

# Chapter 7

## Innate and Adaptive Immune Cell Metabolism in Tumor Microenvironment

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**Abstract** During an immune response, leukocytes undergo major changes in growth and function that are tightly coupled to dynamic shifts in metabolic processes. Immunometabolism is an emerging field that investigates the interplay between immunological and metabolic processes. The immune system has a key role to play in controlling cancer initiation and progression. Increasing evidence indicates the immunosuppressive nature of the local environment in tumor. In tumor microenvironment, immune cells collectively adapt in a dynamic manner to the metabolic needs of cancer cells, thus prompting tumorigenesis and resistance to treatments. Here, we summarize the latest insights into the metabolic reprogramming of immune cells in tumor microenvironment and their potential roles in tumor progression and metastasis. Manipulating metabolic remodeling and immune responses may provide an exciting new option for cancer immunotherapy.

**Keywords** Immunometabolism • Tumor microenvironment • Dendritic cell • T cell • Macrophage

### 7.1 Introduction

As cancers edit and escape this initial immune detection, they also generate an immunosuppressive microenvironment which restricts T-cell infiltration, activation, and effector function both through direct repression (via cytokines, nutrients restriction, etc.) and the recruitment of immunosuppressive populations [1].

Tumors display altered metabolism relative to benign tissues [2, 3]. The common feature of this altered metabolism is the increased glucose uptake and fermentation of glucose to lactate. This phenomenon is observed even in the presence of completely functioning mitochondria and, together, is known as the “Warburg effect”

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[4]. These changes support abnormal survival and growth of malignant cells by providing energy, macromolecular precursors, and reducing equivalents [5]. Recently, increasing evidence indicates that cancer cells subvert the metabolic characteristics of the tumor microenvironment to shape immune responses within tumors [6]. Papers by Chang et al. [7] and Ho et al. [8] show that Warburg metabolism enables tumor cells to restrict glucose availability to T cells, suppressing anti-tumor immunity.

Immunometabolism is an emerging field of investigation dissecting the contribution of key metabolic pathways to immune cell development, fate, and behavior [9, 10]. The present article aims at understanding immune cells' metabolism in tumor microenvironment and its potential role in tumor progression and metastasis. Characterizing the reciprocal metabolic interplay between immune and cancer cells will provide a better understanding of treatment efficacy and resistance and also help develop a new strategy for cancer immunotherapy.

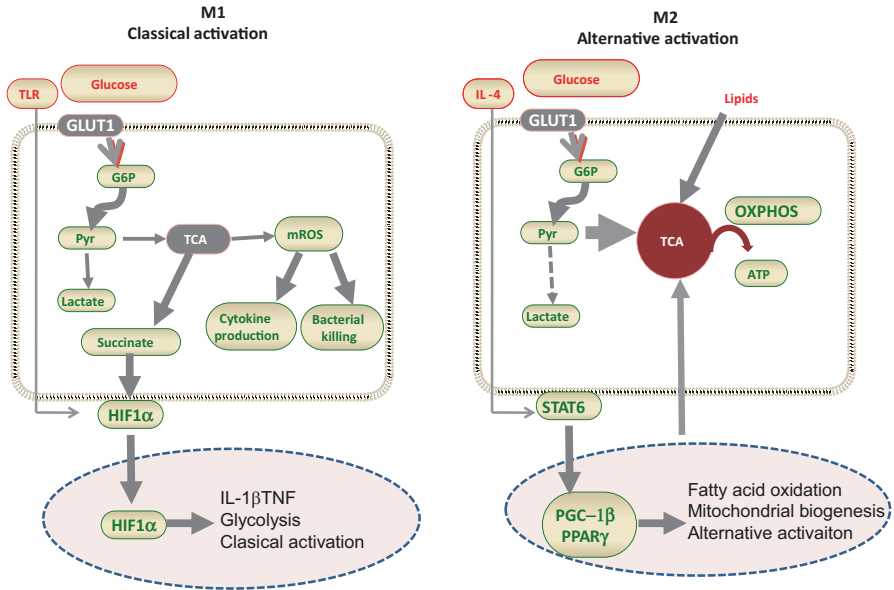
## 7.2 Innate Cells in the Tumor Microenvironment

### 7.2.1 Macrophage

Tumor-associated macrophages (TAM) are among the most abundant inflammatory cells in tumors. Within the same tumor, the coexistence of two distinct TAM subpopulations has been shown, both derived from tumor-infiltrating inflammatory monocytes: M2-like MHC-II<sup>low</sup> TAM that reside in the hypoxic regions of the tumor and perform angiogenic, immunosuppressive, and protumoral activities and M1-like MHC-II<sup>high</sup> TAM that are present in the normoxic tumor regions and possess proinflammatory and antitumoral characteristics [11, 12]. Recent studies on intracellular metabolism in macrophages provide new insights on the functioning of these critical controllers of innate and adaptive immunity [13, 14]. The metabolic reprogramming of M1 and M2 has been summarized in Fig. 7.1.

#### 7.2.1.1 Classically Activated Macrophages (M1)

M1 macrophage metabolism is characterized by aerobic glycolysis, fatty acid synthesis, and a truncated tricarboxylic acid (TCA) cycle. Although the importance of glycolysis in inflammatory activation of macrophages was first noted almost a century ago, its biochemical and bioenergetic importance had not been appreciated until recently. The activated M1 macrophages have high rates of glucose and glutamine uptake and lactic acid production. However, neither glycolysis nor glutaminolysis are necessary for ATP generation. The decreased respiration and a broken TCA cycle of M1 macrophages lead to accumulation of succinate, citrate, and nitric oxide (NO), which support the production of key M1 cellular products or act as signals to alter immune function [15, 16]. The endogenous metabolites can adopt



**Fig. 7.1** Metabolic reprogramming of macrophages in tumor microenvironment. Macrophages in a tumor microenvironment have been characterized as M1- and M2-polarized subtypes. In M1 macrophages, there is decreased respiration and a broken Krebs cycle, leading to accumulation of succinate and citrate, which act as signals to alter immune function. Downstream of TLR signaling, mitochondrial ROS (mROS) can also support the function of M1. In M2 macrophages, the Krebs cycle and oxidative phosphorylation are intact and FAO is utilized. Type 2 cytokine, such as IL-4, activates STAT6 transcription factor. Then STAT6 promotes the metabolic transition to oxidative metabolism by inducing genes of FAO and mitochondrial biogenesis. In addition, STAT6 transcriptionally induces PGC-1 $\beta$ , PPAR $\gamma$ , which synergize with STAT6 to enhance oxidative metabolism. GLUT1 glucose transporter 1, G6P glucose 6-phosphate, HIF-1 $\alpha$  hypoxia-inducible factor 1 $\alpha$ , IL-1 $\beta$  interleukin-1 $\beta$ , IL-4 interleukin-4, OXPHOS oxidative phosphorylation, Pyr pyruvate, PGC-1 $\beta$  PPAR $\gamma$  coactivator-1 $\beta$ , PPAR peroxisome proliferation-activated receptor, ROS reactive oxygen species, STAT signal transducer and activator of transcription, TCA tricarboxylic acid cycle, TLR Toll-like receptor, TNF tumor necrosis factor

regulatory roles that govern specific aspects of inflammatory response, as recently shown for succinate, which regulates the proinflammatory interleukin-1 $\beta$ (IL-1 $\beta$ ) hypoxia-inducible factor-1 $\alpha$ (HIF-1 $\alpha$ ) axis. Furthermore, Lampropoulou V et al. [17] report that itaconate modulates macrophage metabolism and effector functions by inhibiting SDH-mediated oxidation of succinate. Collectively, these studies demonstrate that endogenous metabolite-derived signals might be important integrators and effectors of host immunity in tumor microenvironment. Both the increased mitochondrial oxidation of succinate via succinate dehydrogenase (SDH) and an elevation of mitochondrial membrane potential combine drive mitochondrial reactive oxygen species (ROS) production [13]. Blocking ROS production by uncoupling mitochondria inhibits this inflammatory phenotype. Therefore, remodeling of the TCA cycle is a metabolic adaptation accompanying inflammatory macrophage activation.

### 7.2.1.2 Alternatively Activated Macrophages (M2)

The metabolic signature of M2 macrophages is characterized by fatty acid oxidation (FAO) and an oxidative TCA cycle. Tumor-promoting M2 macrophages are induced under the influence of interleukin-4(IL-4), IL-13, IL-10, and macrophage colony-stimulating factor (M-CSF) and lack the cytotoxicity of M1 macrophages. M2 macrophages appear to contribute to immune suppression through the production of IL-10 and transforming growth factor- $\beta$  (TGF- $\beta$ ) [18]. Myeloid-derived suppressor cells are immature myeloid-lineage cells that also can be immunosuppressive in the tumor microenvironment. Major mechanisms of suppression include the expression and functional activity of arginase [19] and the nitrosylation of surface proteins on infiltrating T cells, including the T-cell receptor (TCR) [20]. Gene expression profiling studies of human melanoma have revealed arginase transcripts expressed in a subset of non-T-cell-infiltrated tumors [21], so myeloid-derived suppressor cells may be a component of the phenotype of T-cell exclusion.

Processes that drive the glycolytic switch in M1 macrophages are downregulated in M2 macrophages. One example is that M1 macrophages express u-PFK2, an isoform of phosphofructokinase-2 that is highly active, promoting glycolysis [15]. In contrast, M2 macrophages express a different isoform, PFK FB1, which is much less active [22, 23]. Another example is that the activation of the key metabolic regulator pyruvate kinase M2 (PKM2) attenuated an LPS-induced proinflammatory M1 macrophage phenotype while promoting traits typical of an M2 macrophage [24]. The activation of PKM2 using two well-characterized small molecules, DASA-58 and TEPP-46, inhibited LPS-induced HIF-1 $\alpha$  and IL-1 $\beta$ , the important genes involved in glycolysis. The mechanistic target of rapamycin complex 2(mTORC2) operated in parallel with the IL-4R $\alpha$ -signal transducer and activator of transcription 6(Stat6) pathway to facilitate increased glycolysis during M2 activation via the induction of the transcription factor IRF4. IRF4 expression required both mTORC2 and Stat6 pathways, providing an underlying mechanism to explain how glucose utilization is increased to support M2 activation [25].

Since macrophage metabolism is certainly connected to its functionality, metabolic reprogramming of M2-like TAM might be a new strategy to repolarize TAM toward an antitumoral phenotype and thus dampen tumor growth and metastasis. Although a system-level understanding of TAM metabolism is currently absent and rather limited, there is an emerging evidence that unraveling the TAM phenotype might lead to the identification of alternative, novel targets for TAM-directed intervention.

### 7.2.2 Dendritic Cell

Dendritic cells (DCs) display different phenotypes and activity in tumors and exhibit distinct pro-tumorigenic and anti-tumorigenic functions. DCs are supposed to play a key role in inducing and maintaining the antitumor immunity. However, their

antigen-presenting function is jeopardized in tumor microenvironment. Under circumstance, DCs are polarized into tolerogenic phenotype with immunosuppressive function, which limit antitumor activity of effector T cells (TE). The metabolic switch of DCs from the anti-tumorigenic phenotype to the tolerogenic phenotype has been summarized in Fig. 7.2.

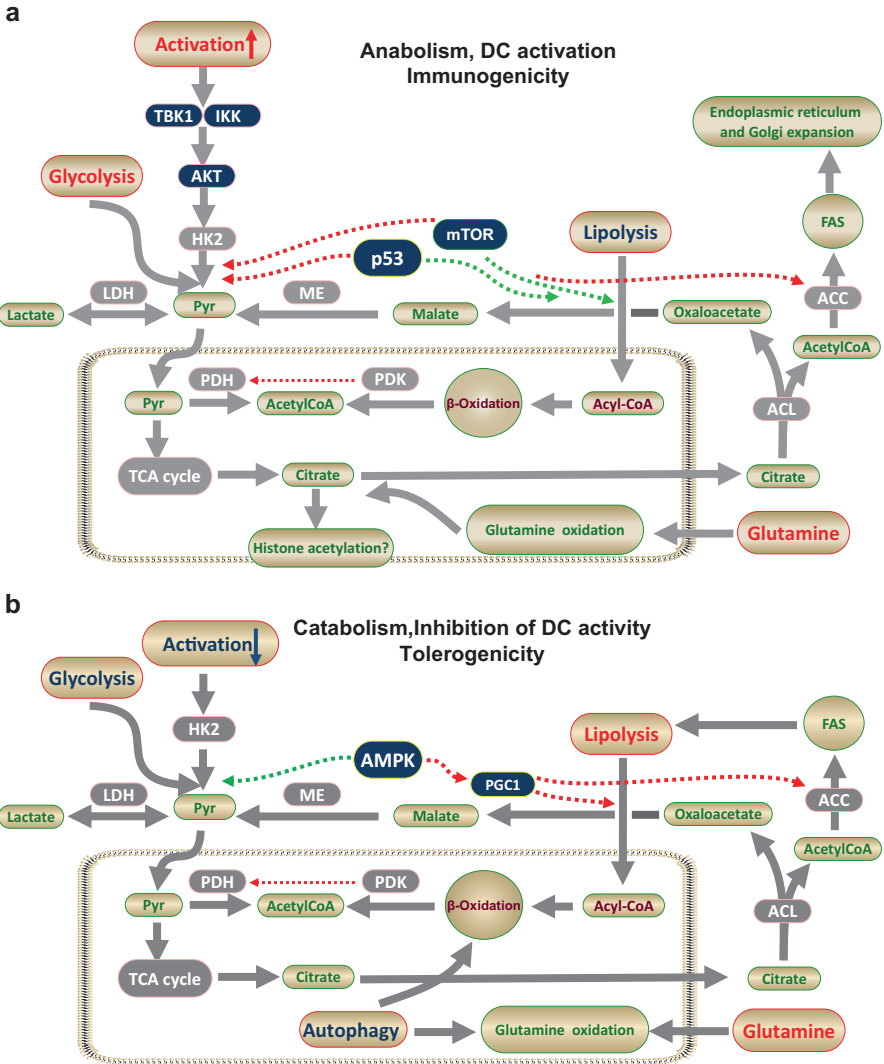
### 7.2.2.1 Immunogenic DCs

Catabolic metabolism centered around mitochondrial oxidative phosphorylation (OXPHOS) is associated with cellular longevity and quiescence of DCs, whereas cellular activation and proliferation are accompanied by a switch to glycolytic metabolism to support anabolic pathways needed for biosynthesis [26]. The phosphatidylinositol 3'-kinase/Akt(PI3K/Akt) pathway, which could be antagonized by the adenosine monophosphate (AMP)-activated protein kinase (AMPK), is required for DC maturation [27]. The switch from OXPHOS to glycolysis is a direct consequence of TLR-induced inducible nitric oxide synthase (iNOS) expression that through the production of NO poisons the mitochondrial respiratory chain in an autocrine fashion [28]. Bart Everts et al. [29] found that DC glycolytic flux increased within minutes of exposure to TLR agonists and that this served an essential role in supporting the de novo synthesis of fatty acids for the expansion of the endoplasmic reticulum and Golgi required for the production and secretion of proteins that are integral to DC activation. Signaling via the kinases TBK1, IKK $\epsilon$ , and Akt was essential for the TLR-induced increase in glycolysis by promoting the association of the glycolytic enzyme HK-II with mitochondria [29] (Fig. 7.2a).

### 7.2.2.2 Tolerogenic DCs

In contrast to immunogenic DCs, tolerogenic DCs favor OXPHOS and FAO. Tolerogenic DCs, as opposed to immunogenic DCs, are maturation resistant and express increased levels of immunoregulatory factors, important for controlling regulatory T-cell (Treg) responses [30]. Proteomic analysis of human DCs treated with immunosuppressive drugs dexamethasone and vitamin D3 revealed increased expression of genes associated with mitochondrial metabolism and OXPHOS along with enhanced tolerogenic phenotypes [31–33](Fig. 7.2b). The direct inhibition of glycolysis in TLR-activated DCs favors the induction of forkhead box P3(Foxp3)-expressing helper T cells [29]. Consistent with a role for peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) coactivator 1 $\alpha$ (PGC1 $\alpha$ ) in regulating DC activation, resveratrol, a drug favoring catabolic metabolism through activation of the histone deacetylase (HDAC) sirtuin 1, inhibits the expression of hypoxia-inducible factor 1 $\alpha$ (HIF1 $\alpha$ ) and enhances PGC1 $\alpha$  expression in DCs, which render these cells more tolerogenic phenotypes [34–37].

Plasmacytoid dendritic cells (pDCs) are a distinct lineage of DCs that are more specialized for cytokine production, particularly type I interferon (IFN) production.



**Fig. 7.2** Metabolic reprogramming of dendritic cells in tumor microenvironment. Metabolic switch from anabolic metabolism to catabolic metabolisms consistent with DC function transferring from immunogenicity to tolerogenicity. **(a)** Demonstrates activated DCs that need metabolic reprogramming to provide the bioenergetic and biosynthetic support. The PI3K/Akt/mTOR pathway, which could be antagonized by the AMPK, is required for DCs maturation. Signaling via the kinases TBK1, IKK $\epsilon$ , and Akt was essential for the TLR-induced increase in glycolysis by promoting the association of the glycolytic enzyme HK-II with mitochondria. Acetyl-CoA converted from citrate is a major acetyl donor for the acetylation pathway. **(b)** Illustrates that catabolic metabolism centered around OXPHOS is associated with cellular longevity and quiescence of DCs. Enhances catabolic metabolism through activation of the histone deacetylase (HDAC) sirtuin 1, inhibits the expression of hypoxia-inducible factor 1 $\alpha$  (HIF1 $\alpha$ ), and upregulates PGC1 $\alpha$  expression in DCs. In contrast to immunogenic DC, autophagy and the oxidation of fatty acids and glutamine render these cells more tolerogenic phenotypes. AMPK AMP-activated protein kinase, ATP adenosine triphosphate, ACC acetyl-CoA carboxylase, ACL ATP citrate lyase, AKT protein kinase B, FAS fatty acid synthesis, GLUT1 glucose transporter 1, G6P glucose 6-phosphate, HK hexokinase, LDH lactate dehydrogenase, mTOR mammalian target of rapamycin, PDH pyruvate dehydrogenase, PDK pyruvate dehydrogenase kinase, Pyr pyruvate, PGC-1 PPAR $\gamma$  coactivator-1, TBK1 TANK-binding kinase 1, IKK $\epsilon$  inhibitor of nuclear factor- $\kappa$ B kinase subunit- $\epsilon$ , TCA tricarboxylic acid cycle

In vivo pDCs depletion delayed tumor growth, showing that tumor-associated pDC provides an immune-subversive environment, most likely through Treg activation, thus favoring tumor progression [38]. Wu et al. [39] recently report that through an autocrine type 1 IFN receptor-dependent pathway, induced changes in pDCs of cellular metabolism are characterized by increased FAO and OXPHOS. Direct inhibition of FAO and of pathways prevented full pDC activation [39].

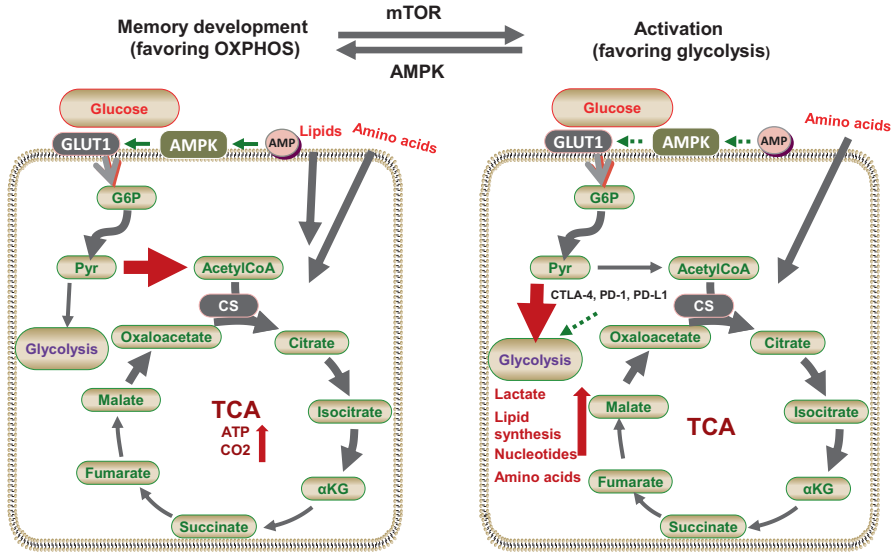
How the effects of fatty acid synthesis differ so markedly in DCs isolated from tumors compared with those TLR-activated DCs is an important unanswered question. However, scientists speculate that accumulated fatty acids are supporting FAO and therefore tolerogenicity in the cancer setting [40]. These adaptations allow tolerogenic DCs a metabolic advantage in low-glucose, lactate-rich environments; they resist suppression of DCs function and proliferation in competition of nutrients.

### 7.3 Adaptive Cells in Tumor Microenvironment

Among the tumor-infiltrated lymphocytes (TILs), T cells are the most abundant cells [41]. CD8<sup>+</sup> T cells have a central role in antitumor immunity, but their activity is suppressed in the tumor microenvironment. Reactivating the cytotoxicity of CD8<sup>+</sup> T cells is of great clinical interest in cancer immunotherapy.

#### 7.3.1 Effector T Cells (TE)

Most tumor cells express antigens that can mediate recognition by host CD8<sup>+</sup> T cells [41]. Resting CD8<sup>+</sup> T cells undergo dynamic shifts in cell metabolism and switch from an oxidative metabolism to aerobic glycolysis upon activation. This transition is essential to support growth and differentiation into cytotoxic T cells capable of dividing every 6–8 h and of producing inflammatory cytokines and the cytolytic granules perforin and granzyme-B [42]. Chang et al. [7] report that glucose consumption by tumors metabolically restricts T cells, leading to their dampened mTOR activity, glycolytic capacity, and IFN- $\gamma$  production. Targeting aerobic glycolysis in the tumor has increased the supply of glucose to TILs, thus boosting their effector function. The checkpoint blockade antibodies against CTLA-4, PD-1, and PD-L1 restore glucose in tumor microenvironment, permitting T-cell glycolysis and IFN- $\gamma$  production [7](Fig. 7.3). Recently, Ho et al. [8] uncovered the glycolytic metabolite phosphoenolpyruvate (PEP) as a new metabolic checkpoints for T-cell activity. PEP plays an important role in sustaining T-cell receptor-mediated Ca<sup>2+</sup>-nuclear factor of activated T cell (NFAT) signaling and effector functions by repressing sarco/ER Ca<sup>2+</sup>-ATPase (SERCA) activity. Together these data suggest that both nutrients and substrates concentration in a local microenvironment can have a marked impact on immune cell function.



**Fig. 7.3** Metabolic reprogramming of T lymphocytes in tumor microenvironment. Upon activation, lipid oxidation is downregulated in resting T cells, and glycolysis increases along with glutamine oxidation, in order to produce biosynthetic precursors required for rapid cell growth and proliferation. The checkpoint blockade antibodies against CTLA-4, PD-1, and PD-L1, which are used clinically, restore glucose in tumor microenvironment, permitting T-cell glycolysis and IFN- $\gamma$  production. At the end of an immune response, the cells that survive to become memory T cells revert back to lipid oxidation with increased capacity for efficient energy generation. The mTOR pathway plays a key role in these metabolic shifts. Mechanistically, rapamycin treatment reduced mTORC1 activity and led to increased AMPK phosphorylation that correlated with an increased ability of CD8<sup>+</sup> T cells to perform lipid oxidation. AMPK AMP-activated protein kinase, ATP adenosine triphosphate, CTLA-4 cytotoxic T-lymphocyte-associated protein 4, GLUT1 glucose transporter 1, G6P glucose 6-phosphate, mTOR mammalian target of rapamycin, PD-1 programmed cell death protein 1, PDL-1 programmed death-ligand 1, Pyr pyruvate, TCA tricarboxylic acid cycle

The lipid requirements of membrane synthesis are essential for TE function as well. Inhibiting cholesterol esterification in CD8<sup>+</sup>T cells led to potentiated effector function and enhanced proliferation [42, 43]. Inhibiting cholesterol esterification increases the plasma membrane cholesterol level of CD8<sup>+</sup>T cells, which causes enhanced T-cell receptor clustering and signaling as well as more efficient formation of the immunological synapse [43, 44]. Sterol regulatory element-binding protein (SREBP) is another critical target for meeting the heightened lipid requirements of membrane synthesis during blastogenesis [45].

TE function has been regulated by transcriptional or posttranscriptional mechanisms. Aerobic glycolysis (the Warburg effect) is a metabolic hallmark of activated T cells and has been implicated in augmenting effector T-cell responses, including expression of the proinflammatory cytokine IFN- $\gamma$ , via 3' untranslated region (3'UTR)-mediated mechanisms [46]. Another study shows that lactate dehydrogenase A (LDHA) is induced in activated T cells to support aerobic glycolysis but



promotes IFN- $\gamma$  expression independently of its 3'UTR. Instead, LDHA maintains high concentrations of acetyl-coenzyme A to enhance histone acetylation and transcription of Ifng. Ablation of LDHA in T cells protects mice from immunopathology triggered by excessive IFN- $\gamma$  expression or deficiency of regulatory T cells [47].

T-cell exhaustion is characterized by the stepwise and progressive loss of T-cell functions [1]. The exhausted T cells acquired a distinct metabolic profile from that of TE and TM cells. Two related studies by Bengsch et al. [48] and Scharping et al. [49] indicate dysfunctional mitochondria are identified as a key correlate of CD8<sup>+</sup> T-cell exhaustion. PD-1 pathway blockade resulted in transcriptional rewiring and reengagement of effector circuitry in the exhausted CD8<sup>+</sup> T cells' epigenetic landscape [50]. Manipulating glycolytic and mitochondrial metabolism might enhance checkpoint blockade outcomes [51].

### 7.3.2 Memory T Cell (TM)

A successful immune response relies not only on the ability of T cells to extensively proliferate and attain effector function but also to form long-lived memory T cells that can respond again to future antigen encounter. There is intense interest in understanding how long-lived cellular immunity is generated. CD8<sup>+</sup> cytotoxic T cells further differentiate into long-lived quiescent memory CD8<sup>+</sup> T cells (TM). TM cells require efficient energy generation to support basic cellular functions and prevent cell death [52, 53]. Pearce et al. reported that CD8<sup>+</sup>TM cells possessed substantial mitochondrial spare respiratory capacity (SRC). SRC is the extra capacity available in cells to produce energy in response to increased stress or work and as such is associated with cellular survival. The mTOR pathway plays a key role in this metabolic remodeling. Tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6), an adaptor protein in the TNF-receptor and interleukin-1R/Toll-like receptor superfamily, is known to be required for the transition from effector to TM cells [52, 53]. TM cells express high levels of the mitochondrial lipid transporter-carnitine palmitoyltransferase 1a (CPT1a), and retroviral CPT1a expression enhanced CD8<sup>+</sup> TM generation in an adoptive transfer model.

Mitochondrial dynamics underlies TM fate. TE cells have punctate mitochondria, while TM cells possess fused networks. By altering cristae morphology, fusion protein Opa1 and fusion in TM cells are required for electron transport chain (ETC) complex associations favoring OXPHOS and FAO [54]. Enforcing fusion in TE cells imposes TM cell characteristics and enhances antitumor function. Thus, targeting mitochondrial dynamics may offer a novel adjuvant strategy to therapeutically influence cancer therapy.

Elevating L-arginine levels induced global metabolic changes including a shift from glycolysis to oxidative phosphorylation in activated T cells and promoted the generation of central memory-like cells endowed with higher survival capacity and, in a mouse model, antitumor activity [55]. During the process, three transcriptional regulators (BAZ1B, PSIP1, and TSN) sensed L-arginine levels and promoted T-cell survival [55].

### 7.3.3 *Regulatory T Cells (Treg)*

Treg cells respond to immune and inflammatory signals to mediate immunosuppression. The evidences show maintaining lineage and survival integrity of Treg cells require simultaneous response to both environmental signals and metabolic homeostasis [56, 57]. Treg cell-specific deletion of *Atg7* or *Atg5*, two essential genes in autophagy, leads to loss of Treg cells and greater tumor resistance. Mechanistically, autophagy deficiency upregulates metabolic regulators mTORC1 and c-Myc and glycolysis, which contribute to defective Treg function. Furthermore, mTORC1 acts as a fundamental “rheostat” in Treg cells to link immunological signals from TCR and IL-2 to lipogenic pathways and functional fitness and highlight a central role of metabolic programming of Treg cell suppressive activity in immune homeostasis and tolerance [57].

Tissues with low-glucose and high-lactate concentrations frequently require immune responses to be more pro-tolerant, avoiding unwanted reactions against self-antigens or commensal bacteria. Angelin et al. report that the Treg transcription factor *Foxp3* reprograms T-cell metabolism by suppressing *Myc* and glycolysis, enhancing OXPHOS, and increasing nicotinamide adenine dinucleotide oxidation [58]. These adaptations allow Tregs a metabolic advantage in low-glucose, lactate-rich environments. This metabolic phenotype may explain how Tregs promote peripheral immune tolerance during tissue injury but also how cancer cells evade immune destruction in the tumor microenvironment.

Why do TE, TM, and Treg cells adopt markedly different types of metabolism upon activation? One possibility is that the shift to glycolysis in TE may be optimally suited to the rapid, short-term bursts of activation that are required at sites of infection or inflammation, whereas FAO in TM may be better able to energetically support cell survival, as T cells continue to fight virus infection or tumors over a long time period. Understanding T cells metabolism may therefore lead to novel approaches for selective immune modulation in cancer and autoimmune diseases.

## 7.4 Remaining Questions and Bottleneck

Intense ongoing investigation of immune cell metabolism is yielding an exponentially growing amount of information. Armed with new information and a comprehensive understanding of how metabolism dictates immune cell fate, researchers may discover novel therapeutic strategies for treatment of tumor. For example, as we discussed in Sect. 7.3, the long-term survival and antitumor immunity of adoptively transferred CD8<sup>+</sup>T cells is dependent on their metabolic fitness. Scientists utilized a lipophilic cationic dye tetramethylrhodamine methyl ester (TMRM) to identify and isolate metabolically robust T cells based on their mitochondrial membrane potential ( $\Delta\Psi_m$ ) [59]. Transfer of these low- $\Delta\Psi_m$  T cells was associated with superior long-term in vivo persistence and an enhanced capacity to eradicate

established tumors compared with high- $\Delta\Psi_m$  cells. The use of  $\Delta\Psi_m$ -based sorting to enrich for cells with superior metabolic features was observed in CD8<sup>+</sup> and CD4<sup>+</sup> T-cell subsets.

At present, there are still obvious obstacles in reaching clinically effective conclusions about immunometabolism in tumor: the vast metabolic heterogeneity within a tumor and the lack of metabolic resemblance between tissue culture and in vivo conditions [60]. The in vitro studies performed cannot faithfully recapitulate the conditions of tumor local environment [61]. Therefore, the use of a reductionist approach to investigations, by focusing on specific nutrients, can produce misleading information which hampers reaching further conclusions. As a starting point, developing standardized techniques allowing assessment of the metabolism of human physiology is critical.

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