

REVIEW

Metabolic dynamics instruct CD8⁺ T-cell differentiation and functions

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Cytotoxic CD8⁺ T cells are a key element of the adaptive immune system to protect the organism against infections and malignant cells. During their activation and response, T cells undergo different metabolic pathways to support their energetic needs according to their localization and function. However, it has also been recently appreciated that this metabolic reprogramming also directly supports T-cell lineage differentiation. Accordingly, metabolic deficiencies and prolonged stress exposure can impact T-cell differentiation and skew them into an exhausted state. Here, we review how metabolism defines CD8⁺ T-cell differentiation and function. Moreover, we cover the principal metabolic dysregulation that promotes the exhausted phenotype under tumor or chronic virus conditions. Finally, we summarize recent strategies to reprogram impaired metabolic pathways to promote CD8⁺ T-cell effector function and survival.

Keywords: T cells · metabolism · T-cell differentiation · T-cell exhaustion · infection

Introduction

CD8⁺ T cells are one of the most crucial components of the adaptive immune system, and play a key role in response to pathogens and tumors. Upon antigen stimulation, naive CD8⁺ T cells get activated and differentiate into effector cells and a small subset of memory cells can form after antigen clearance. Emerging evidences support that metabolic reprogramming not only provides energy and biomolecules to support pathogen clearance, but is also tightly linked to T-cell differentiation [1,2]. The dynamic metabolic profiles and the exposure to pathological microenvironmental impact CD8⁺ T-cell functions and differentiation program. Metabolic programming is, thus, considered to be one of driving players to regulate T-cell biology and to ultimately tailor the adaptive immune protection. In this review, we summarize the metabolic profiles in CD8⁺ T cells and highlight the metabolic switch between different differentiation states. In addition, we

cover current knowledge about metabolic dysregulation that supports the establishment of the exhaustion state in response to chronic antigen exposure.

Metabolic reprogramming during CD8⁺ T-cell activation and differentiation

Overview of metabolic profiles in differentiation states

Naive CD8⁺ T cells are activated in response to the coordination of three signals, including TCR (signal 1), costimulation (signal 2), and inflammatory cytokines (signal 3) [3]. After activation, those pathogen-specific CD8⁺ T cells undergo massive expansion and differentiation into effector T cells, which contribute directly to pathogen clearance. Then, the majority of CD8⁺ T cells undergo contraction phase and die by apoptosis. Eventually, a small percentage of T cells survive and form different memory subsets, which can provide long-term protection [4]. Interestingly, emerging evidence reveals that different differentiation

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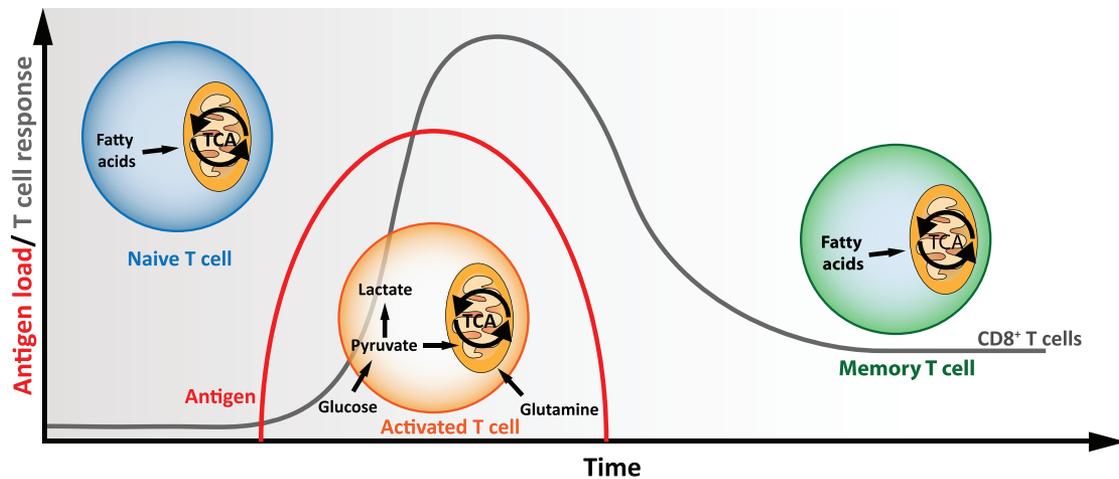


Figure 1. Metabolic profiles of CD8⁺ T cells during an immune response. Upon acute infection, the activation of naive CD8⁺ T cells triggers massive expansion and differentiation into effector T cells, which contribute directly to pathogen clearance. TCR signaling stimulates aerobic glycolysis to support the intense cell proliferation. Following the elimination of the antigen, the majority of CD8⁺ T cells undergoes the contraction phase. However, a small percentage of T cells survive and form memory subsets, which can provide long-term protection. These cells rely on fatty-acid oxidation and oxidative phosphorylation for their maintenance.

states of CD8⁺ T cells require distinct metabolic supplies to support their bioenergetic demands and epigenetic programming. In general, naive CD8⁺ T cells are maintained in a quiescent phenotype, which is characterized by low metabolic activities and catabolic metabolism [5]. Since naive CD8⁺ T cells have limited demands for biomolecular production, they predominantly rely on mitochondrial OXPHOS to produce ATP and sustain homeostasis [6,7]. The long-term survival and metabolic regulation of naive CD8⁺ T cells depend on signals induced by self-ligands/TCR and IL-7 [8–10]. To prevent the atrophy of quiescent T cells, IL7-IL7R signaling promotes basal glucose catabolism by regulating the expression of glucose transporter 1 (GLUT1) through activation of AKT, which plays a central role in glucose uptake [11,12]. Glucose then acts as the crucial nutrient to fuel oxidative phosphorylation (OXPHOS) (Fig. 1).

Upon acute infection, pathogen-specific CD8⁺ T cells undergo activation and expansion with metabolic transition from catabolism to anabolism, where extracellular nutrients are utilized to promote biosynthesis and ATP production for proliferation and effector function [13,14]. These metabolic features are characterized by the increase of aerobic glycolysis, glutaminolysis, as well as mitochondrial biogenesis [15] (Fig. 1). During this process, glucose is utilized for aerobic glycolysis and pentose phosphate pathway to facilitate synthesis of amino acid, NADPH, nucleotide, and ribosome. Moreover, the metabolites from glycolysis and glutaminolysis enter tricarboxylic acid cycle, which supports mitochondrial metabolism and OXPHOS, and mitochondrial dynamics also instruct T-cell differentiation program [16]. Of note, emerging works reveal that metabolic programs intertwine with intracellular signaling to tailor the functions of effector CD8⁺ T cells [14,17] (Fig. 2). TCR engagement combined with CD28 costimulation triggers PI3K-AKT signaling, which in turn, facilitates GLUT1-controlled glucose uptake [18,19]. The expression of GLUT1 is also upregulated by transcription fac-

tors such as Nuclear Factor of activated T-cells 1 (NFAT1) [20]. AKT can further induces the activity of mammalian Target of Rapamycin (mTOR), the central driver of anabolism, to promote the glycolytic flux by its downstream transcription factors [17,21]. Diverse glycolytic enzymes, including hexokinase 2 and pyruvate dehydrogenase, are also tuned following activation [20,22]. Amino acids promote T cell activation and effector features through multiple mechanisms. First, increased glutamine uptake and glutaminolysis catabolize the glutamine to support essential biosynthesis [23]. Among the glutamine transporters, Slc32a1 and Slc32a2 are upregulated via transcription factor Myc in an AKT-mTOR dependent manner. Second, system L transporter Slc7a5 (also known as LAT1) controls the uptake of large neutral amino acid, such as leucine uptake, and supports protein synthesis in T cells. The loss of Slc7a5 and deprivation of leucine impair the activation of mTOR and expression of c-Myc, which results in impairment of effector T-cell differentiation [23]. Third, methionine uptake is essential to sustain S-adenosylmethionine synthesis, which provide substrate for DNA and histone methylations and subsequently impact on epigenetic program during T-cell differentiation [24].

During the contraction phase, some antigen-specific CD8⁺ T cells differentiate into memory phenotype and enter a more quiescent state, which displays catabolic metabolism. However, memory CD8⁺ T cells are metabolically different from naive CD8⁺ T cell, which has been speculated to allow different metabolic niche and support a stronger reaction after re-encountering the same antigen [15,25,26]. The survival of memory cells is maintained by IL-7 and IL-15, as well as mitochondrial metabolism [27–29] (Fig. 2). Previous studies in mouse models showed that memory cells present elevated spare respiratory capacity, which is the extra amount of ATP that can be produced by OXPHOS upon high energetic demand, increased mitochondrial content, and altering cristae that favor the proximity of electron trans-

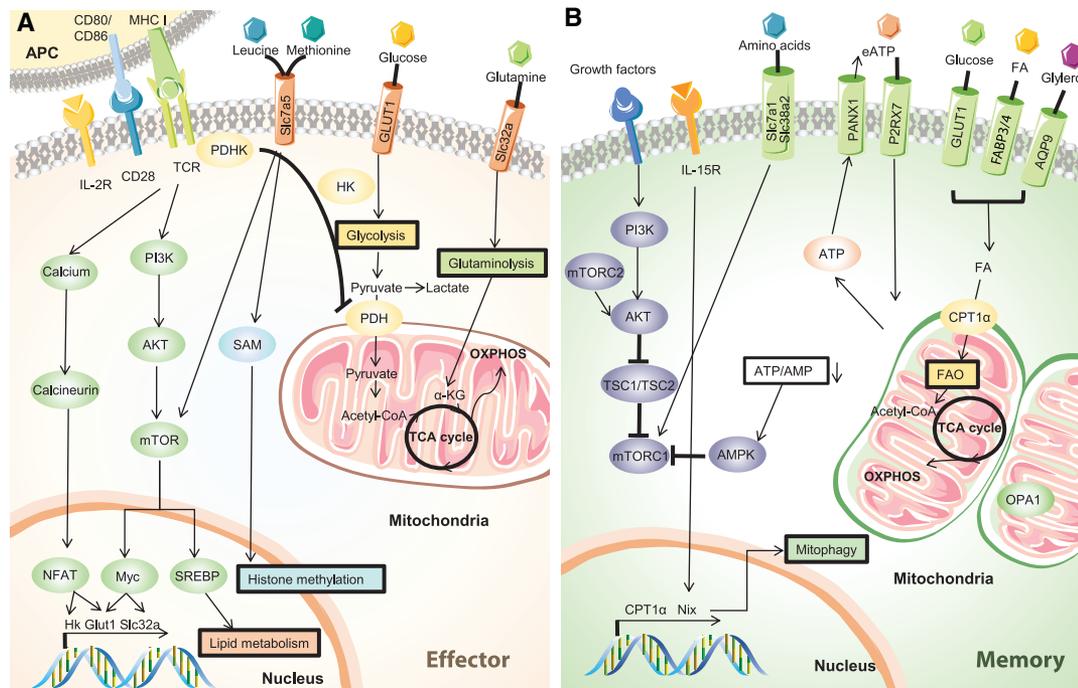


Figure 2. Metabolic reprogramming in CD8⁺ T-cell activation and memory formation. (A) Metabolic changes in CD8⁺ T cells upon activation. Upon antigen recognition, stimulatory signals T-cell receptor (TCR) and costimulation CD28 induce the activation of PI3K-AKT pathway, mTOR, and the calcium release in the cytosol. This pathway result in the upregulation of the transcription factors NFAT and Myc that promote glycolysis and glutaminolysis. (B) Metabolic regulation during memory CD8⁺ T-cell formation. After antigen clearance, mTORC1 is inhibited which leads to the downregulation of aerobic glycolysis. Fatty acids are used as main substrates for energy production: fatty acids enter the mitochondria through CPT1- α and are transformed in acetyl-CoA by fatty-acid oxidation. Acetyl-CoA enters then the tricarboxylic acid cycle to support the oxidative phosphorylation and energy production. Mitochondrial fitness is also closely regulated by proteins involved in mitophagy like Nix and also by inner mitochondrial membrane protein Optic atrophy 1 (OPA1) that promotes mitochondrial fusion.

port chain proteins in memory CD8⁺ T cells [16,27]. Additionally, IL-15 signaling facilitates the expression of carnitine palmitoyltransferase Ia (CPT1a), which is a rate-limiting enzyme that transports fatty acid into mitochondria and promotes fatty-acid oxidation [27]. Of note, the contribution of CPT1a in supporting memory T-cell survival remains to be confirmed due to the potential off-target effect caused by etomoxir [30]. Meanwhile, arginine metabolism plays an important role in regulating memory T-cell differentiation [31]. T cells cultured with increased L-arginine exhibit reduced glycolysis, increased OXPHOS, and central memory phenotype, which are mediated in part by arginine sensors in the nucleus [31].

Since mitochondria controls numerous metabolic programs that can impact T-cell activation and differentiation, it is reasonable to imagine that regulatory circuit controlling mitochondrial dynamics has to be finely tuned for supporting T-cell activation and differentiation. In support of this, dynamin-related protein 1 (DRP1) that controls mitochondrial fission has been shown to enhance anabolic metabolism, whereas loss of DRP1 leads to a more quiescent state in CD8⁺ T cells [16]. Besides, absence of mitochondrial fission protein DRP1 promotes memory formation by dictating mitochondrial fusion [16]. In line with this observation, the deletion of Optic atrophy 1 (Opa1), an inner mitochondrial membrane fusion protein, results in a strong impairment in

the development of central memory CD8⁺ T cells [16,32]. Moreover, the impairment of mitophagy, a process that degrades dysfunctional mitochondria [33], leads to impaired memory T-cell formation. In addition, the increased expression of Nix, which is a key mitophagy molecule, promotes effector memory formation, but not central memory formation [33].

Metabolic program intertwines with differentiation program of T-cell exhaustion

Differentiation program of T-cell exhaustion

In the context of tumors and chronic viral infection, such as HBV, HCV, and HIV, the immune system fails to control the inflammation and to clear the antigen efficiently, which leads to a prolonged and persistent antigen exposure in the organism. The continued stimulation in CD8⁺ T cells is believed to skew the T cells into a particular differentiated state known as “exhaustion” [34–36]. This state is characterized by the loss of effector cytokine production, impaired proliferation, upregulation of inhibitory receptors including programmed cell death protein-1 (PD-1), T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), and lymphocyte activating-3, and altered transcriptional and

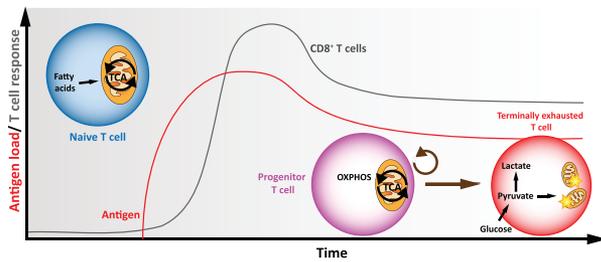


Figure 3. Metabolic deficiency in exhausted CD8⁺ T cells in chronic environment. Upon antigen persistence, T cells form an intermediate progenitor subset that presents memory-like characteristics like self-renewal and tcf-1 expression. This progenitor subset irreversibly gives rise to the short-lived terminally exhausted subset, which is associated with a glycolytic gene signature as well as mitochondrial damage.

epigenetics profiles [34,37,38]. The establishment of the exhausted phenotype has been described as a progressive differentiation process, starting from the precursors of memory cells [39]. The consequence of this process is a heterogenic pool of exhausted T cells [40]. Tremendous efforts have been made in the past few years to unveil this heterogeneity with the hope to better understand various responses to immune checkpoint blockade [41,42]. A CD8⁺ T-cell lineage, characterized by the expression of the T-cell factor-1 (TCF-1) and named as “progenitor exhausted T cells” or “memory-like T cells,” displays stem-like properties including self-renewal and the ability to differentiate into terminally exhausted cells (Tex) [40,43,44,45] (Fig. 3). Interestingly, progenitor exhausted T cells (Tpex) have been reported to increase their proliferation, expansion, and further differentiation in effector and terminally exhausted T cells upon PD-1 blockade treatment, which make them a particularly attractive therapeutic target in treating cancer and chronic viral infections [40,43,45,46]. Recent work in mice further refined the current understanding of this differentiation process by introducing four subsets of progenitor, intermediate and terminally exhausted T cells linked in a hierarchical development pathway [47]. Those subsets are defined by their CD69 and Ly108 markers and by the expression of TCF-1, TOX, and T-bet which coordinate the development of this lineage. Of note, it has also been shown that only the TCF-1⁺ Tpex population, but not the terminally exhausted T cells, is able to replenish T cells upon transfer in another infected mouse, suggesting that the presence of Tpex population supports a long-term protection in the diseases where pathogens and tumor cells cannot be eliminated [40]. However, a cooperation between progenitor and exhausted T cells has been shown to be crucial for the long-term control of the viral load and tumors [48]. These observations suggest that maintaining a balance in this lineage is critical to keep the infection and tumor in check.

Mitochondrial dysfunction in exhausted T cells

Given the high interest toward the therapeutic potential of targeting progenitor T cells [40,43,45,46], understanding the metabolic profile and how metabolic program orchestrate T-cell differentia-

tion could provide opportunities to promote formation of progenitor T cells and potentially enhance immune checkpoint blockade via metabolic interventions. In the early phase of chronic lymphocytic choriomeningitis virus infection, virus-specific CD8⁺ T cells have been shown to present depolarized mitochondria, causing bioenergetic defects and loss of effector function [38]. Interestingly, overexpression of the peroxisome proliferator-activated receptor coactivator-1 α (PGC1- α) could lead to lower depolarized mitochondrial content and rescue effector function, suggesting that metabolic manipulation could present a great therapeutic opportunity. These findings are supported by human studies showing that exhausted T cells extracted from HBV-infected patients, presented a strong decreased expression of genes involved in the mitochondrial biogenesis and fitness [49]. Moreover, these HBV-specific CD8⁺ T cells also accumulated depolarized mitochondria. This accumulation leads to strong production of mitochondrial reactive oxygen species, which is known to modulate T-cell effector functions [50,51]. PD-1 signaling also contributes to the impaired bioenergetic profile by repressing aerobic glycolysis, promoting fatty acid oxidation, and compromising mitochondrial fitness by repressing the expression of PGC1- α [38,52]. Furthermore, PD-1 signaling was shown to reduce the length and number of mitochondrial cristae, in human CD8⁺ T cells, which also promotes the apparition of dysfunctional mitochondria [53]. This suggests that PD-1 blockade could be used to reinvigorate exhausted T cells by promoting glucose uptake and mitochondrial fitness [38,54].

Competitive and immunosuppressive microenvironment

Similar to virus-specific CD8⁺ T cells in chronic viral infections, tumor-infiltrating lymphocytes also become exhausted in the tumor microenvironment because of persistent antigen stimulation, the exposure to nutrient deficiency, and various stress signals [55,56] and the accumulation of dysfunctional mitochondria [57]. Activated T cells require high amounts of nutrients as source of energy and building blocks for their proliferation and effector function. Nutrients competition and deprivation is known to impact human T-cell differentiation, metabolic profile, and shift the survival balance [58,59]. Notably, nutrient shortage has been shown to promote apoptosis through the Bcl-2 family members, Puma and Noxa [58]. Furthermore, the tumor microenvironment contains immunosuppressive molecules that can also impair T-cell metabolism and promote exhaustion. For example, cholesterol present in the mouse tumoral microenvironment is taken up by the cells which increase ER stress and XPB1 pathway, resulting in declined antitumor activity [60]. Reprogramming of the methionine recycling machinery by tumor cells, in hepatocellular carcinoma of mice, increases the methionine metabolites S-adenosylmethionine and 5-methylthioadenosine levels in the environment, leading to T-cell dysfunction [61]. Other environmental cues can drive T-cell exhaustion like persistent IL-2 stimulation in the tumoral microenvironment. It was reported that

continuous IL-2 presence triggered persistent activation of STAT-5 which leads to metabolic changes in murine T cells by overexpressing the tryptophan hydroxylase 1. This enzyme catalyzes the conversion of tryptophan to 5-hydroxytryptophan, which activates AhR nuclear translocation and then an overexpression of inhibitory receptors and an inhibition of cytokine production in T cells [62].

Recent work reports that ER stress, in human ovarian cancer, engages the IRE1- α -XBP1 pathway in T cells which decreased mitochondrial respiration and effector functions [63]. Mechanistically, the activation of the IRE1- α -XBP1 signaling limited glutamine uptake by repressing the abundance of glutamine carriers and, thus, limiting fuel necessary to engage mitochondrial metabolism in a glucose-deficient tumoral microenvironment [63]. The stimulator of IFN genes pathway (STING) is another stress-response pathway and has the ability to sense foreign or self-DNA aberrantly localized in cytosol and respond by the induction of type I IFNs [64,65]. Moreover, STING expression has been reported to be largely decreased in CD8⁺ T cells from cancer patients compared with healthy donors. In the same study, the authors further unveiled that the STING cascade enhances TCF-1 expression by inhibiting Akt activity and the maintenance of progenitor CD8⁺ T cells in a type I IFN-dependent manner [66]. These findings are consistent with previous results demonstrating that Akt signaling promotes CD8⁺ T-cell effector differentiation by facilitating aerobic glycolysis, while inhibiting memory-precursor formation [67,68]. In that regard, it has been shown that enhanced aerobic glycolysis was a characteristic of terminally exhausted T cells and a glycolytic gene signature was increased in Tex compared to Tpex derived from human melanomas [69]. Since mitochondrial impairment can also promote dependency on aerobic glycolysis to meet cellular metabolic demands, it is possible that Tex cells display a higher glycolytic score as a result of mitochondrial defects. In line with this postulate, several groups recently uncovered that CD8⁺ TILs accumulating depolarized and dysfunctional mitochondria displayed characteristics of terminally exhausted T cells such as diminished TCF-1 expression, increased expression of PD-1 and TIM-3, and a loss of cytokine production [70,71]. Moreover, forcing the accumulation of depolarized mitochondria is sufficient to trigger the apparition of an exhausted phenotype in vitro [70].

Taken together, these recent findings point out the metabolic differences in different T-cell differentiation states during exhaustion. These observations are suggesting that exhausted CD8⁺ T cells from different stages of exhaustion present distinct metabolic profiles to support metabolic demands of each differentiation state. The metabolic preference and mitochondrial fitness seem to be crucial for their function and long-term persistence. We could speculate that the immune system, in response to overwhelming challenges, instructs the formation of this particular T-cell lineage to control as best as possible the infection load and to adapt the metabolic features supporting this new organization structure. The progenitor exhausted subset does not engage into heavy proliferation and cytokine production, but maintain a basal self-renewal capacity and differentiation capacity to become

effector and terminally exhausted T cells. Thus, those progenitors may have metabolic needs similar to memory T cells to cope with their long-lived ability. These distinct metabolic demands in Tpex and Tex could spare metabolic niche to simultaneously support the survival and functions of both subsets, a critical step for fighting the persisting infection and tumors. Another related question is how can the TCF-1⁺ Tpex population be maintained in tumors where T cells receiving persistent stimulation and the environmental stress. Different groups tried to follow the differentiation and the localization of this lineage and reported pools of Tpex in niches within tumor draining LNs. They found a stable reservoir of tumor-specific TCF-1⁺ CD8⁺ T cells in dLN and those migrated toward the tumor [72,73]. In presence of the tumoral microenvironment, these cells progressively became terminally differentiated. These findings indicate that nutritional changes happening in LNs or as a result of the metabolic crosstalk between T cells and APCs could guide T-cell differentiation in the context of prolonged antigen exposure.

Metabolic reprogramming

Emerging strategies have been focusing on reprogramming the metabolic profile and flexibility of virus- and tumor-specific T cells to improve their function and persistence in response to metabolic stress. Targeting the mitochondrial fitness and biogenesis is one of the most promising strategies (Fig. 4). In that regard, using the mitophagy process could be beneficial for exhausted cells. Mitophagy is the selective removal of damaged mitochondria to maintain mitochondrial health and turnover [74,75]. Yu et al., showed that stimulating mitophagy by providing the NAD precursor nicotinamide riboside [76] reduced the accumulation of depolarized mitochondria in a drp-1-dependent manner and decreased mitochondrial reactive oxygen species levels [70]. Furthermore, nicotinamide riboside treatment on melanoma-bearing mice boosted the effector cytokines production ability of TILs and improved tumor control [70]. Inhibiting mitochondrial regulators, such as REGNASE-1, has been shown to promote the mitochondrial fitness, in a BATF-dependent manner, and the formation of long-lived effector CD8⁺ T cells and result in better tumor control [77]. In addition, preserving the mitochondrial quality by *N*-acetylcysteine, an antioxidant, is able to protect T cells from mitochondrial ROS-induced mitochondrial damage. As a result of this action, *N*-acetylcysteine treatment could restore T-cell proliferation and effector function and elicit a memory-cell associated transcriptome [69]. Mitochondrial activity of terminally exhausted T cells can also be rescued by 4-1BB stimulation, which is a costimulatory molecule expressed on exhausted T cells. The 4-1BB stimulation supported mitochondrial fusion and biogenesis by activating p38-MAPK and PGC1- α in mice [78]. 4-1BB induced mitochondrial fitness enhancement could improve tumor control in an adoptive cell therapy model and synergized with anti-PD-1 treatment. Given the promising results of 4-1BB stimulation, this strategy has been further used for CAR-T-cell generation. Incorporation of the intracellular domain of 4-1BB

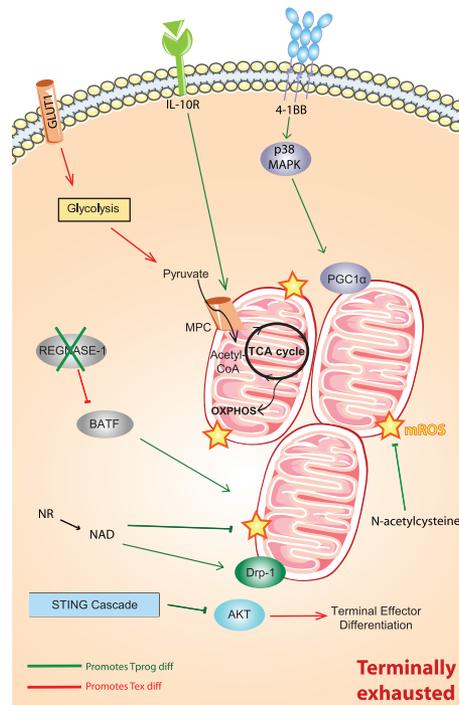


Figure 4. Metabolic reprogramming of terminally exhausted T cells. Different strategies could be used to specifically target the metabolism and the mitochondrial fitness of terminally exhausted T cells. The deletion of mitochondrial regulator REGNASE-1 can increase mitochondrial quality in a BATF-dependent manner. Preserving mitochondrial health against the accumulation of mitochondrial ROS by N-acetylcysteine can also restore T-cell effector function and memory-like phenotype. Mitochondrial fusion and biogenesis can be rescued through p38-MAPK and PGC1- α activation by 4-1BB stimulation. Treatment with IL-10-Fc fusion protein was able to reprogram the metabolism of terminally exhausted TILs by promoting oxidative phosphorylation by the mitochondrial pyruvate carrier MPC.

showed enhanced central memory phenotype associated with increased respiratory capacity, fatty acid oxidation, mitochondrial biogenesis, and partially preventing exhausted phenotype [79,80]. Our recent work also reported that treating tumor-bearing mice with an IL-10-Fc fusion protein could reprogram the metabolism of terminally exhausted TILs by promoting the mitochondrial pyruvate carrier-dependent oxidative phosphorylation [81]. This resulted in the enhancement of the expansion and the effector function of terminally exhausted cells and contributed to better tumor control in a progenitor T cell-independent manner. Interestingly, this therapeutic mode of action is independent of progenitor cells, unlike currently used therapies that rely heavily on progenitor subset stimulation like anti-PD-1 therapy and subsequently could be used in a complementary manner to boost at the same time progenitor and terminally exhausted T cells.

Conclusions

Delineating how the metabolic profile in CD8⁺ T cells evolves during the course of immune response against infection and tumors has been a major interest in the immunology field. However, the

main metabolic regulators that trigger those transitions remain unclear, which hampers our progress to optimize long-term immunization against pathogens by harnessing immunometabolic regulations. Moreover, the dysregulated metabolic activities have been shown to disarm CD8⁺ T-cell responses and orchestrate the exhaustion program in response to chronic infection or cancers. Recent studies gave a glimpse on how targeting metabolic program in CD8⁺ T cells can improve CD8⁺ T-cell function in chronic viral infections and tumor control. However, given the diversity of metabolic states in different tissues and the impacts caused by metabolic status of subjects, it remains challenging to exploit our knowledge in immunometabolism of CD8⁺ T cells to customize treatments. One could also expect metabolic discrepancies in T cells between chronic infection and tumors since the tumor microenvironment exhibits more complicated metabolic stress and communications. We postulate that environmental cues further disrupt the metabolic profile of T cells in tumors compared to chronic infection, potentially causing faster and stronger terminal differentiation in tumor site resulting in accumulation of depolarized mitochondria and alter lactate metabolism. This could be the reason why we can observe niches of TCF1⁺ T_{PEX} in draining LNs. In that regard, metabolic reprogramming designs should take into consideration nutrients access, localization, and environmental cues that CD8⁺ T cells will undergo. In addition, most studies about T-cell metabolism are conducted by using mouse models and no direct comparison has been made between the metabolic profile in murine and human T cells which may lead to translational issues. In conclusion, a full consideration on the interplay between intrinsic and extrinsic metabolic programs and a better understanding of the different specific metabolic status in tumor and infection, and in human and mouse, could pave a critical step for us to tailor desirable CD8⁺ T-cell immune responses.

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Abbreviations: **CPT1a:** carnitine palmitoyltransferase Ia · **DRP1:** dynamin-related protein 1 · **mTOR:** mammalian Target of Rapamycin · **Opa1:** Optic atrophy 1 · **PD-1:** programmed cell death protein-1 · **TIM-3:** T-cell immunoglobulin and mucin-domain containing-3 · **TCF-1:** T-cell factor-1 · **Tex:** terminally exhausted cells · **Tpex:** progenitor exhausted T cells · **PGC1- α :** proliferator-activated receptor coactivator-1 α · **STING:** stimulator of IFN genes pathway

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