



Mini-review

Survival of the fittest: Cancer challenges T cell metabolism

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ABSTRACT

T cells represent the major contributors to antitumor-specific immunity among the tumor-infiltrating lymphocytes. However, tumors acquire ways to evade immunosurveillance and anti-tumor responses are too weak to eradicate the disease. T cells are often functionally impaired as a result of interaction with, or signals from, transformed cells and the tumor microenvironment, including stromal cells. Among these, nutrients use and consumption is critically important for the control of differentiation and effector mechanisms of T cells. Moreover, Treg cells-skewing conditions often coexist within the cancer milieu, which sustains the notion of immune privileged tumors. Additionally, cancer cells contend with tumor infiltrating lymphocytes for nutrients and can outcompete the immune response. PD1- and CTLA-4 based immunotherapies partially remodel cell metabolism leading the way to clinical approaches of metabolic reprogramming for therapeutic purposes. Here we shortly discuss T cell fates during anti-tumor immune responses and how signals within tumor microenvironment influence T cell metabolism, altering functions and longevity of the cell.

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Introduction

T cell activation is instrumental to mount an antigen-specific immune-response. In this process the T cell receptor (TCR) recognizes its cognate antigen presented by the major histocompatibility complex (MHC) on antigen presenting cells. Once activated, T cells grow, enter a vigorous proliferation cycle and commit to mediate diverse effector functions, ranging from cytokine secretion to cell-contact cytotoxicity. These antigen-specific effector mechanisms are indispensable for pathogen clearance and tumor immunosurveillance.

It has become clear that tight control of cellular metabolism is crucial for almost every step of T cell activation [1]. Metabolic processes ensure that T cells sustain the increased energy needs that are associated with a protective immune response. Especially the environment, for example the tumor ecosystem, which is characterized by specific nutrients and altered stromal cells, may

become conditioning. Harsh environmental cues can modulate T cell functionality [2] and metabolic reprogramming of T cells by tumors have been shown to dampen anti-tumor immunity [3]. Thus, therapeutic intervention with the objective to interfere with T cell energy metabolism and the identification of metabolic checkpoints are promising for the development of targeted immunotherapies.

In this review we briefly discuss the current metabolic aspects of T cells, cancer and mesenchymal cells, and provide a comprehensive, levelled view on the metabolic crosstalk between T and tumor cells. We will comment on how this is connected to the outcome of an immune response especially in the context of a favorable anti-tumor attack and focus on potential therapeutic opportunities.

T cell bioenergetics

Naïve T cells have relatively low metabolic demands and feed their energy obligations mainly by oxidative phosphorylation (OXPHOS) [4,5] (Fig. 1). This is the most efficient process to produce cellular energy by producing 30–36 molecules of ATP out of one molecule of glucose. In contrast, glycolysis generates only 2 molecules of ATP per glucose unit [6]. Both pathways are physiologically

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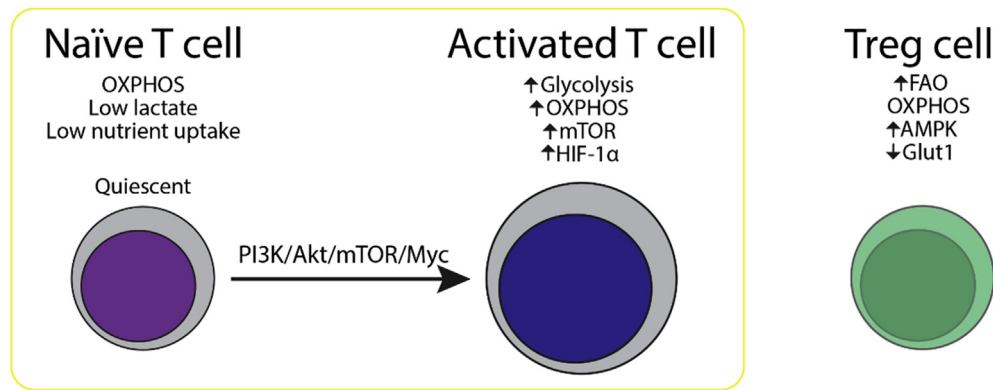


Fig. 1. T cells adapt their metabolism upon activation and differentiation. Naïve T cells do not proliferate or acquire effector functions. These cells have minimal energetic requirements, which are sufficient to support survival. Thus, the naïve T cell state is characterized by a low nutrient consumption (glucose) and OXPHOS. When activated, metabolic changes through the PI3K/Akt/mTOR axis and Myc accompany the new effector functions and the need of rapid proliferation. Glycolysis and OXPHOS typify the activation state: the former supports cytokine secretion, the latter proliferation. Treg cells produce energy by lipid oxidation (FAO) and OXPHOS.

involved in different aspects of the T cell response and shifting the balance between them can result in a different outcome.

The main fuel of T cells is glucose, which is catabolized through the glycolytic cascade to pyruvate. Pyruvate can either be reduced to lactate to regenerate NAD^+ in active T cells, or converted by pyruvate dehydrogenase to acetyl-CoA to nourish the tricarboxylic acid (TCA) cycle for further oxidation in naïve and memory T cells. Generally, T cells that display a high glycolytic activity tend to be short-lived and active. In contrast, T cells with low glycolytic metabolism are inclined to differentiate into long-lived memory cells [7] (Fig. 1).

Sequential substrate oxidation in TCA cycle forms the reducing equivalents NADH and FADH_2 , which transfer their electrons to the mitochondrial electron transport chain to drive OXPHOS and ATP production. The TCA is a central metabolic network which provides precursors for energy production and biosynthesis. To this extent, the act of filling up the TCA cycle with TCA intermediates (for example oxaloacetate) is referred as to anaplerosis. Conversely, cataplerosis refers to the depletion of TCA metabolites to feed biosynthetic pathways (i.e. oxaloacetate for gluconeogenesis). There is a substantial flux of four- and five-carbon intermediates that branch in via anaplerosis, or out via cataplerosis, from the TCA cycle. Particularly, cataplerotic reactions are necessary for T cells as they foster many anabolic pathways such as the synthesis of fatty acids and amino acids [8]. Notably, glutaminolysis has been shown to be crucial for T cell growth and proliferation, since it fuels the TCA cycle in the form of α -ketoglutarate [8]. Thus, glutaminolysis supports mitochondrial metabolism even when pyruvate is preferentially converted into lactic acid as a result of aerobic glycolysis. However, ultrastructural analysis by electron microscopy revealed that T cell differentiation is associated with differential mitochondrial shape. In particular, differentiated T cells showed fissid mitochondria and anabolic phenotype, whereas memory T cell have fused mitochondria coupled to a catabolic signature [9]. However, the molecular signal and events that drive these mitochondrial dynamics are not entirely clear. Thus, further studies on the initiatory mechanisms and their role in determining T cell fate are needed.

Intermediates produced during glycolysis and TCA cycle can also serve as building blocks for biosynthetic processes [10]. Anabolic pathways are particularly important in the T cell activation process because they sustain incremental biomass accumulation for cellular growth and proliferation [11]. In this scenario, the T cell must preserve the integrity of a balanced metabolism to comply with a variety of (non-)immunological cues, by counteracting their needs for energy and biosynthesis to execute their effector functions.

A view on cancer metabolism

Cancer cells are able to evade immune recognition in a dynamic process known as cancer immunoediting, in which immuno-surveillance and tumor cell elimination are overcome by the natural selection of tumor variants in response to the immune pressure. This in turn can ultimately lead to tumor escape and outgrowth. The selection process is fostered by the cancer's genetic instability in combination with its fast growth rate and the selective pressure of the immune system, which results in tumor cells that no longer express detectable tumor antigens. In addition, the tumor tissue can reinstate a functional dormancy within the tumor infiltrating T cells (TILs). The study of the unrestrained tumor growth has been at the forefront of research for years [12]. Intensive efforts are underway to identify mechanisms that limit adaptive anti-tumor responses and exploit their vulnerabilities for efficient treatments, since metabolic alterations seem to be absolutely required for malignant transformation [13].

During cancer development, cells first suffer and tolerate a plethora of synergistic intrinsic and extrinsic stresses, which includes (epi-) genomic instability, immune pressure, organic metabolites availability, bloodstream access and hypoxia. This consequently impacts tissue homeostasis and creates a corrupted metabolic niche, where dynamically adapting cancer cells act as major shapers. Notwithstanding, cancer (stem-) cells are able to bypass this phase and counteract the bio-environmental tension via differentiation of various intra-tumoral clones [14], which acquire specific metabolic adaptations [15].

Moreover, solid tumor progression is influenced by mesenchymal cells such as cancer-associated fibroblasts (CAFs) and endothelial cells. CAFs are activated fibroblasts, which have been corrupted by the inflammatory tumor conditions [16]. CAFs are metabolically reprogrammed to aerobic glycolysis and acquire specific stromal metabolic features, which support tumor growth at multiple levels [15]. For example, it has been shown that CAFs from various cancer types harbor high levels of hypoxia-induced HIF-1 α and lactate transporters MCT1 and MCT4 [17–21]. Because of the increased glycolytic activity this results in a high secretion of lactate into the tumor environment which has been shown to have further tumor promoting effects. Both, lactate and cell intrinsic HIF-1 α change the inflammatory phenotype of tumor infiltrating M1 macrophages to tolerogenic tumor supporting M2 macrophages [22]. Stromal cells contribute to lactate levels within the tumor environment and the increased concentrations have been implicated in supporting cell invasiveness and metastasis [23]. Despite that, CAFs are known to secrete multiple tumor promoting factors

including cytokines, chemokines, signaling ligands and matrix modifying enzymes [16].

Chronic proliferation is intimately linked to neoplastic transformation and involves adjustment of energy metabolism. The view that rapidly proliferating cells use glycolysis for their anabolic energy requirements is based on observations with activated lymphocytes, whereas differentiated cells rely primarily on mitochondrial OXPHOS [24]. In particular, aerobic glycolysis distinguishes cancer from non-transformed resting cells. As a consequence, cancer cells themselves produce large amount of lactate regardless of the availability of oxygen, which is known as the 'Warburg effect' [25]. Although this 'glycolytic phenotype' is rather energetically inefficient, cancer cells boost their energy metabolism by upregulating glucose transporters such as Glut1, which substantially increases glucose import into the cytoplasm [26] to sustain their limitless growth and clonogenic potential. The glycolytic upregulation can be further maintained by hypoxia in solid tumors [26]. Indeed, a recent pan-cancer *in silico* analysis that studied genomic aberrations alongside transcriptional changes in 10 different human cancer types, identified a core group of candidate metabolism regulating genes (including GADPH, SQLE, DHCR7) that are strongly associated with tumor hypoxia [27]. Nonetheless, it should be noted that cancer cells can quickly adapt their energy metabolism to the environmental constraints and can switch from the glycolytic pathway to an oxidative metabolism and *vice versa* [28].

It is interesting to note that physiologically the presence of oxygen would limit glycolysis as the result of the 'Pasteur effect' [29]. In normoxic conditions, a unit of glucose is completely oxidized in a sequential way through glycolysis, TCA cycle and OXPHOS, which generates maximal 30–36 molecules of ATP per glucose. The fully active respiratory chain provides abundant ATP and citrate which both act as allosteric inhibitors of phosphofructokinase 1 (PFK1) [30], the rate limiting enzyme in glycolysis. Allosteric inhibition of PFK1 by these metabolites is the molecular basis of the 'Pasteur effect'. Currently, it is not entirely known how cancer cells circumvent this regulatory circuit to sustain their high aerobic glycolysis rate. At the same time, cancer cells make use of the side-product of glycolysis, e.g. lactate, which might counteract oxidative stress (reactive oxygen species, ROS) [31,32]. Control of ROS within cancer cells is crucial for their survival and it has been shown that cellular antioxidants are important for cancer cell initiation and progression [33,34]. Furthermore, the high glycogenic capacity of tumors promotes glucose restriction in the local microenvironment [2,35,36], decreases the intracellular pH and promotes toxic, pro-tumorigenic effects. Also, some tumors possess intra-tumoral cellular subtypes that work symbiotically: one subpopulation is glucose-dependent and secretes lactate in the microenvironment, while the second subtype imports and utilizes the lactate produced by neighboring cancer and stromal cells as main fuel for the TCA cycle through the lactate dehydrogenase [37]. In that respect it is important to shed light on the control of the energy metabolism at a molecular level in cancer cells and investigate how this differs from non-transformed or neighboring cells. It would be attractive to target cancer cell specific metabolic switches, as blocking these metabolic fluxes would impede the metabolic adaptation of the tumor.

Metabolic intersection of T cell function and the tumor ecosystem

Upon cognate recognition of MHC-peptide complex, the bio-energetic expenditure of T cells increases dramatically over the resting state. Activated T cells are faced with the metabolically challenging task to transition from a marginal metabolic activity to

a highly metabolic active state characterized by cellular growth and high cell division rate [38]. Along with TCR engagement, costimulatory signals and cytokines initiate the process of metabolic reprogramming. This deep energetic rewiring requires a significant uptake of extracellular nutrients which are cardinal to metabolically assist the development of T cell effector functions [8,39,40] (Fig. 1). Indeed, receptors for glucose transporters and amino acids are upregulated in activated T cells, matching with a concomitant induction of aerobic glycolysis and glutaminolysis [41]. This drastically increases the uptake of glucose, glutamine and oxygen consumption. Intracellularly, several pathways are implicated in metabolic reprogramming and its regulation is under transcriptional and translational control [40]. The mechanistic target of rapamycin (mTOR), which operates downstream of the PI3K/Akt signaling (Fig. 2) and the transcription factor Myc play a major role in this process [8,42].

The increased metabolic activity upon T cell activation induces the production of ROS by the mitochondria. Our group has recently shown that ROS has to be balanced by the antioxidant glutathione (GSH) to ensure metabolic reprogramming of T cells [43]. Controlling ROS by GSH ensures the integrity of the T cells energy metabolism and ensures proper activation of mTOR, NFAT and the expression of Myc [43]. mTOR and Myc have been linked to cellular metabolism and regulate the expression of metabolic enzymes, the glucose transporter (Glut1), amino acid transporters and the T cell proliferative burst [8,44]. Physiologically, Myc promotes early T cell growth, glycolytic and glutaminolytic metabolism [8]. Myc is also a major oncogenic driver of cancer cells and guides a similar metabolic program in malignant cells as in T cells [43,45,46]. It was reported that Myc initiates ROS production with concomitant DNA damage and tumorigenic initiation [47]. Other studies showed that low level of ROS can promote cell proliferation *in vitro* [48]. Notably, ROS signaling influences T cell activation, proliferation and effector function [49]. However, ROS accumulation has to be tightly controlled by anti-oxidants and critical levels of ROS co-drive metabolic reprogramming through Myc, which is important for immune homeostasis [43].

Other genes that are involved in metabolic reprogramming in T cells exert a role in tumors. For example, enhanced expression of HIF-1 α promotes glycolysis and sustains survival of malignant cells [50]. HIF-1 α is induced by hypoxia, which is a characteristic of many tumors and associates with poor prognosis [51]. HIF-1 α has been reported to positively regulate Th17 cell and negatively affect regulatory T (Treg) cell responses, which would be counterintuitive in hypoxic environments [52,53]. However in a different study, HIF-1 α was found to facilitate Treg cell mediated suppression in the hypoxic environment of the gut during experimental colitis [54]. While it seems to be established that hypoxia and HIF-1 α impact the balance between inflammatory and regulatory T cell subsets more studies on actual tumors and TILs are required to clarify the function of hypoxia and HIF-1 α .

Similar to Myc, HIF-1 α has been reported to promote a general metabolic program that regulates nutrient transport and induces metabolic enzymes [55]. However, in contrast to Myc, HIF-1 α is dispensable for activation-induced T cell metabolic reprogramming [8].

Competition for nutrients in the tumor environment determines T cell functionality

Decreased nutrient contents within the tumor microenvironment can significantly dampen T cell effector function or skew the T cell lineage commitment to ultimately create a favorable tolerogenic setting that promotes tumor growth [35,36,56] (Fig. 3). When metabolites are restricted by exacerbated consumption, both

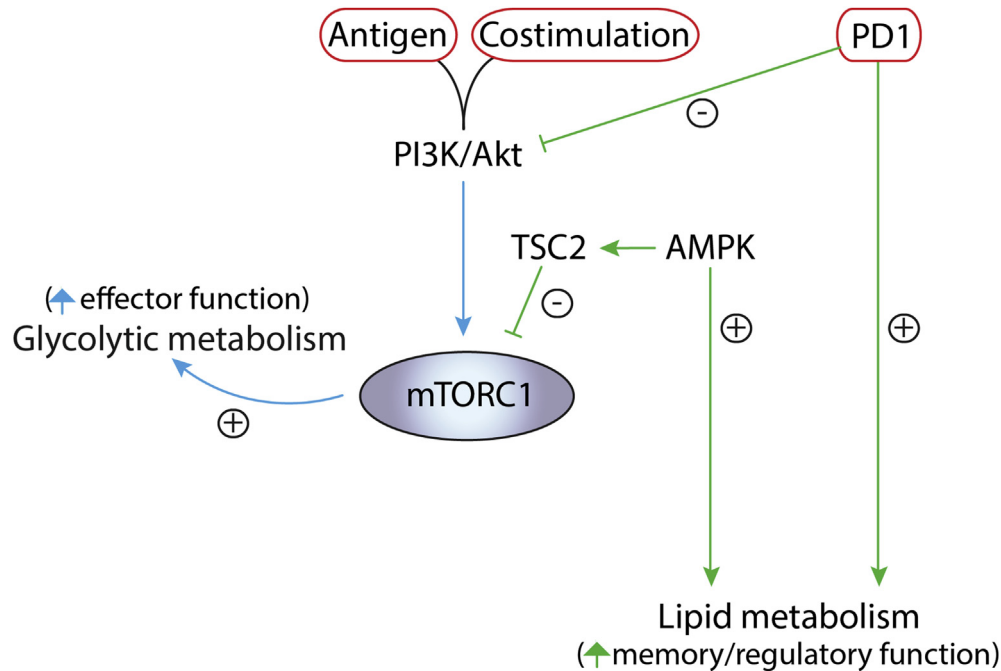


Fig. 2. mTOR controls metabolism and function of T cells. Antigen stimulation and co-stimulation engage the PI3K/Akt/mTORC1 pathway. T cell differentiation is influenced by the activation status of mTORC1 and differentially supports glycolysis or FAO (AMPK pathway). PD1 can support Treg cell skewing conditions in the tumor settings.

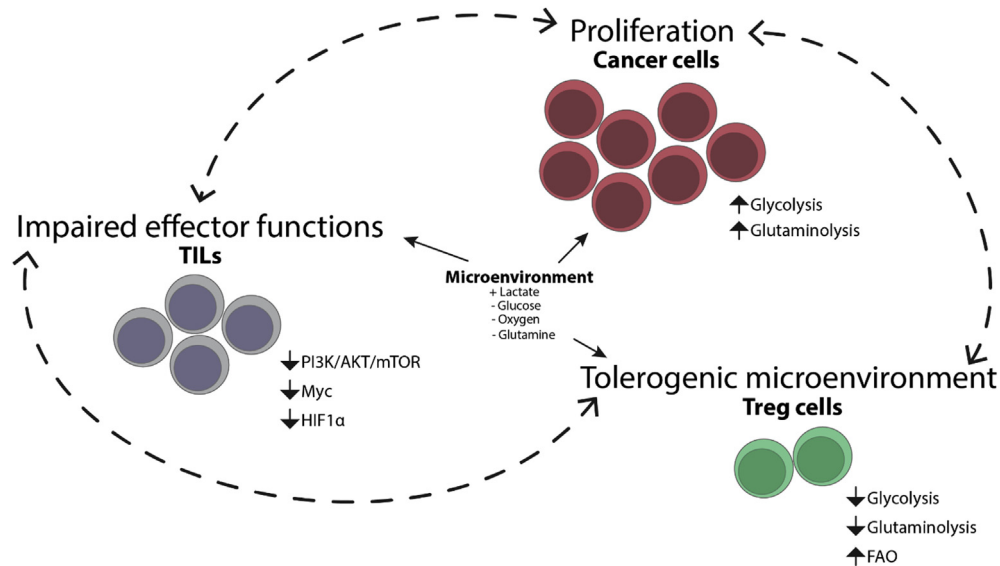


Fig. 3. TILs and tolerogenic microenvironment in the tumor ecosystem. Proliferation of cancer cells is mainly associated to the increase of their glycolytic rate. Among others, the Warburg effect promotes glucose deprivation and hypoxia which has a negative effect on crucial metabolic pathways, transcriptional factors and effector molecules in TILs. Thus, T cell effector functions are suppressed. At the same time the activity of Treg cells promotes the establishment of a tolerogenic tumor microenvironment, favoring cancer growth.

cancer and TILs compete for the same nutrients and may struggle to sustain their metabolism. Ultimately, the competition for nutrients can result in a metabolic rivalry, which can significantly contribute to tumor evasion from the immune system. Indeed, glutamine limitation shifts the balance of Th1 cells towards tolerogenic Treg cells [57]. This finding might correlate with the high Treg cells infiltration and poor prognosis observed in ovarian cancer [58]. However, the contribution of Treg cell in tumor progression is not entirely clear and is controversially discussed [59]. On this note, glutamine availability increases mTOR activation, which facilitates the expression of important transcriptional regulators of immunometabolism such as IRF4, HIF-1 α and Myc [60]. The transcription

factor IRF4 is a key regulator of a molecular network which is induced by the TCR in an affinity-dependent manner. IRF4 functions as a dose-dependent regulator of the metabolic reprogramming of activated T cells, thus acting as a “rheostat”, translating TCR affinity into transcriptional programs that links metabolic function with the clonal selection and effector differentiation of T cells [61,62].

At the molecular level, low energy (and nutrient) sensing is associated with an increase in the AMP/ATP ratio, which in sequence activates AMPK and skews towards oxidative metabolism, disfavoring cell proliferation [46,63–65]. Mechanistically, AMPK phosphorylates the Tuberous Sclerosis Complex 2 (TSC2), increasing

its GTPase-activity which, in turn, inhibits mTOR signaling [66]. Therefore, low cellular energy levels result in a decreased mTOR activity, which redirects T cell differentiation commitment from effector to regulatory properties [67,68] (Fig. 2). Taken into account that Treg cells prefer oxidative metabolism (Fig. 1), it is tempting to speculate that the decreased energy availability within the tumor environment is sensed by the AMPK-mediated pathway, which lowers anti-tumor activity of TILs. In this view, immunotherapies aiming at altering T cell metabolism to stabilize their effector functions are a worthwhile tumor treatment option.

As mentioned earlier, the nutrient composition in the tumor microenvironment can lead to tumor evasion. For instance, recent studies have demonstrated that glucose-depleted microenvironments contribute to diminished T cell anti-tumor responses [35,36,69]. However, different T cell subset may also reflect specific alteration of the tumor and withhold a prognostic value. To this extent, previous studies examined the abundance of different T cell subsets (CD4⁺, CD8⁺, Treg and memory) in colorectal cancer specimens and found that different densities affect patient survival. For example high density of memory (CD45RO⁺) and Treg cells (FoxP3⁺) were associated with improved overall survival [70]. How different T cell subsets affect the metabolic behavior of cancer cells themselves is currently not known and remains of high interest for the scientific community.

Cancer immunotherapies targeting metabolism

There is accumulating experimental evidence that an altered metabolic activity induces aberrant immune responses, which can be promoted by the tumor and its microenvironment. It has become apparent that tumors can co-opt tools of metabolic restrictions as mechanism of immune resistance. Consequently, hopes are high that manipulation of immune cell metabolism can effectively strengthen anti-cancer immunity, which is currently considered promising for the development of novel anti-cancer immunotherapies [15].

One of the mechanisms of immune resistance includes immune-inhibitory pathways, termed immune checkpoints, which normally mediate immune tolerance, prevent autoimmunity and mitigate collateral tissue damage in the course of the inflammatory process [71]. In tumor or neighboring stromal cells, inhibitory ligands and receptors that regulate T cell effector functions are commonly overexpressed [72]. Therefore, a core concept of cancer immunoescape is that transformed cells have developed ways to evade the immune system by taking advantage of peripheral tolerance mechanisms. Immune-checkpoint inhibitory receptors have been actively studied in the context of pre-clinical and clinical studies to augment antitumor immune responses. Initially, genetic targeting in mice led the way to an efficient anti-cancer strategy. Genetic deletion of the inhibitory receptor cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) induced T cell hyper reactivity and identified CTLA4 as a negative regulator of T cell activation [73]. Based on these results a blocking antibody against CTLA4 had been generated, which has shown to induce tumor rejection *in vivo* [74]. Ipilimumab was then developed as a fully human anti-CTLA4 monoclonal antibody and has been successfully evaluated in several clinical trials [75]. Next to ipilimumab, the immune-checkpoint blocking antibody nivolumab has been currently approved for anti-cancer therapies [76]. Nivolumab is an antagonistic antibody against the programmed cell death protein 1 (PD1), which is upregulated on exhausted T cells and dampens T cell activity [77]. 20–25% of the treated patients developed strong anti-tumor responses to non-small-cell lung cancer, melanoma, or renal-cell cancer with negligible side effects [78].

In general, PD1 is expressed on a large proportion of TILs from many different tumors [79,80] and is commonly a hallmark of exhausted or anergic T cells that have experienced high levels of stimulation or insufficient CD4⁺ T cell help [77]. As mentioned earlier, PD1 ligands are commonly upregulated on the tumor cell surface from many different human tumors [81]. PD1 ligation on T cells interferes with early T cell activation and phosphorylation of TCR, CD3 ζ and ZAP70 is blocked [82–84]. Numerous signaling effects are triggered by PD1 ligation in T cells, and have been reviewed elsewhere [85]. However, PD1 ligation also induces metabolic changes. Recently it was shown that T cell adapt their cellular metabolism from glycolysis and glutaminolysis to increased fatty acid oxidation (FAO) in response to PD1 ligation [86]. The increased rate of FAO is due to the concomitant upregulation of the carnitine palmitoyl transferase (CPT1A) and the induction of lipolysis, which results in increased availability and utilization of free fatty acids for energy generation [86]. Non-activated naïve T cells do not show increased FAO, which indicates that PD1 triggering does not simply shut down cellular signaling but also engages a specific metabolic program that has been associated with the increased life span of PD1-stimulated T cells [86]. This metabolic state is similar to the metabolism observed in non-proliferative memory T cells and Treg cells (Fig. 1) and emphasises that tumors use this pathway to specifically dampen an efficient anti-tumor immune response.

Of note, expression of PD1 itself is also responsive of the metabolic state of T cells. PD1 expression is upregulated by declining glucose concentrations, which is typically found in the microenvironment of glycolytic tumors [35,87]. In the highly glycolytic tumor environment the concentration of glucose is drastically reduced, and forces TILs to compete with the tumor for glucose uptake. This fierce competition metabolically restricts T cells and has been associated with a striking reduction of an anti-tumor response [35,36] (Fig. 3). In support to that, promoting cancer cell metabolism by increasing the glycolytic capacity did not stimulate tumor cell proliferation but decreased T cell dependent tumor rejection *in vivo* [35]. Interestingly, treatment with immune-checkpoint blocking antibodies against CTLA-4, PD1 or PD-ligand 1 (PD-L1) increased the glucose concentration within the tumor microenvironment, most likely by immune-mediated killing of tumor cells [35]. However, checkpoint blockade increased the

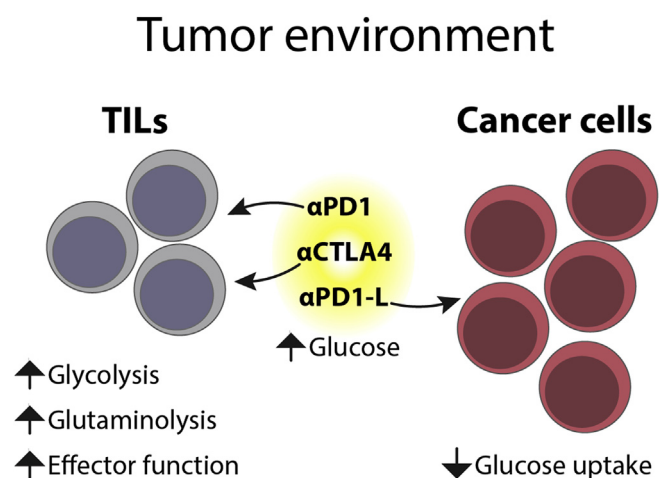


Fig. 4. Checkpoint inhibitors boost anticancer immunity and refuel the metabolic engine. Immune checkpoints can be blocked by antibodies, resulting in enhanced antitumor immunity and better clinical outcomes (please see main text for details). CTLA4 and PD1 increase the metabolic fitness by increasing glycolysis, glutaminolysis and effector functions of TILs. PD-L1 ligation on tumor cells decreases glucose uptake.

glycolytic activity and metabolic fitness of TILs [35]. Due to this metabolic boost (Fig. 4), TILs are better equipped to compete with cancer cells for essential nutrients and checkpoint blockade have been shown to increase the glucose consumption in multiple studies [83,86,88,89]. *Ho et al.* have found that limitations in the glycolytic metabolite phosphoenolpyruvate (PEP) are crucial to restrict an anti-tumor response under glucose limiting conditions *in vivo* [36]. Restoring PEP levels by the expression of PEP carboxykinase 1 in T cells led to a reduced tumor growth and increased survival of tumor bearing mice [36]. This further supports the findings that metabolic reprogramming of increased glucose utilization of TILs can boost anti-tumor immunity in a glucose-deprived tumor microenvironment (Fig. 4).

Interestingly, some evidence also suggests that blocking PD-L1 has a direct effect on cancer cells themselves. This treatment reduced the glycolytic capacity of tumor cells and increased glucose levels in the environment [35]. This might further contribute to the beneficial outcome of an anti-PD-L1 treatment. More recent studies showed that anti-PD-1 treated T cells have an increased mitochondrial mass and produce more ROS. Artificially increased ROS were shown to have synergistic effects with PD1 blockade on tumor-growth inhibition [90]. Additionally, activation of both mTOR and AMPK was also observed in those cells with high mitochondrial energy metabolism [90]. Altogether, these findings suggest that interfering with T cell metabolism might be promising to treat tumors.

Next to PD1, CTLA4 is considered the 'chief' pathway of the immune checkpoint inhibitors, as it stops potentially autoreactive T cells at the initial stage of naïve T cell activation [73,91]. The signaling changes by CTLA4 co-stimulation have been associated with reduced metabolic responses in T cells. Indeed Frauwirth and colleagues have shown that anti-CTLA4 antibody impaired Glut1 expression, glucose uptake and glycolysis by decreasing Akt activity [39]. Based on that, CTLA4 engagement interferes with mTOR activation and promotes T cell anergy [92].

Additionally, both PD1 and CTLA4 are highly expressed in Treg cells, which are enriched in many tumors [93]. The PD1 pathway is associated with increased proliferation of Treg cells through inhibition of the Akt-mTOR axis and the induction of FAO [86,94] (Figs. 1 and 2). In this scenario, PD1 blockade may also enhance antitumor immune responses by limiting the proliferation and immune-suppressive capacity of Treg cells. Although CTLA4 has been identified as a pivotal effector mechanism for Treg cell-mediated tolerance more than 15 years ago [95,96], future studies need to elucidate the exact intracellular mechanism [97]. Furthermore, data on the effect of CTLA4 in Treg cells metabolism are missing, although mTORC1-dependent lipid metabolism has already provided a direct link to the upregulation of surface CTLA4 and ICOS (inducible T cell costimulator), which are key intrinsic receptors for Treg cell-mediated suppression [98].

Checkpoint blockade can support effective T cells metabolism (Fig. 4), which has been shown to support anti-tumor responses on multiple levels. Although much more work is needed to fully characterize these molecular mechanisms, anticancer agents or antibodies that systemically target metabolic processes hold promise for future clinical research. However, the majority of these drugs are still in the pre-clinical stage [15,99].

In the best scenario, novel anti-cancer drugs should generate strong anti-tumor T cell responses and in parallel should harbor toxicity for cancer cells themselves. Metformin, for instance, possesses direct clinical efficacy in colorectal, breast and other types of cancer [100,101]. Multiple mechanisms of action have been described for metformin [102]. For example, the inhibition of mTORC1 via AMPK activation by metformin dampens HER2 and Akt expression, which interferes with tumor growth [103,104]. *In vivo*

studies have also shown that metformin facilitates HIF-1 α degradation with a subsequent increase in oxygen consumption on hepatocellular carcinoma xenografts [105]. In turn, metformin also promotes Treg cells development and memory formation [68,106]. Furthermore, this strategy paves a way to developing a step-wise approach combining metabolism-targeting drugs together with adoptive transfer of metabolically pre-conditioned T cells or checkpoint inhibitors. Following this pre-emptive metabolic reshaping of immunological interventions, we could make cancer immunotherapy more effective in the clinic.

Conclusion

Every biological process depends on a functional metabolism and cellular energy. In that respect immunometabolism is no exception, and its regulation is crucial for T cell biology. Moreover, interfering with T cell metabolism is especially attractive for therapeutic application as the different T cell subsets differ in their metabolic properties, which are closely related to their function. Along these lines, the constant turnover of multiple cell types influences the metabolic signatures of cancer through its different stages. In this evolving context it is hard to conceive a "master" regulatory pathway or metabolite.

However, metabolic profiles of these malignant cells can be quite instructive. In fact, each disease can assume a metabolic signature, and since all cells share the common metabolic machinery which is often "tuned" as a result of altered inflammatory chain reactions, reshaping the energetic features of immune subsets or the proximal environment may lead to a treatment for many inflammatory diseases including autoimmunity and cancer. Unfortunately, corresponding normal tissues rely on the same common metabolic denominator for physiologic functions. Therefore, careful examination has to be provided to identify metabolic targets without impacting crucial functions of healthy cells in the long run.

The investigation of immunometabolism in human diseases is currently ongoing and is associated with high hopes for scientists related to the field. Even though metabolic pathways represent exciting druggable targets, the metabolic manipulation of T cell is still not ready for clinical testing as effective immunotherapy and the potential for off-target effects might also be high. However, the efforts to develop an effective treatment may broaden the scope of targeting metabolism for disease intervention from cancer interference to prevention, by e.g. dietary interventions.

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