NK and T cell effector functions to boost immune defenses against disease. Targeting mechanisms to enhance NK cell-mediated innate responses can improve T cell functions during infection [6]. Conversely, boosting T cell activity can support NK cell effector responses [7]. There are also analogous subsets of NK and T cells regarding cell function, which are relevant to autoimmune diseases. For example, different T cell subsets can promote or mitigate aspects of autoimmunity. Generally, Th17 effector cells promote autoimmunity while regulatory T (Treg) cells suppress these responses [8–13]. Similarly, NK cells have a dual role in autoimmune disease, where aberrant NK cell activation promoting pathogenesis, while regulatory NK subsets maintain tolerance [14, 15]. The knowledge of T cell metabolism outweighs (but may inform) how metabolism may influence NK cell function.

Immunometabolism

Cellular metabolism encompasses both the catabolic and anabolic pathways to generate and use adenosine triphosphate (ATP) molecules, respectively. Features of these metabolic pathways have been reviewed elsewhere in detail [16]. It is commonly reported that glucose is the principle fuel source used by immune cells [16]. Circulating glucose can directly fuel tissue metabolism through the tricarboxylic acid (TCA) cycle or anaerobic glycolysis, but this can also occur indirectly via circulating lactate [17]. Glucose can be catabolized to pyruvate through glycolysis, which rapidly generates ATP and nicotinamide adenine dinucleotide (NADH) molecules. Pyruvate is then converted to lactate (anaerobic glycolysis) or acetyl-CoA, which is oxidized in the mitochondria by the tricarboxylic acid (TCA) cycle (aerobic glycolysis), fueling oxidative phosphorylation (OXPHOS). The catabolism of other biomolecules, such as glutamine via glutaminolysis and fatty acids via fatty acid oxidation (FAO), can also fuel the TCA cycle and OXPHOS. The TCA cycle is a series of oxidation steps which generate redox reagents NADH and flavin adenine dinucleotide ($FADH_2$) that provide electrons for OXPHOS. OXPHOS is a series of redox reactions that generates a proton gradient across the inner mitochondrial membrane resulting in ATP production. Depending on the metabolic needs of the cell, intermediates in the TCA cycle can participate in anabolic pathways including fatty acid (FAS) and amino acid synthesis.

Immune cells adopt a range of functional profiles depending on the environment. Both NK cells and T cells rapidly mount an effector response to inflammatory signals, curtail their response when the inflammatory signal is cleared, and retain basal functionality, longevity, and readiness to respond in the interim of inflammation. A shift in these diverse functional states requires plasticity of energetic metabolism. Therefore, it is not surprising that immune cells in different functional states adopt distinct metabolic signatures. Further, it has become clear that cell metabolism can dictate immune cell function.

NK and T cell metabolism

Metabolic flux of different substrates can regulate T cell function and characterizes different subsets including effector, memory, and Treg cells. However, less is known about how specific metabolic pathways influence NK cell function. There is some evidence that the metabolic requirements for activation and effector function in NK cells demonstrate parallels with the immunometabolism of T cells. Given the similarities between these cells including a common lymphoid lineage, shared effector functions, and ability to form memory populations, it is logical that the state of knowledge in T cell metabolism could inform on comparative knowledge gaps and new areas of research in NK cell metabolism. Much of the evidence reviewed on cellular development, activation, effector function, memory, and immune suppression is based on cell biology assessment and mouse models to understand the underlying molecular mechanisms. Nevertheless, identifying common metabolic pathways between NK and T cells would identify metabolic nodes that could be exploited to alter both innate and adaptive immunity.

Cell development

Metabolic changes during T cell development

In the thymus, T cell development begins with progenitor cells committed to the T cell lineage that becomes CD4/8 double negative (DN) thymocytes. DN thymocytes undergo rearrangement of the TCR gene forming a pre-TCR via beta selection (Fig. 1, outlined in black) [18]. DN thymocyte proliferation coincides with increased glycolytic flux, increased expression of the glucose transporter, Glut1 and activation of PI3K-AKT signal transduction [18, 19]. Subsequently, DN thymocytes transition to double positive (DP) thymocytes [18, 19], undergo maturation, coinciding with a decrease in the rate of cellular proliferation. DP thymocytes become increasingly metabolically quiescent, as genes involved in lipid biosynthesis, oxidative–reductive reactions, and carbohydrate metabolism are down-regulated [20]. This more quiescent metabolic state continues as DP thymocytes mature to single positive (SP) naïve T cells which exit the thymus. Naïve T cells rely primarily on FAO and OXPHOS to efficiently sustain basal energetic needs (Fig. 1) [21–24].
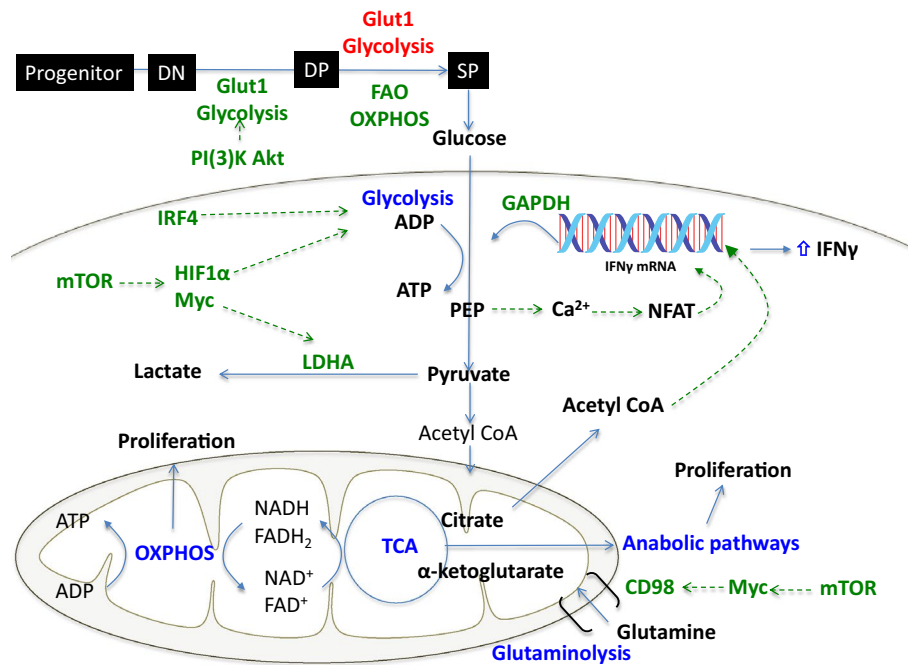
Fig. 1 Metabolic regulation during T cell development and effector functions. Following beta selection, double negative (DN) thymocytes undergo a proliferative phase, during which Notch signaling via the PI(3)K-Akt pathway increases Glut1 expression and glycolysis rates to support proliferation and transition to double positive (DP) thymocytes. As DP thymocytes mature, proliferation rates decrease, and they become more metabolically quiescent. This state is characterized by a reduction in Glut1 expression and glycolysis and a greater reliance on fatty acid oxidation (FAO) and oxidative phosphorylation (OXPHOS) during the transition to single positive (SP) T cells. Resting, SP naïve T cells primarily use FAO for energy generation via OXPHOS. Metabolic regulation of T cell effector function involves enhanced glycolysis, and conversion of pyruvate to lactate is mediated by mTOR and interferon regulatory factor 4 (IRF4). mTOR up-regulates HIF1α and Myc which in turn promote glycolysis and expression of lactate dehydrogenase A (LDHA). mTOR signaling through Myc also up-regulates expression of the glutamine transporter CD98 and consequent glutamine uptake and glutaminolysis. Both glucose and glutamine oxidation through the tricarboxylic acid (TCA) cycle are essential for T effector cell proliferation by fueling ATP production via OXPHOS and providing substrates for anabolic pathways. Elevated rates of glycolysis are critical for T cell IFN-γ production as enzymes and intermediates of the glycolytic pathway play critical roles in the regulation of IFN-γ production. The glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) inhibits translation of IFN-γ mRNA by binding to the 3′UTR region of IFN-γ mRNA. Increased rates of glycolysis diverts GAPDH away from IFN-γ mRNA binding for use in the glycolytic pathway, thus facilitating IFN-γ translation. LDHA promotes IFN-γ transcription by facilitating mitochondrial export of citrate and subsequent conversion to acetyl-CoA which promotes IFN-γ transcription through histone acetylation. The glycolytic intermediate phosphoenolpyruvate (PEP) up-regulates IFN-γ transcription by sustaining elevated levels of cytosolic Ca^{2+} through inhibition of endoplasmic reticulum Ca^{2+} uptake. Cytosolic Ca^{2+} induces nuclear factor of activated T cells (NFAT) signaling which promotes IFN-γ transcription. IFN-γ production is, however, independent of oxidative phosphorylation. Red indicates down-regulated pathways; green indicates up-regulated pathways. (Color figure online)

There is good evidence for the requirement of glucose metabolism in the early stages of T cell proliferation and development, particularly via glucose transport through Glut1. For example, using LckCreGlut1$^{fl/fl}$ mice, Glut1 deficient DN thymocytes have reduced DP thymocytes and lower levels of mature CD4 SP T cells [13]. Glut1 deficiency appears not to alter later stages of T cell development, since DN thymocytes in the initial development stages (stages DN 1–3) are unchanged in mice that lack Glut1, but there is a trend for reduced DN thymocytes in the 4th developmental stage [13]. As DN3 and DN4 thymocytes have the highest expression of Glut1 and undergo extensive proliferation, this suggests Glut1 is required to support this proliferative phase [13]. Despite a role for glucose transport, not all aspects of anaerobic glycolysis appear strictly required for thymocyte development, since deficiency of lactate dehydrogenase A (LDHA), the enzyme which converts pyruvate to lactate, did not affect the frequency of DN, DP, mature CD4 SP or CD8 SP T cells [21]. It should be carefully considered that glucose can also be used in aerobic metabolism via pyruvate entry into the TCA cycle. Altogether, these studies demonstrate that cellular glucose transport is required for normal T cell development in mice. Proliferation stages of T cell development coincide with increased rates of glycolysis, but not necessarily strictly involving or requiring all aspects of anaerobic glycolysis.

Metabolic changes during NK-cell development

Murine NK-cell maturation state is defined by the expression of the cell-surface markers CD11b and CD27. Immature CD11b^{lo}CD27^{hi} NK cells transition to an intermediate CD11b^{hi}CD27^{hi} stage, and then CD27 declines resulting in the expression pattern of CD11b^{hi}CD27^{lo} in the mature NK-cell subset (Fig. 2, outlined in black) [25]. As immature NK proceed down the maturation process, cells progressively rely less on glycolysis and cellular glucose uptake. It is important to consider that immature NK cells have increased cell size and granularity when comparing coincident changes in cell metabolism and maturity status. Nevertheless, immature NK cells have increased glucose transporter and nutrient receptor expression compared to mature NK cells [26]. Furthermore, transcript analysis also has shown that pathways associated with cell growth were down-regulated in mature cells, whilst FAO and aerobic metabolism metabolic pathways are up-regulated as NK cell mature (Fig. 2, outlined in black) [26].

The metabolic regulator mTOR is a key node integrating cell NK metabolism and development. mTOR can integrate cell growth signals to cellular metabolism by promoting cell proliferation, anabolic responses, and anaerobic glycolysis [27]. Activation of mTOR signaling is progressively down-regulated as NK cells mature, consistent with reduced glucose uptake during maturation [26]. Mice with an NK cell-specific mTOR deletion (NK-mTOR^{−/−}) have compromised NK cell development due to impaired transition from immature to intermediate subsets. Consequently, NK-mTOR^{−/−} mice have lower mature NK cells in peripheral organs [26]. The involvement of mTOR in NK cell maturation, together with changes in metabolism during maturation, indicates one way that cell metabolism is linked to development. However, a comprehensive, functional assessment of metabolic pathways during NK cell maturation has not been reported.

Comparison of NK cell and T cell metabolism during development

Many parallels exist between the metabolic signatures of developing lymphocytes. Both immature NK and T cells have higher reliance on glucose metabolism compared to
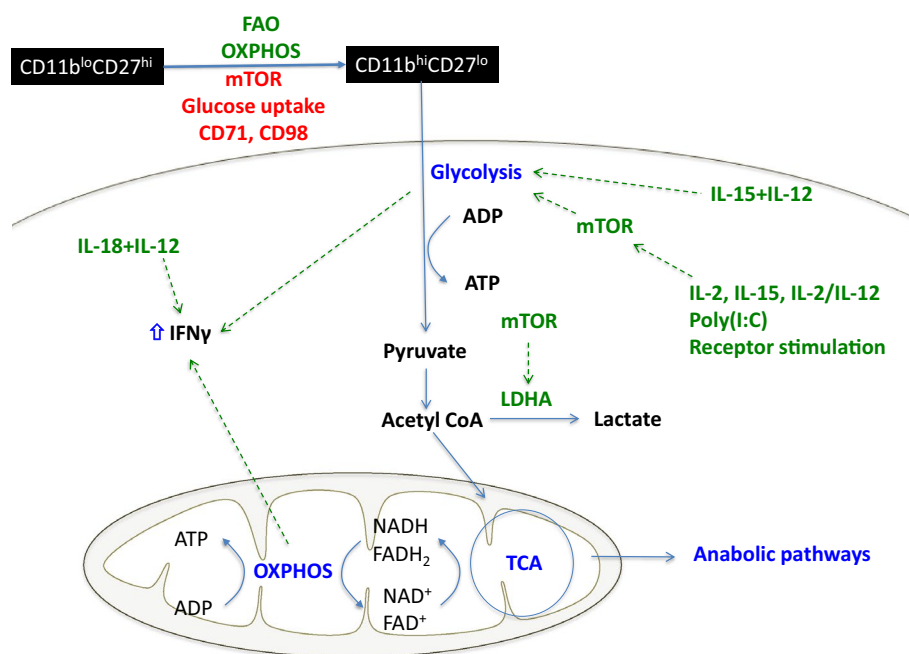

Fig. 2 Metabolic regulation during NK-cell development, activation, and effector function. NK cells develop in the bone marrow from CD11b^{lo}CD27^{hi} immature precursors to CD11b^{hi}CD27^{hi} intermediates and finally CD11b^{hi}CD27^{lo} mature NK cells. As NK cells mature mTOR expression, glucose uptake, and nutrient receptor expression decrease while expression of genes involved in FAO and OXPHOS increase. The metabolic regulation of NK cell effector functions such as IFNγ production depends on the method of NK cell activation. Activation of NK cells via IL-2, IL-15, IL-2/IL-12, Poly(I:C), or activation receptor-mediated stimulation activates mTOR which up-reg-ulates glycolysis through numerous mechanisms including increased Glut1 and hexokinase 2 expression, and supports anoxic glycolysis through up-regulation of LDHA. Conversely, IL-15/IL-12 stimulation up-regulates glycolysis in an mTOR-independent mechanism. Overall, up-regulation of glycolysis and/or oxidative phosphorylation (OXPHOS) is critical for activation-induced NK cell IFNγ production and degranulation via most activation methods; however, IL-18/IL-12-induced NK cell effector functions are independent of glycolysis and OXPHOS. Red indicates down-regulated pathways; green indicates up-regulated pathways. (Color figure online)

their mature counterparts, which likely supports more rapid cell proliferation. Both cell types become metabolically quiescent as they acquire a mature phenotype, relying mainly on FAO and OXPHOS to support a change in energetic needs. There is evidence that glucose transport via Glut1 expression is critical for T cell development. There is evidence that mTOR can regulate NK cell development, but the direct role of glycolysis and Glut1 in regulating NK cell development requires further clarification.

Cell activation

Metabolic regulation of T cell activation (T effector cells)

While mature naïve cells are skewed towards a more metabolically quiescent status, T cells can undergo profound metabolic reprogramming upon activation to support proliferation and effector functions (Fig. 1, intracellular). Effector T cells have augmented glycolysis and OXPHOS, indicating greater cellular metabolism than naïve T cells. During this overall increase in cellular metabolism, effector T cells have a greater reliance on aerobic and anaerobic glycolysis at the relative expense of energy derived from OXPHOS upon activation [21–24]. The importance of extracellular glucose transport and subsequent utilization is evident in Glut1-deficient mature T cells which have reduced proliferation upon activation [13]. However, T effector cells are not entirely dependent on glucose to fuel ATP production. In vitro experiments culturing murine CD4 T cells in galactose instead of glucose, which limits glycolysis, only partially reduced proliferation. These results indicate that other compounds contribute to OXPHOS-derived ATP to support proliferation [22]. In addition, there is evidence that T effector cells do not rely solely on glucose catabolism to fuel aerobic glycolysis as substrates beyond glucose (and lipids) have been shown to fuel OXPHOS. One study demonstrated that murine effector T cells utilize glutamine to maintain intracellular ATP levels in glucose-limiting conditions [28]. This increase in glutamine oxidation is facilitated by increased expression of glutamine transporters such as CD98, which is analogous to increased glucose use facilitated by increased Glut1 levels [13, 23, 29].

Up-regulation of glucose and glutamine transport and catabolism produces intermediates that can fuel anabolic pathways involved in cell growth, proliferation, and effector functions. Effector T cells up-regulate numerous anabolic pathways to help achieve theses cellular responses, including augmented fatty acid synthesis (FAS) [11, 23, 30]. Increased glucose catabolism supplies short-chain carbon molecules that can be shunted through the TCA cycle and citrate pyruvate shuttle system, promoting FAS

[11]. To demonstrate that lipid synthesis is critical in effector cell differentiation, one group showed that inhibiting the FAS rate-limiting enzyme acetyl-CoA carboxylase 1 (ACC1), impaired Th17 differentiation in both murine and human T cells [11]. Furthermore, T cell-specific ACC1-knockout mice were protected against Th17-mediated autoimmune encephalomyelitis. Thus, upon activation, T effector cells increase overall metabolic activity, with enhanced glucose and glutamine catabolism to rapidly generate energy and feed anabolic pathways.

Numerous studies have highlighted key metabolic regulators of T cell activation-induced metabolic reprogramming (Fig. 1, intracellular). Up-regulating Glut1 and glucose uptake is an important hallmark of T effector cell differentiation. One study demonstrated that Glut1 deficiency in both murine and human T cells impaired anaerobic glycolysis and glycolytic capacity upon activation [13]. Unlike naïve T cells, Glut1 expression promotes T effector survival. Another study showed Glut1 expression and glycolysis enhanced T effector cell differentiation, since aged Glut1 transgenic mice had enriched CD4 T effector cell subsets [29]. Furthermore, they demonstrated that T effector cell differentiation in vitro was impaired in the absence of glucose.

As a key regulator of glycolysis, mTOR plays a critical role in T effector cell metabolism. mTOR activity is up-regulated upon T cell activation and exerts numerous functions to promote glycolysis. mTOR up-regulates hypoxia-inducible factor 1-alpha (HIF1α), which stimulates glycolysis, resulting in Th17 differentiation (Fig. 1, highlighted in green on left) [31]. mTOR also activates the transcription factor Myc which promotes glycolysis, glutamine oxidation, and CD98 expression (Fig. 1, highlighted in green bottom right) [23]. In line with these roles, mTOR activation is critical for T effector cell differentiation as treatment with the mTOR inhibitor rapamycin inhibited this process [29]. In addition to mTOR integration of cellular nutrient/energy status and even cellular oxygen sensing by mTOR, transcription factors can influence metabolic control of T cell activation. For example, interferon regulatory factor 4 (IRF4) can act T effector cell glycolytic switch point. IRF4 knockout CD4 T cells have reduced glucose uptake, and impaired glycolysis and OXPHOS upon activation [32]. IRF4 appears to be an important immunometabolism relay that can help integrate the metabolic status of immune cells to activation status (and effector function), but IRF4 also can propagate immune responses into whole body and tissue metabolic outcomes such as lipid storage versus oxidation and insulin sensitivity [33–35]. Altogether, these studies highlight the critical role of metabolic reprogramming for T effector cell differentiation.

Metabolic regulation of T effector cell function

Cellular metabolic status also impacts T cell effector function (Fig. 1). T cell activation is characterized by a shift in relative energy reliance from OXPHOS to aerobic glycolysis [22]. Changes in the cellular metabolism upon activation of T effector cells are not entirely dependent on glucose to fuel ATP production from aerobic glycolysis. However, limiting supply or catabolism of either glucose or glutamine reduces proliferation of T effector cells in vitro and in vivo [23]. This suggests that both glucose and glutamine are essential for T effector cell growth and proliferation. In addition, the transcription factor Myc supports glucose and glutamine catabolism upon cellular activation. Genetic deletion of Myc impaired in vitro and in vivo T cell proliferation, suggesting its role in driving catabolism and proliferation [23]. Up-regulation of anabolic pathways is also critical for supporting T effector cell proliferation. Indeed, ACC1-knockout T cells had impaired proliferation in vivo in response to antigen stimulation, suggesting ACC provision of malonyl CoA for FAS is required for CD8 T cell proliferation during an activating stimulus [30]. In addition to glucose flux, amino acid metabolism is essential for T cell activation. In activated T cells, TCR:antigen engagement increases the expression of amino acid SLC7 transporters and cellular uptake of leucine and glutamine, where ERK/MAPK signaling has been implicated [36–38]. Inhibiting amino acid uptake and subsequent metabolism can alter T cell function. For example, blocking l-leucine uptake in human primary T cells using the inhibitor JPH203 or siRNA-mediated reduction in SLC7a5 ex vivo reduced production of cytokines such as IFN-γ, IL-4, and IL-17 [37]. CD8 T cells deficient in SLC7a5 have impaired antigen responses and do not undergo proliferative expansion [38]. Glutamine deficiency also impairs secretion of cytokines such as IL-2, IFN-γ and also impairs T cell proliferation [36]. Ma et al. recently showed serine uptake is required for optimal CD4 and CD8 T cell expansion in vivo and in vitro [39]. Altogether, these studies demonstrate that amino acid uptake and utilization is required for optimal effector T cell proliferation and function.

Amino acid metabolism also plays a role in Th1 and Th17 cellular differentiation. Elevated levels of glutamine and its metabolite alpha-ketoglutarate promote Th1 and Th17 differentiation, where mTORC1 signaling has been implicated [40]. Th1 and Th17 differentiation, but not Th2 or T-reg, is impaired in SLC1a5 and SLC7a5 deficient CD4 T cells, which transport glutamine and l-leucine, respectively [38, 41]. Interestingly, T-reg differentiation is promoted during glutamine deficiency [40]. Therefore, these results suggest that amino acid metabolism is required for proper CD4 T cell differentiation, and Th1 and Th17 subsets may depend on mTOR signaling.

Augmented IFN-γ production is a critical effector function of activated T cells. Numerous studies have examined the roles of glycolytic enzymes and intermediates in regulating cytokine expression. A seminal study by Chang et al. revealed that IFN-γ production is regulated by glycolysis, independent of cellular metabolism via OXPHOS. Culturing T cells in galactose, which limits glycolysis, impaired IFN-γ secretion while inhibiting OXPHOS did not [22]. This study found that glycolysis regulated IFN-γ post-transcriptionally, since increased translation occurred when cells were cultured in glucose rather than galactose. Furthermore, it was revealed that glycolysis controlled IFN-γ production through the glycolytic enzyme glyceraldehyde-3 phosphate dehydrogenase (GAPDH). GAPDH inhibits IFN-γ translation by binding to the 3′UTR mRNA region. During glycolysis, GAPDH is diverted from IFN-γ mRNA binding, enabling its translation [22].

In addition, a GAPDH-independent mechanism of IFN-γ regulation by anaerobic glycolysis has been uncovered [21]. LDHA regulates T cell IFN-γ production in a 3′UTR-independent mechanism. By sustaining high rates of pyruvate conversion to lactate, the cell's reliance on OXPHOS and TCA cycle for energy is reduced, facilitating mitochondrial export of citrate for conversion to acetyl-CoA (Fig. 1, center). Cytosolic acetyl-CoA leads to histone acetylation and consequently IFN-γ transcription. An additional regulator of T cell IFN-γ production is the glycolytic intermediate phosphoenolpyruvate (PEP). PEP promotes effector T cell IFN-γ expression by inhibiting the endoplasmic reticulum calcium transporter SERCA [42]. Inhibition of SERCA sustained cytosolic calcium levels, promoting nuclear factor of activated T cells (NFAT) signaling, resulting in IFN-γ transcription. Altogether, these studies reveal that enzyme and intermediates of glycolysis can control T cell IFN-γ production via multiple and redundant mechanisms.

Numerous TCR-independent methods of stimulation have also been used implicate glycolysis as a critical regulator of T cell IFN-γ production, including IL-18/IL-12 cytokine administration or IL-2/IL-36 stimulation of dual-costimulated T-cells. However, the method of stimulation may determine the relative importance of glycolysis in T cell effector function. One study described a glycolytic-independent mechanism for IFN-γ production following TLR2 stimulation of antigen-experienced CD8 T cells [43]. Interestingly, IFN-γ production was unaffected by glycolysis inhibition via 2-deoxyglucose (2DG), but dependent on OXPHOS. More information is required to understand how T cell metabolism influences effector function from diverse ligands and how metabolic flux can influence effector functions beyond IFN-γ.

Metabolic regulation of NK cell activation

NK cell activation induces metabolic changes reminiscent to T effector cells. Similar to T cells, NK cell activation prompts cellular proliferation and effector functions, which necessitate profound metabolic demands. As a result, activated NK cells increase metabolic flux through both glycolysis and OXPHOS, with a proportionally increased reliance on glycolysis relative to OXPHOS (Fig. 2) [26, 44, 45]. Elevated glycolysis upon activation is supported by enhanced glucose uptake and Glut1 expression [44, 45]. The evidence to date suggests that NK cell activation is fueled by glucose as a substrate. However, the reliance on glucose flux through aerobic and anaerobic fates may be different in NK cells compared to T cells. For example, one study showed that glucose-derived OXPHOS was required for NK receptor-mediated activation and that inhibiting glycolysis via 2DG or OXPHOS reduced intracellular levels of ATP to comparable levels in NK cells [46]. This is contrasted by T cells, where glutamine oxidization supplements ATP levels in glucose-deficient conditions [28]. Nevertheless, glucose catabolism in NK cells feeds anabolic pathways similar to T cells. One study shows that murine NK cells up-regulate genes involved in protein and lipid biosynthesis in response to IL-15 stimulation or mouse cytomegalovirus (MCMV) infection [26]. In addition, upon IL-2/IL-12 stimulation, ^{13}C-glucose-tracing demonstrated that NK cells up-regulate glucose-fueled FAS and amino acid synthesis [47]. An important future area of research will be determining how different activating ligands such as IL-15, IL-12/18 or IL-2/36 alter effector function in NK cells versus T cells.

Two critical nodes have been shown to regulate NK cell metabolic reprogramming upon activation. NK cell up-regulation of both glycolysis and OXPHOS is regulated by a Srebp-mediated transcriptional program that up-regulates glucose metabolism through increased activity of a citrate–malate shuttle [47]. In addition, mTOR regulates NK cell metabolic reprogramming upon activation. mTOR activity is up-regulated in both mouse and human NK cells following various methods of stimulation, including Poly(I:C), IL-15, IL-2/IL-12, and IL-2 [26, 44, 45, 48] (Fig. 2, highlighted in green on right). Genetic or chemical inhibition (via rapamycin) of mTOR impairs activation-induced increase in NK cell size, glucose uptake, and nutrient uptake receptor expression [26, 44, 45]. Furthermore, mTOR activation in IL-15-stimulated NK cells correlated with increased expression of genes related to cell metabolism [48]. Inhibiting mTOR also directly suppressed murine NK cell activation-induced anaerobic glycolysis and the up-regulation of rate-limiting glycolytic enzymes, hexokinase 2 and LDHA, and Glut1 following IL-2/IL-12 stimulation [44]. While mTOR is a critical regulator of glycolysis, NK cells can up-regulate anaerobic glycolysis using an mTOR-independent mechanism following IL-12/IL-15 stimulation [45]. One important consideration is the dose and duration of ligand exposure. For example, prolonged treatment with a high dose of IL-15 eliminated the reliance on changes in metabolic flux for NK-cell receptor stimulation [46]. Thus, NK cell activation induces metabolic changes, but their regulation may depend on how they are activated.

Metabolic regulation of NK cell effector functions

Since metabolism underpins aspects of NK cell activation, it is logical to elucidate whether these metabolic changes directly regulate NK cell effector functions. Similar to T cells, activation induces NK cell proliferation, which requires profound bioenergetic demands. Thus, it is likely that an increase in energy production via glycolysis and OXPHOS, along with production of biomolecules through anabolic pathways sustain NK cell proliferation. There is still a dearth of information on metabolic control points of NK cell proliferation. Nevertheless, in support of this concept, deletion of mTOR or inhibition of Srebp impairs NK cell proliferation [26, 47].

NK cells are potent IFN-γ producers following activation. There is evidence implicating mTOR, Srebp, and both glycolysis and OXPHOS in NK cell IFN-γ production following stimulation (Fig. 2). For example, mTOR deletion in NK cells impaired IFN-γ secretion in response to Poly(I:C) stimulation [26]. Blocking mTOR also prevented NK cell IFN-γ production following Poly(I:C) and IL-2/IL-12 stimulation [44]. Likewise, inhibition of Srebp impaired IL-2/IL-12-induced IFN-γ production [47]. Blocking glycolysis using 2DG or galactose in culture also impaired NK cell IFN-γ secretion upon IL-2/IL-12 stimulation [44]. Furthermore, culturing NK cells in either glucose-free media or with 2DG inhibited cytokine production following activation receptor stimulation [46]. Despite these similar nodes of immunometabolism, the evidence to date suggests that NK cells have a differential reliance on OXPHOS compared to T cells. NK cells rely on OXPHOS for IFN-γ production following activation with IL-2, or IL-12/IL-15 stimulation [45, 46]. It is not yet clear if this is a cell intrinsic or ligand-derived difference in metabolic control of effector function compared to T cells.

While mTOR, Srebp, glycolysis, and OXPHOS are critical for NK cell IFN-γ production following stimulation, the metabolic control of cytokine production depends on the method of activation. Human NK cells produced IFN-γ independent of mTOR following in vitro IL-12/IL-15 activation, but was mTOR dependent following IL-2 stimulation [45]. However, limiting glycolysis by culturing cells in galactose reduced IFN-γ expression in CD56^{bright} NK cells following IL-12/IL-15 stimulation. This indicates an mTOR-independent mechanism of glycolytic control in IFN-γ production

[45]. One study reported that NK cells produced IFN-γ following short term stimulation with IL-12/IL-18 independent of both OXPHOS and glycolysis [26, 46]. These findings demonstrate that NK cells differ from T cells, which are dependent on glycolysis for IL-12/IL-18-induced IFN-γ secretion [49]. However, both studies assessed short term (1–6 h) IL-18/IL-12 NK cell stimulation and the metabolic requirements may change with extended (18–24 h) stimulation. Duration is important in determining the metabolic requirements for NK cell IFN-γ secretion. For example, treatment with IL-15 for 72 h eliminated cellular reliance on glycolysis and OXPHOS for receptor-mediated IFN-γ production [46]. While glycolytic intermediates and enzymes are critical for T cell secretion, these findings indicate they are not necessarily involved in the NK cell IFN-γ response.

A multitude of activation, inhibitory, and cytokine receptors enable NK cells to integrate a range of non-specific inflammatory signals that regulate activation and IFN-γ production as an early innate response. Distinct molecular mechanisms are responsible for IFN-γ secretion by NK cells, depending on the mechanism of activation [50]. Furthermore, different combinations of activation signals synergize to induce enhanced NK cell IFN-γ production compared to a single activation signal [51]. The specific metabolic requirements of NK cells to produce IFN-γ based on activation method further demonstrates the complexity in examining mechanisms underlying this effector function. Nevertheless, targeting conserved changes in energy flux may simplify strategies aimed at purposefully modifying NK cell effector function.

Comparing the metabolic regulation of NK cell and T cell activation and effector functions

Both T cells and NK cells require more energy upon activation and undergo fundamental metabolic reprogramming. Although there are different metabolic needs and responses induced by the plethora of potential activating ligands, one commonality appears to be use of glucose to fuel augmented glycolysis and OXPHOS, including a relative shift toward glycolysis in both cell types upon activation (Tables 1, 2). This metabolic shift is likely in response to increased energy demands for ATP and anabolic responses within a short period of time. Similar anabolic pathways are up-regulated by activated T cells and NK cells. However, while FAS is critical for differentiation and proliferation of T effector cells, it was surprisingly not required for NK cell growth and proliferation [47]. While many similar metabolic pathways are up-regulated upon T cell and NK cell activation, there appears to be cell intrinsic discrepancies in metabolic control points, particularly in response to diverse activating signals. mTOR up-regulates T cell glycolysis through HIF1α and Myc activation, but it is not yet clear how mTOR up-regulates glycolysis in NK cells and it is not known if HIF1α and Myc are common nodes of metabolic control in both NK and T cells.

Control of metabolic pathways and effector functions in NK cells depends on the stimulation method and there are a multitude of ligands to consider. While mTOR promotes NK cell glycolysis following numerous activation methods, IL-12/IL-15 activation up-regulates glycolysis

Table 1 Summary of major metabolic fuels used by T cell and NK cell subsets

	Glucose	Glutamine	Exogenous FAs	Endogenous FAs	References
T effector	✔	✔			[11, 13, 21–23, 28, 29, 31, 32, 49, 52]
T memory	✔			✔	[53]
Treg			✔		[11]
Activated NK	✔				[26, 44–47]
Memory NK	Poorly defined				
Regulatory NK	Poorly defined				

Table 2 Summary of major metabolic pathways in T cell and NK cell subsets

	Anaerobic glycolysis	OXPHOS	FAS	FAO	PPP	Glutaminolysis	References
T effector	✔	✔	✔		✔	✔	[11, 13, 21–23, 28–32, 54]
T memory		✔	✔	✔			[53–57]
T regulatory		✔		✔			[11, 12, 29, 58, 59]
Activated NK	✔	✔	✔				[26, 44–47]
Memory NK	Poorly defined						
Regulatory NK	Poorly defined						

in an mTOR-independent mechanism. Since mTOR and glycolysis can regulate NK and T cell IFN-γ production, the mechanism of action for this effector may be comparable between both cell types. Glycolytic transcriptional regulation of IFN-γ is mediated in T cells by LDHA. However, whether this occurs in NK cells is unknown. The role of GAPDH in post-translational regulation of IFN-γ is well established in T cells, but is likely not imperative for NK cells [21, 44]. Therefore, further research outlining mechanistically how mTOR and glycolysis regulate NK cell IFN-γ transcription is needed.

Similarities in T cell and NK cell metabolism upon activation reflect comparable energetic requirements of both cell types to support proliferation and effector function. The variability in metabolic regulation of NK cell IFN-γ production is likely attributed to the various stimuli to which NK cells respond when mounting an innate response. The evidence to date suggests that adaptive immune responses by T cells rely heavily on glycolysis to produce IFN-γ. Glycolytic intermediates and enzymes are directly involved in controlling T cell IFN-γ production. The metabolic mechanisms underpinning innate NK cell IFN-γ production are poorly characterized and appear to engage both glycolysis and OXPHOS, but this is likely dependent on the method of activation. Nevertheless,

metabolic pathways play a fundamental role in controlling both T cell and NK cell activation and effector functions.

Memory

Metabolic regulation of memory T cells

During an inflammatory response, T effector cells are highly energetic to support their proliferation and effector functions. At the peak of an immune response, nutrient competition and absence of stimulatory signals occur when resolution of inflammation is initiated. During this time, the T cell population contracts and generates a memory subset [60]. A landmark study by Pearce et al. identified that the transition to memory T cells is metabolically regulated. A metabolic switch from anoxic glycolysis in T effector cells to FAO is required to generate a memory population (Fig. 3) [55]. To augment FAO, memory T cells increase the expression of the rate-limiting enzyme carnitine palmitoyl transferase 1 (CPT1) [56]. CPT1 inhibition by etomoxir in memory cells reduced rates of OXPHOS, suggesting FAO primarily drives OXPHOS-derived ATP [56].

With enhanced reliance on FAO to meet metabolic demands, memory T cells have increased mitochondrial mass, copy numbers, and spare respiratory capacity (SRC)
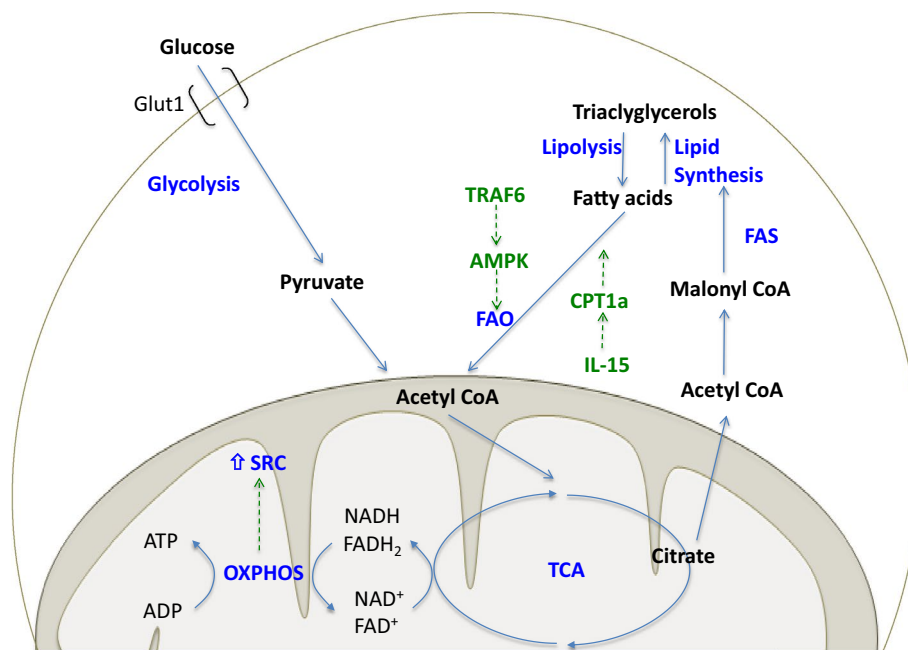

Fig. 3 Regulation of memory T cell metabolism. Memory T cells primarily use fatty acid oxidation (FAO) as their main energy source, which is up-regulated by tumor necrosis factor receptor-associated factor 6 (TRAF6) and IL-15. TRAF6 promotes memory T cell fatty acid oxidation (FAO) through activation of adenosine monophosphate-activated kinase (AMPK) activation. IL-15 promotes FAO by up-regulating the FAO rate-limiting enzyme carnitine palmitoyl transferase 1 (CPT1) and inducing mitochondrial biogenesis, both of which support the increased spare respiratory capacity (SRC) of memory T cells. Liberated fatty acids feed into the tricarboxylic acid (TCA) cycle to support oxidative phosphorylation (OXPHOS). Green indicates up-regulated pathways. (Color figure online)

[56]. These dynamic mitochondrial changes are associated with the metabolic effects of the cytokine IL-15, which also promotes memory cell survival. Buck et al. showed that effector T cells have punctate mitochondria, while memory T cells have fused mitochondria networks, which is associated with improved OXPHOS efficiency [54]. These mitochondrial and metabolic differences in memory T-cells are mediated by enhanced expression of mitochondrial dynamin-related protein (Drp)-1 and optic atrophy (Opa)-1 fusion protein, respectively. Mitochondrial fission in effector T cells results in reduced OXPHOS efficiency and reliance on anoxic glycolysis, while fused mitochondria in memory T cells improves OXPHOS efficiency [54].

Inhibiting FAO reduced SRC in both in vitro cytokine-generated and in vivo virus-induced memory T cells, suggesting FAO augments SRC [56]. Furthermore, FAO and SRC promoted memory T cell survival both in vitro and in vivo [56]. This efficient energy-producing metabolic signature is advantageous during the contraction phase, when nutrients and inflammatory signals are limited [56]. Therefore, SRC enables memory cells to generate more ATP, increase mitochondrial membrane potential, and promote survival upon re-stimulation compared to activated naïve T cells [56]. Lastly, FAO and changes to mitochondrial dynamics are critical for the development, longevity, and secondary response of memory T cells.

Memory T cells do not consume large amounts of exogenous lipids, indicating these fatty acids are not primarily utilized for FAO. Culturing memory T cells in lipid-depleted media did not affect rates of FAO, OXPHOS, nor SRC [53], further suggesting endogenous fatty acids may fuel FAO in these cells (Fig. 3). In addition, inhibiting FAS impaired memory T cell survival. In vitro experiments using ^{13}C-labeled glucose revealed that glucose-derived carbon from catabolic events is synthesized into fatty acids to fuel FAO by memory T cells [53]. Furthermore, culturing memory T cells in low glucose conditions reduced OXPHOS and SRC [53]. Glutamine supplementation did not restore OXPHOS or SRC in low glucose conditions, indicating that glucose primarily fuels FAS in memory T cells [53].

Storage and release of fatty acids from triacylglycerols is imperative for memory T cells. Inhibition of the triacylglycerol synthesis enzyme diacylglycerol acyltransferase reduced SRC [53]. Additionally, silencing the lysosomal acid lipase enzyme, which hydrolyzes lipids to fatty acids, impaired memory T cell development and survival [53]. Overall, these studies demonstrate that memory T cells utilize glucose transport and catabolism to fuel lipid synthesis and, in turn, oxidize de novo synthesized lipids to fuel OXPHOS.

Several key regulators are involved in the metabolic reprogramming of memory T cells (Fig. 3). The adaptor protein tumor necrosis factor receptor-associated factor 6 (TRAF6), which inhibits antigen-specific T cell activation

[61], regulates memory T cell development by activating adenosine monophosphate-activated protein kinase (AMPK). AMPK is an intracellular nutrient sensor that can increase FAO [55]. Mice with TRAF6 deficient T-cells mount a robust primary T cell response, but had impaired memory generation. Increasing AMPK-mediated FAO restored memory T cell generation in mice with TRAF6-deficient T cells. AMPK and mTOR are both intracellular nutrient sensors, with opposing roles in metabolic regulation [27]. AMPK up-regulates FAO and OXPHOS to efficiently generate ATP during low nutrient conditions, while mTOR promotes growth and proliferation through enhanced glycolysis and anabolic pathways [27]. Interestingly, mTOR may negatively regulate CD8 memory T cell development. Inhibiting mTOR augmented levels of antigen-specific CD8 memory T cells, in mice following lymphocyte choriomeningitis infection, and in non-human primates vaccinated with modified vaccinia virus [62]. IL-15 also provides a metabolic node that is critical for memory T cell generation. IL-15 increased mitochondrial biogenesis and CPT1 expression, resulting in enhanced memory T cell FAO, SRC, and OXPHOS [56]. Thus, certain cytokines (IL-15) and drugs (metformin or rapamycin) can tip the balance of catabolic and anabolic signals that regulate memory T-cells.

Metabolic regulation of memory NK cells

While classically characterized as innate immune cells, studies show that NK cells can develop memory, enabling enhanced responsiveness upon re-stimulation. Memory NK cells are induced by infection (i.e., cytomegalovirus (CMV) [63]), or by cytokine stimulation (i.e., IL-12/IL-15/IL-18 [3]). Memory development has fundamental implications in the immune response to infections. More importantly, memory NK cells also have translational implications, as improved longevity and potent secondary response can be exploited for NK cell immunotherapies to enhance persistence and function [64].

There is a dearth of information that describes key metabolic regulators involved in generating NK cell memory. The evidence to date links defective mitochondria, autophagy and key metabolic nodes in NK cell memory. It is thought to engage protective mitophagy responses in a subset of cells that become long-lived (i.e., memory). It has been shown that activation of AMPK or inhibition of mTOR promotes mitophagy-dependent NK memory cells as NK cells transition from contraction to the memory phase [65]. It is not yet clear which metabolic pathways or intermediates are involved, but given that AMPK and mTOR are key nodes of cell catabolism and anabolism, it is likely that glucose and/or OXPHOS flux regulates NK cell memory formation through control of mitophagy. Since autophagy/mitophagy can regulate lipid metabolism in other cell types, FAO may

also be integral in memory development [66]. Since metabolic pathways contribute to T cell memory, characterizing metabolic pathways in NK cells transitioning to memory cells and determining their role in regulating development and enhanced secondary response should be assessed. Uncovering underlying metabolic mechanisms in NK cell memory would determine how memory is regulated and lead to strategies improving persistence and activation potential for vaccine or adoptive NK cell therapies.

Limiting inflammation

Metabolic regulation of regulatory T cells and regulatory NK cells

The role of regulatory T cells (Tregs) is to limit inflammatory responses and maintain tolerance to self-antigens. To support these functions, Tregs have opposing metabolic needs from effector cells (Fig. 4). Treg generation is highly dependent on mitochondrial FAO, but hindered by enhanced glycolysis. For example, inhibiting FAO impaired Treg differentiation, but not T effector cells [29]. Tregs also rely more on OXPHOS and have greater mitochondrial mass and ROS production than T effector cells [58]. In contrast to T effector cells, Treg differentiation is enhanced by restricting glycolysis or its precursors such as using low glucose including 2DG in cell culture [29, 31]. While Tregs and memory cells both rely on FAO and increased mitochondrial mass, Tregs primarily use exogenous fatty acids for FAO and have low levels of FAS compared to memory subsets. In fact, blocking FAS by inhibiting ACC1 favors Treg differentiation [11].

Interestingly, Treg proliferation and its suppressive function have distinct metabolic needs [12]. In response to TLR signals, Tregs up-regulate Glut1 expression and anaerobic glycolysis to support proliferation and inhibit suppressive functions. In turn, suppressive Tregs utilize lipid oxidation coincident with reduced glycolysis. Together, these studies demonstrate a critical role for FAO and OXPHOS in Treg generation and suppressive function.

Numerous regulators of Treg function also modulate cell metabolism. AMPK plays an integral role in integrating nutrient status and Treg metabolism by increasing FAO, where AMPK activation promotes Treg differentiation both in vivo and in vitro (Fig. 4) [29]. Likewise, mTOR inhibition promotes Treg differentiation [29], whereas its activation through TLR signaling inhibits its suppressive function [12]. The transcription factor FOXP3 is influenced by cell metabolism and is a master regulator of Treg development and function [67]. FOXP3 transcriptional controls are linked to reduced expression of glycolytic enzymes and increased expression of FAO enzymes. FOXP3 activation coincides
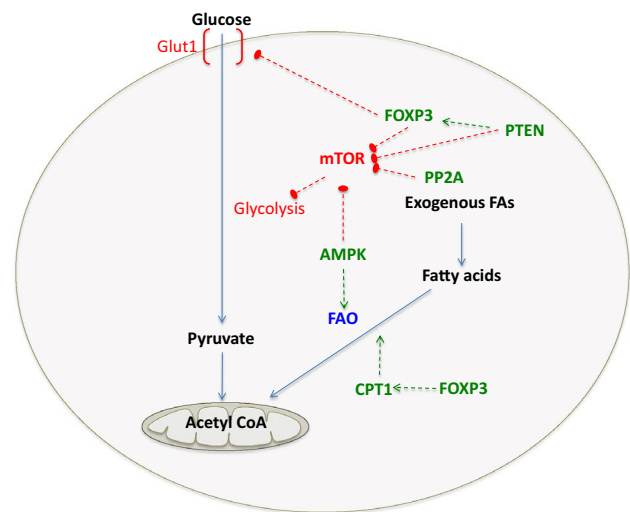

Fig. 4 Regulation of regulatory T cell (Treg) metabolism and suppressive function. Regulatory T cells oxidize exogenous fatty acids via fatty acid oxidation (FAO) and oxidative phosphorylation (OXPHOS) as a main source of energy. Numerous regulators of Treg suppressive function promote a Treg metabolic profile by inhibiting glycolysis and promoting fatty acid oxidation (FAO). Adenosine monophosphate-activated kinase (AMPK) promotes Treg differentiation and FAO, while inhibiting mTOR. FOXP3 inhibits glycolysis by inhibiting mTOR activity, Glut1 expression, and expression of glycolytic enzymes, but promotes FAO by increasing expression of the FAO rate-limiting enzyme carnitine palmitoyl transferase (CPT1). PTEN signaling supports Treg function by inhibiting mTOR signaling and consequent glycolysis while stabilizing FOXP3 expression. Similarly, PP2A promotes Treg function through inhibition of mTOR and glycolysis. Red indicates down-regulated pathways; green indicates up-regulated pathways. (Color figure online)

with reduced mTOR activity, glucose uptake, anaerobic glycolysis, and promotes mitochondrial lipid oxidation and rate of oxygen consumption. In essence, FOXP3 is a node in Treg development and function by tipping the metabolic balance toward FAO.

PTEN is an intracellular phosphatase that supports Treg function and confers protection from autoimmunity. Mice with a Treg-specific PTEN deletion have impaired function, resulting in systemic autoimmunity and lymphoproliferative disease [59]. PTEN supports suppressive function by inhibiting mTOR signaling, stabilizing FOXP3 expression, and promoting FAO. PTEN deficient Tregs have impaired mitochondrial function, characterized by reduced mitochondrial mass, and membrane potential (Fig. 4). Furthermore, PTEN$^{-/-}$ Tregs up-regulated glycolysis following TCR stimulation. PP2A is another intracellular phosphatase that is important for Treg function by suppressing mTOR activity [68]. Treg-specific PP2A$^{-/-}$ cells have increased basal and maximal anaerobic glycolytic rates comparable to WT cells, mediated by mTOR.

Very little is known about regulatory NK cells. Regulatory NK cells have a role in limiting autoimmune disease,

whereas aberrant NK cell activation can contribute to its pathogenesis [14, 15] However, the underlying metabolic regulation of these distinct functional NK cell subsets and whether metabolic manipulation of these NK cell subsets can affect the course of autoimmune diseases, as it does in T cells, are important unanswered questions.

Inhibitory signals alter T cell metabolism

In addition to specialized regulatory cells, signals that help resolve T cell effector function counterbalance prolonged or excessive inflammation. Changes in cellular metabolic flux can integrate inhibitory T cell signals. For example, nutrient availability (at the peak of T cell activation) may promote inflammatory resolution, where AMPK is activated in a low glucose local environment [69].

Numerous inhibitory compounds alter T cell metabolism, including indoleamine 2,3-dioxygenase (IDO), an enzyme involved in tryptophan catabolism [70, 71]. IDO inhibits T cell Glut1 expression and glucose uptake [72]. Furthermore, IDO down-regulated FAS enzymes ATP-citrate lyase and ACC1, which are critical for T effector function [71]. Programmed death-1 (PD1) is a checkpoint receptor expressed by T cells that inhibits effector function, maintains tolerance, and limits the inflammatory response [73]. The pathological up-regulation of PD1 occurs within a tumor or during chronic viral infection, resulting in impaired T effector functions [74, 75]. PD1 ligation promotes conversion of T cell metabolism from an anaerobic glycolytic state toward a more oxidative state upon activation [76]. Additionally, PD1 ligation inhibited glucose and glutamine uptake, and subsequent catabolism, while up-regulating lipolysis and FAO. Similarly cytotoxic T-lymphocyte-associated antigen 4 (CTLA4), another checkpoint receptor, also inhibits glycolytic metabolism, but without up-regulating FAO [76]. Therefore, inhibitory signals suppress T effector function by suppressing T cell glycolytic and anabolic pathways.

Limiting NK cell effector functions

Metabolic flux can also inhibit NK cell function. The evidence to date supports a role for AMPK and mTOR pathways in limiting NK cell function. One study reported that AMPK activity correlates with impaired function in senescent NK cells with high inhibitory receptor KLRG1 expression [77]. Not only do KLRG1^{bright} NK cells have spontaneous AMPK activation, but KLRG1 ligation activates AMPK which negatively regulates NK cell IFN-γ production, cytotoxicity, and proliferation [78]. Immunosuppressive cytokines, such as transforming growth factor-beta (TGF-β) can inhibit NK cell function. Deletion and TGF-β signaling reduced NK cell cytotoxicity and IFN-γ production both in vitro and in vivo [79]. TGF-β signaling inhibited IL-15-induced up-regulation of both anaerobic glycolysis and OXPHOS, an effect that occurs via mTOR inhibition [79]. Taken together, these results suggest that TGF-β inhibits IL-15-induced NK cell effector functions through mTOR inhibition, demonstrating that metabolic pathways can mediate inhibitory NK cell signals.

Comparison of NK cell and T cell inhibition

Similar metabolic by-products, inhibitory signals, and intracellular regulators known to promote Treg suppressive function or inhibit T effector functions also suppress NK cell effector functions. For instance, lactic acid secreted by tumor cells impaired both NK and T cell anti-tumor responses, including IFN-γ production, promoting tumor growth. Furthermore, elevated levels of lactic acid inhibit intracellular ATP levels in both cell types upon activation [80]. In addition, IDO and PD1, which mediate inhibitory signals that coincide with changes in metabolic flux in T cells function, also impair NK cell effector functions [81, 82]. However, the effects of IDO and checkpoint inhibitor pathways on NK cell metabolism are ill-defined. Similar to T cells, the intracellular phosphatase PTEN negatively regulates NK cell cytotoxicity. Overexpression of PTEN reduced murine and human NK cell cytotoxicity by suppressing the PI3K/AKT pathway, a known mTOR activator. Inhibition of PTEN enhances NK cell cytotoxicity against tumor targets [83, 84]. PP2A activity also affects NK cell function. Promoting PP2A activation in NK cells suppressed cytotoxicity, granzyme B expression, and IFN-γ production [85, 86]. Inhibiting PP2A increased NK cell cytotoxicity of metastasis in a B16 melanoma model [87]. Since both PTEN and PP2A enhanced T cell mitochondrial oxidation and mitigate anaerobic glycolysis to sustain Treg function, it is plausible that similar metabolic processes are engaged to alter NK cell cytotoxic function.

T effector cell inhibition or enhanced Treg suppressive function is associated with a metabolic conversion from glycolytic and anabolic pathways to lipid oxidation. NK cell inhibition is regulated by inhibiting mTOR or activating AMPK, indicating similar metabolic reprogramming in T cells may regulate NK cell inhibition. While energy-sensing molecules inhibit NK cell function, it is not yet clear if distinct metabolic pathways are altered in response to specific inhibitory signals. Additionally, determining the metabolic state and regulation of NK cell subsets in immunosuppressive states, including senescence and tumor microenvironments is positioned to advance cell-based therapeutic strategies.

Endocrine and pharmacological modifiers of T and NK cell metabolism

Endocrine factors such as insulin play a critical role in glucose uptake and glucose flux in many cell types, including, lymphocytes [88]. The metabolic consequences of insulin are facilitated by the insulin receptor-dependent signaling and glucose transporters [89]. Induction of the insulin signaling cascade in activated T cells occurs following CD28 signaling upon antigen presentation, leading to PI3K-dependent Akt phosphorylation, up-regulation of GLUT1 and subsequent increase in glucose uptake, glycolysis and lactate production [24]. Using an inducible insulin receptor-knockdown and adoptive transfer approach, Fischer et al. demonstrated that silencing the insulin receptor reduced glucose transport, glycolysis, migration, and IFN-γ expression, and enhanced apoptosis in activated CD4 T cells [88]. Insulin receptor deficiency also reduced proliferation rates in both activated CD4 and CD8 T lymphocytes when compared to controls. In CD8 T-cells, knockdown of the insulin receptor also reduced cytotoxic alloantigen responses. Furthermore, insulin receptor deficiency did not impair thymocyte development, nor Treg number and suppressive capacity [88]. Therefore, insulin does not equally affect all T cell subsets. Overall, these findings suggest that the insulin signaling cascade contributes to effector T cell glucose flux. Similar to effector T cells, NK cells express the insulin receptor, but little is known regarding its role in NK cell physiology [90]. Further work is needed to determine whether NK and T cells share physiological commonalities regarding the role of insulin.

Rapamycin and metformin are two compounds that have profound metabolic effects and can influence immune cell metabolism. Rapamycin was discovered as an antifungal metabolite with immunosuppressive and anti-proliferative effects in mammalian cells and is a well-known inhibitor of mTOR [91]. In CD8 T cells, inhibiting mTOR with rapamycin transcriptionally regulates T cell fate by promoting memory cell formation [92]. Enhanced mTOR kinase activity with IL-12 treatment and antigen stimulation in naïve CD8 T cells induced the expression of the transcription factor T-bet, which leads to short-lived effector cell formation. Conversely, inhibiting mTOR with rapamycin treatment dampened T-bet expression, while enhancing eomesodermin expression, a transcription factor known to promote memory cell precursors. Rapamycin has historically been considered an immunosuppressive drug, but its immunostimulatory effects on memory CD8 cells were revealed upon adoptive transfer of rapamycin treated CD8 T cells, which had enhanced antigen-recall responses [62]. Enhanced memory cell formation due to rapamycin treatment was also shown in both murine and primate models virally infected with acute lymphocytic choriomeningitis and vaccinia, respectively [62]. These results demonstrate that mTOR is involved in determining CD8 T cell fate, whereby rapamycin treatment favors a memory phenotype.

Few studies have examined the effects of rapamycin on NK cell function. Splenic NK cells treated with rapamycin significantly decreased IFN-γ production, glucose uptake, and inhibited IL-2/IL-12-mediated up-regulation of granzyme B expression [44]. Similarly, rapamycin treated human NK cells had decreased glucose uptake and IFN-γ production, along with impaired CD98 expression, which is a subunit of the neutral amino acid transporter LAT-1 [45]. Rapamycin-induced mTOR inhibition has also been shown to inhibit NK cell proliferation, degranulation, and cytotoxicity [79]. Overall, these studies demonstrate that rapamycin treatment may not only affect NK cell metabolism by inhibiting glucose uptake and possibly affect amino acid transport but can impair NK cell cytotoxic function.

Metformin is commonly prescribed during prediabetes and type 2 diabetics since can alter lipid and glucose metabolism and acts as an insulin sensitizer by reducing hepatic glucose production and promoting glucose uptake in skeletal muscle [93]. The insulin-sensitizing effects of metformin are linked to AMP-activated protein kinase-acetyl-CoA carboxylase-mediated changes in lipid metabolism and the balance between lipogenesis and FAO in metabolic tissues such as the liver [94, 95]. Since AMPK activation and increased FAO also promote Treg differentiation, it is not surprising that metformin treatment of naïve T cells impairs Th17 differentiation [96]. In a murine model of asthma, in vivo treatment with metformin decreased Glut1 expression and increased the number and percentage of Tregs [29]. Similarly, mice treated with metformin had significant improvements in clinical severity and mortality during acute graft versus host disease, which was associated with metformin-mediated reductions in Th1/Th17 and increased Th2 and Treg populations [96]. In vitro experiments also showed that Th17 cells treated with metformin can skew towards characteristics of Treg cells, demonstrating its therapeutic potential in modulating CD4 T cell differentiation relevant to autoimmune diseases. The effects of metformin treatment on NK cell metabolism and function is currently unknown. Therefore, further work is needed to determine if metformin alters NK cell function and whether these effects are mediated through changes in cellular metabolism.

Conclusion

T cells and NK cells share common metabolic nodes that influence cell development, activation, effector, memory and regulatory function. Coaxing changes in cell metabolism

may have consequences that span innate and adaptive immune responses. Cell intrinsic or ligand specific differences in how metabolic flux controls cell fate in NK and T cell subsets should be considered.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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