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Metabolism of immune cells in cancer

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Abstract

Through the successes of checkpoint blockade and adoptive cellular therapy, immunotherapy has become an established treatment modality for cancer. Cellular metabolism has emerged as a critical determinant of the viability and function of both cancer cells and immune cells. In order to sustain prodigious anabolic needs, tumours employ a specialized metabolism that differs from untransformed somatic cells. This metabolism leads to a tumour microenvironment that is commonly acidic, hypoxic and/or depleted of critical nutrients required by immune cells. In this context, tumour metabolism itself is a checkpoint that can limit immune-mediated tumour destruction. Because our understanding of immune cell metabolism and cancer metabolism has grown significantly in the past decade, we are on the cusp of being able to unravel the interaction of cancer cell metabolism and immune metabolism in therapeutically meaningful ways. Although there are metabolic processes that are seemingly fundamental to both cancer and responding immune cells, metabolic heterogeneity and plasticity may serve to distinguish the two. As such, understanding the differential metabolic requirements of the diverse cells that comprise an immune response to cancer offers an opportunity to selectively regulate immune cell function. Such a nuanced evaluation of cancer and immune metabolism can uncover metabolic vulnerabilities and therapeutic windows upon which to intervene for enhanced immunotherapy.

Work over the past several decades has shown that activated immune cells employ many metabolic pathways attributed to cancer cells[1-3] (Fig. 1). This convergence of metabolic adaptations creates a fundamental competition for nutrients required by cancer cells and immune cells within the tumour microenvironment (TME). However, we are coming to find

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Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

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fundamental differences between the metabolic programmes of cancer cells and immune cells, as well as between different immune cells. Understanding these differences can reveal specific metabolic vulnerabilities and, consequently, novel targets for therapeutic approaches aimed at metabolic programming in order to enhance cancer immunotherapy.

Although the ability of cancer cells and tumour tissue to upregulate glycolytic catabolism of glucose to form lactate, even in oxygen-replete conditions (aerobic glycolysis), a process known as the 'Warburg effect', has been considered a hallmark of malignancy, it has become increasingly clear that cancer metabolism is heterogeneous, and that cancer cells can engage in a broad range of metabolic programmes to meet the demands of growth and proliferation, and that in addition to aerobic glycolysis, mitochondrial respiration is fundamentally important in this regard[4-7]. Predictably, highly metabolically active cancer cells (Fig. 1) impart profound effects on the TME, leading to nutrient depletion, hypoxia, acidity and the generation of metabolites that can be toxic at certain concentrations. A significant amount of glucose from the TME is metabolized through aerobic glycolysis, generating high amounts of lactate and H^+, thereby lowering the intratumoural pH. That said, it is likely that the balance between lactate-generating glycolysis and oxidative phosphorylation (OXPHOS) is dependent on the degree of hypoxia, which can be both heterogeneous and wide ranging within the TME. It is instructive to note that in moderately hypoxic regions, CO_2 derived from mitochondrial respiration is hydrated by extracellular carbonic anhydrase enzymes, forming HCO_3^- and H^+. Thus, oxidative metabolism can be a significant and often overlooked source of extracellular acidification within the TME.

Given the recent establishment of cancer immunotherapy, including the use of blocking antibodies against immune checkpoint pathways and adoptive cell therapy with chimeric antigen receptor T cells (CAR T cells), several recent studies have begun to establish the relationship of tumour-intrinsic metabolism to successful immunotherapy. For instance, it has been reported that increased glycolytic metabolism in melanoma cells is associated with resistance to adoptive T cell therapy and checkpoint blockade[8,9]. Other studies have shown that signalling through immune checkpoint proteins on tumour cells, including PD1 and B7-H3, was responsible for increased glucose depletion within the TME[10-12]. Interestingly, some immunosuppressive checkpoint pathways are actually induced as a direct consequence of tumour acidification[13]. Further, immune checkpoint blockade can dampen glycolysis of tumour cells, restore glucose in the TME and permit T cell glycolysis and cytokine production[14]. Several recent studies have demonstrated that targeting specific aspects of tumour-intrinsic metabolism, such as the hexosamine biosynthesis pathway (HBP) or glutamine metabolism, could foster an immune response and sensitize tumours to checkpoint blockade[15,16].

Because of the emergence of immunotherapy as a pillar of oncologic therapy, it is increasingly vital to understand as much as possible about the metabolic interdependence of infiltrating immune cells and cancer. This Review aims to discuss the following fundamental questions: which metabolic programmes are critical for the function of specific cell subsets involved in the immune response to cancer; how these metabolic programmes might be perturbed within the TME; the implications of metabolic derangements in the TME for

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current immunotherapeutic paradigms; and how metabolic interventions might be leveraged to enhance the antitumour immune response.

The TME and immune contexture

Highly active metabolic pathways that are characteristic of cancer cells (Fig. 1) can create profound changes in the composition of nutrients and other small molecules within the TME. This can have critical effects on the immune response. The high metabolic activity of cancer cells and disorganized vasculature within the TME can contribute to a nutrient-depleted and hypoxic microenvironment, establishing metabolic competition between cancer cells and infiltrating immune cells[14,17,18]. Indeed, the glucose uptake and effector function of antitumour $CD4^+$ T cells has been shown to be inversely proportional to glycolytic activity of cancer cells in mouse models[18], and glucose availability in the TME allows for improved cytokine expression from antitumour $CD8^+$ T cells[14]. Furthermore, transcriptomic analyses of patients with melanoma from The Cancer Genome Atlas revealed that effector T (T_{eff}) cell genes, such as *CD40lg* and *IFNG*, are inversely correlated with *HK2* expression, which encodes the rate-limiting enzyme in the glycolytic pathway[18]. Metabolic programmes active within cells of the TME can also lead to the generation of toxic concentrations of certain metabolites. Elevated levels of adenosine, kynurenine, ornithine, reactive oxygen species (ROS) and potassium, as well as increased acidosis, have all been reported in the TME, and each can have profound effects in suppressing the antitumour immune response.

The immune contexture of the TME comprises a range of distinct cell types[19] (TAbLe 1). Effector cells perform functions aimed at cell killing and can arise from either the innate (non-specific) or adaptive (antigen-specific) arms of the immune system. Antitumour effector cells arising from the adaptive system include $CD4^+$ and $CD8^+$ T_{eff} cells, which orchestrate and carry out antigen-specific killing of cancer cells, respectively. $CD8^+$ T_{eff} cells are critically important in direct tumour cell killing through the induction of apoptosis and cytokine secretion. $CD4^+$ T cells comprise numerous subsets. Some of these subsets, the most well studied of which is the T helper 1 (T_H1) subset, can also provide significant antitumour activity. These antitumour $CD4^+$ T cells, collectively termed conventional $CD4^+$ ($CD4^+{}_{con)v}$ T cells, are distinct from immunosuppressive, pro-tumorigenic $CD4^+$ T cells known as regulatory T (T_{reg}) cells. Although $CD4^+_{conv}$ cells may engage in direct tumour cell killing, they primarily contribute to antitumour immunity through cytokine secretion and assisting in $CD8^+$ T cell activation. Antitumour $CD4^+{}_{conv}$ T cells share significant metabolic characteristics with $CD8^+T_{eff}$ cells. Although less well understood in terms of antitumour immunity, B cells may also perform effector roles in the TME[20]. Importantly, as part of the adaptive immune system, T cells and B cells can give rise to memory cell populations, which can persist long after the resolution of an infection or tumour response. $CD8^+$ memory T (T_{mem}) cells are a crucial aspect of long-term tumour control. Innate cells, such as natural killer (NK) cells and inflammatory macrophages, perform critical antitumour effector functions as well. There are also immunosuppressive cell populations within the TME, including $CD4^+FOXP3^+T_{reg}$ cells, myeloid-derived suppressor cells (MDSCs), anti-inflammatory macrophages and some B cell populations[20]. Through various mechanisms, including cytokine secretion and metabolic derangements, these cells can dampen or

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eliminate the effectiveness of antitumour effector cell populations. Lastly, antigen-presenting cells, such as intratumoural dendritic cells (DCs), have been shown to perform essential roles in maintaining active adaptive immune response within the TME[21,22]. Numerous excellent reviews can be referred to for more detailed discussions of tumour immunology and immunotherapy[19,23-25].

The metabolism of the antitumour response

Glucose metabolism of antitumour effector T cells.

$CD4^+_{conv}$ and $CD8^+$ T_{eff} cells form the critical effector compartment of the antitumour response. When naive $CD4^+$ and $CD8^+$ T cells, which are non-proliferative, recognize their cognate antigen in the context of co-stimulatory signalling, they become proliferative and enact metabolic features to support immense growth[26-28]. Although many early investigations highlighted the upregulation of aerobic glycolysis as a hallmark of T cell activation, it is now clear that upregulated tricarboxylic acid (TCA) cycle metabolism and OXPHOS are also a critical aspect of $CD4^+_{conv}$ and $CD8^+$ T cell activation. Although TCA cycle metabolism is upregulated within 24 h post activation, upregulated aerobic glycolysis appears to be a more rapid event, occurring within 6 h after activation[27-32].

The transcriptional activity of MYC and hypoxia inducible factor 1 (HIF-1) are both upregulated in response to T cell activation and promote metabolic reprogramming[26,29,33,34]. Notably, although HIF-1 is well known to regulate metabolism in response to hypoxia, its activity is also induced in response to T cell activation in the absence of hypoxia. MYC and HIF-1 transcriptional activity leads to upregulation of genes encoding enzymes that promote glycolysis, such as pyruvate kinase (*PKM1*), hexokinase 2 (*HK2*) and *GLUT1* (ReFS[29,34,35]). Pathways emanating from proximal metabolites in the glycolytic pathway are also integral components of T cell activation and function (Fig. 1). The pentose phosphate pathway (PPP) metabolizes glucose-6-phosphate to generate NADPH and ribose-5-phosphate[36]. Glucose shuttling into the PPP is significantly increased upon $CD4^+$ T cell activation[29]. The PPP is the primary cellular source for NADPH, which is required for fatty acid and plasma membrane synthesis in newly activated $CD8^+$ T cells[37]. NADPH is also critical for REDOX homeostasis in proliferating manunalian cells[38-40]. ROS levels in activated T cells need to be finely regulated. Although dysregulated ROS levels can be toxic[39,41,42], ROS play an important role in T_{eff} cell activation, having been shown to promote nuclear factor of activated T cells (NFAT)-dependent IL-2 expression in $CD4^+$ and $CD8^+$ T cells. Another pathway originating from early glycolytic reactions, the HBP, is the primary cellular source of glycosylation substrates, which mediate a variety of effects on a broad range of proteins, including stability, trafficking and function. The HBP relies on metabolism of glucose and glutamine and is responsive to their availability. The main substrate produced by the HBP, UDP-GlcNAc, is critical for effector $CD4^+$ and $CD8^+$ T cell expansion and function[43]. Lastly, the serine–glycine–one-carbon pathway allows cells the ability to generate serine, glycine, NADPH and one-carbon units for use in the folate cycle. T_{eff} cell proliferation and function were dependent on sufficient serine metabolism in vitro and in vivo[44] (Fig. 1).

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Glucose carbons that are not metabolized to lactate or by proximal glycolytic pathways contribute significantly to the TCA cycle in T_{eff} cells[6] (Fig. 1). In highly proliferative cells, intermediates of the TCA cycle are rapidly consumed to serve as building blocks for a broad range of biomolecular syntheses, a process called cataplerosis[45]. For example, citrate can be exported to the cytoplasm to regenerate acetyl-CoA for use in lipid and cholesterol synthesis, both of which are critical for producing membranes in proliferative T_{eff} cells. Other TCA cycle intermediates function as building blocks for biosynthesis of, for example, nucleotides and amino acids, which are in high demand during proliferation. Like cancer cells, T_{eff} cells are highly proliferative and upregulate specific glycolytic programmes, including aerobic glycolysis, PPP, HBP and TCA cycle support, to allow massive cell division and effector functions.

T cells and glucose restriction in the TME.

Glucose limitation within the TME can markedly affect the T cell response. For example, low-glucose conditions (0.1 mM) suppressed the generation of the glycolytic intermediate phosphoenolpyruvate (PEP) in T cells, which disrupted calcium-dependent NFAT signalling in vitro[18]. Compared with control, decreasing the glucose concentration in growth media has been shown to suppress the extracellular acidification rate (a measure of aerobic glycolysis), augment the oxygen consumption rate (a measure of OXPHOS), attenuate mTOR signalling and suppress the effector function of both $CD4^+$ and $CD8^+$ T_{eff} cells[46-48]. Reduced mTOR complex 1 (mTORC1) signalling interfered with T_{eff} cell differentiation and, in the case of $CD4^+$ T cells, specifically favoured the development of immunosuppressive, pro-tumorigenic T_{reg} cells[49]. Interestingly, in $CD8^+$ T cells, mTOR blockade with rapamycin favoured differentiation of long-lived T_{mem} cells, which may play an important role in sustaining antitumour responses[49-51]. Decreasing glucose availability in culture suppressed production of the critical effector molecules interferon-γ (INFγ), IL-17 and granzyme B in T_{eff} cells compared with control growth media[47,48,52,53]. In activated $CD4^+$ T cells cultured in glucose-free media containing the alternative sugar fuel galactose (which suppresses aerobic glycolysis), the glycolytic enzyme GAPDH assumed a moonlighting role, binding the 3′ untranslated region of *Ifng* mRNA and suppressing its translation and T_{eff} cell function[31]. Glucose restriction in media conditioned by primary ovarian cancer cells led to microRNA-mediated suppression of the histone methylase EZH2 (enhancer of zeste homologue 2), leading to decreased NOTCH signalling, suppressed cytokine production and decreased viability of T_{eff} cells[54].

Increasing the glycolytic capacity of mouse sarcoma cells through either pharmacologic treatment with the AKT activator 4-hydroxytamoxifen in co-culture experiments or overexpression of key glycolytic enzymes (for example, *Glut1*, *Hk2* and *Pdk1*) in tumour cells followed by injection into mice led to suppression of $CD8^+$ T cell effector function compared with vehicle-treated tumour cells or empty vector overexpression, respectively[14]. Similarly, compared with wild-type tumours, implanted *Hk2*- overexpressing melanoma cells suppressed $CD4^+$ T cell antitumour effector function and in vivo responses in mouse models[18]. Furthermore, expression of glycolysis-related genes in tumour samples from patients with melanoma and non-small-cell lung cancer was inversely correlated to T cell infiltration[8]. Tipping the metabolic balance can also be accomplished through directly

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manipulating T cell metabolism. For example, overexpression of the glycolytic enzyme PEP carboxykinase in tumour-specific $CD4^+$ T cells improved antitumour responses compared with control vector-transfected T cells in an adoptive T cell model using melanoma-specific T cells[18].

Mitochondrial respiration is also a critical aspect of T_{eff} cell metabolism. Several recent studies have reported that T cells in patients with cancer (compared with healthy controls) and tumour-infiltrating $CD8^+$ T cells in tumour-bearing mice (compared with non-infiltrating $CD8^+$ T cells) displayed decreased mitochondrial mass as well as indicators of mitochondrial dysfunction[55-57]. Mitochondrial fitness of resting peripheral $CD8^+$ T cells was impaired in patients with chronic lymphocytic leukaemia compared with healthy controls[55]. Furthermore, the degree of response in these patients to CAR T cell therapy was negatively correlated to the degree of mitochondrial impairment of infused CAR T cells[55]. Tumour-infiltrating $CD8^+$ T cells from patients with renal cell carcinoma showed dysregulated mitochondrial dynamics and function, including elevated levels of mitochondrial ROS and hyperpolarization, compared with $CD8^+$ T cells from healthy donors[56]. Normal ex vivo activation of these T cells could be rescued with mitochondrial ROS scavengers or pyruvate supplementation. Mitochondrial biogenesis and function are particularly deranged in a subset of dysfunctional tumour-infiltrating $CD8^+$ T cells termed exhausted T cells (box 1). As a whole, these studies demonstrate that cancer itself can lead to derangements in the metabolism of T_{eff} cells, including mitochondrial dynamics, and that a reciprocal relationship exists between the degree of glycolytic activity of cancer cells and the antitumour effector function of infiltrating T cells.

Amino acids and the antitumour T cell response.

Like cancer cells, highly proliferative immune cells, such as activated T cells, are reliant on amino acid metabolism to support protein and nucleotide synthesis. As such, amino acid transporters, including SLC7A5 (also known as LAT1)[58], SLC38A1 (also known as SNAT1), SLC38A2 (also known as SNAT2)[59] and SLC1A5 (also known as ASCT2)[60], have been found to be highly upregulated during T cell activation compared with naive cells in in vitro human and mouse studies[61]. Essential amino acids must be obtained exogenously. For example, leucine was required for mTORC1 signalling, effector function and proper differentiation in effector $CD8^+$ and $CD4^+$ $CD4^+$ T_{conv} cells. Interestingly, deletion of the leucine transporter, *Slc7a5*, in mouse models caused metabolic failure during in vitro activation and cytokine-directed differentiation of $CD4^+$ (T_H1, IL-17-producing T_H17) and $CD8^+$ T_{eff} cells, but had no adverse effect on the differentiation of T_{reg} cells[26,62]. Activated T cells also rapidly metabolize arginine, and exogenous arginine supplementation leads to improved T cell fitness and increased generation of central T_{mem} cells[63]. Serine, tryptophan and cysteine are also vital nutrients for T cell responses and, as such, are important mediators of antitumour immune responses[44,64-66]. Tryptophan is an essential amino acid and its availability within the TME is an important factor in determining strength and quality of the T cell response. Human T cell proliferation and activation were strongly suppressed in tryptophan-free media compared with normal growth media[66,67]. Cancer cells, tumour-associated macrophages (TAMs), MDSCs, suppressive DCs and cancer-associated

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fibroblasts can deplete tryptophan levels through enzymatic activity of indoleamine 2,3-dioxygenase (IDO)[68], which can be expressed at high levels in these cells within the TME. Underlining the importance of this metabolic pathway for tumour growth, IDO expression has been correlated with poor outcomes in patients with several cancer types, including gastric cancer, colorectal cancer, non-small-cell lung cancer and melanoma[69-71].

In proliferating cells, glutamine provides nitrogen for amino acid and nucleic acid synthesis and carbon to replenish TCA cycle intermediates that are syphoned off as building blocks for biosynthesis — a process called anaplerosis (Fig. 1). Cancer cells and some activated immune cells, such as T cells and macrophages, are generally highly glutamine avid[59,72]. The expression of glutamine transporters SLC1A5 and SLC38A1 and/or SLC38A2 was significantly upregulated during in vitro stimulation of murine $CD4^+_{conv}$ T cells[60]. Driven by MYC, glutamine is metabolized by glutaminase (GLS) to glutamate, which may enter the TCA cycle after conversion by glutamate dehydrogenase (GLUD1) to α-ketoglutarate (αKG; also known as 2-oxoglutarate). αKG is subsequently metabolized to succinate and fumarate in the TCA cycle[29]. Interestingly, in settings of glutamine restriction, some cancer cell lines switch to glucose-fuelled anaplerosis, wherein pyruvate is converted by pyruvate carboxylase to oxaloacetate, which enters the TCA cycle[73]. Our group has recently shown that effector $CD8^+$ T cells are also capable of upregulating pyruvate carboxylase activity under conditions of glutamine blockade in vitro[16].

Although effector function and proliferation in differentiated $CD8^+$ T_{eff} cells was suppressed by limiting glutamine in media[59], if glutamine availability was restricted during activation of $CD8^+$ T cells, it altered differentiation towards a long-lived, memory phenotype[74]. This effect on differentiation was shown to be mediated by αKG. αKG and other TCA metabolites, such as succinate and fumarate, can modulate the activity of a wide range of cellular processes, including epigenetic remodelling and the stability of critical transcription factors, such as HIF-1α (ReFS[45,75]).

Lipid metabolism and T cells.

Activated T cells also reprogramme lipid metabolism, upregulating de novo lipid synthesis and cholesterol uptake, which are critical for membrane synthesis and mediated by the transcription factors sterol regulatory element-binding protein 1 (SREBP1) and SREBP2, respectively[37,76]. Proliferation, metabolic reprogramming and antiviral activity were dramatically suppressed in activated mouse $CD8^+$ T cells lacking SREBP1 and SREBP2 functionality. In addition, the cholesterol content in membranes during $CD8^+$ T cell activation and expansion in vitro was, in part, regulated by cholesterol esterification enzyme acetyl-CoA acetyltransferase (ACAT1). *Acat1* knockout $CD8^+$ T cells showed increased membrane cholesterol and improved T cell receptor clustering and signalling, leading to enhanced proliferation, function and improved tumour killing in adoptive-transfer mouse tumour models[76]. Pharmacologic inhibition of ACAT1 with avasimide improved the antitumour effect in mice compared with vehicle-treated control animals. Cholesterol metabolism and antitumour T cell function is an evolving story, however. A recent study by Ma et al. demonstrated that a high cholesterol content in tumours can induce T cell dysfunction by activating the endo-plasmic reticulum stress response[77]. As such, although

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cholesterol is important for T_{eff} cell proliferation and metabolism, the benefit of targeting specific aspects of cholesterol metabolism to improve the antitumour immune response needs further study.

The metabolism of immunologic memory.

Unlike T_{eff} cells, $CD8^+$ T_{mem} cells preferentially rely on OXPEIOS[78-81]. Compared with $CD8^+$ T_{eff} cells, enhanced spare respiratory capacity, a parameter indicative of the ability of cells to upregulate OXPEIOS, is also highly characteristic of T_{mem} cells[78]. Initial studies using etomoxir as an inhibitor of carnitine palmitoyl transferase 1A (CPT1A), a mitochondrial transporter responsible for the import of long-chain fatty acids destined for fatty acid β-oxidation (FAO), implicated FAO as the primary fuel for OXPEIOS in T_{mem} cells. However, more recent work using T cell-specific *Cpt1a* knockout models has called this into question and demonstrated that off-target effects of high-dose etomoxir (200 μM) are likely responsible for the earlier findings[82]. This should not be taken to imply that T_{mem} cells do not use FAO in support of OXPHOS and spare respiratory capacity but, rather, that FAO is not the sole pathway responsible for this metabolic phenotype. Indeed, the expression of CPT1A is consistently upregulated in $CD8^+$ T_{mem} cells compared with T_{eff} cells. Furthermore, a $CD8^+$T cell subset known as tissue-resident memory cells were specifically dependent on fatty acid binding protein 4 (FABP4) and FABP5 to import extracellular fatty acids for FAO and for maintenance of a long-term memory phenotype[83].

Intermediates of the TCA cycle, such as αKG, succinate and fumarate, are particularly important in adaptive memory. Inhibition of 2-oxoglutarate-dependent dioxygenases (2OGDD) through alterations in these TCA metabolites has been shown to increase memory cell differentiation in $CD8^+$ T cells[84,85]. Although glucose, glutamine and fatty acids are the primary nutrient sources fuelling the TCA cycle, a range of other nutrients, such as amino acids and acetate, can also enter the cycle. In particular, acetate metabolism is emerging as an important source of acetyl-CoA in $CD8^+$ T cells and some cancer types[16,86-88]. In the mitochondria, acetate can enter the TCA cycle after it is metabolized by acyl-CoA synthetase short chain family member 1 (ACSS1) to form acetyl-CoA. Alternatively, acetate can be converted to acetyl-CoA by ACSS2 in the cytoplasm, where it can contribute to fatty acid synthesis and acetylation reactions important in epigenetic reprogramming and post-translational modifications. The metabolism of acetate is an important metabolic pathway for promoting the function of memory $CD8^+$T cells[88]. Interestingly, blockade of glutamine metabolism during T cell activation increased T_{mem}cell differentiation and induced acetate metabolism and associated enzymes, including ACSS1 and ACSS2[16,85]. As quiescent cells, T_{mem} cells preferentially rely on OXPHOS relative to aerobic glycolysis and have significant mitochondrial reserve that is required to upregulate OXPHOS further upon antigen activation. T_{mem} cells can adapt several distinct nutrient sources to fuel this metabolic programme.

Hypoxia and the antitmnour T cell response.

Although tumours are highly heterogeneous, high levels of metabolic activity and associated oxygen consumption, as well as disorganized, poorly functioning vasculature, can generate hypoxic regions with median oxygen saturation levels <2 % (compared with a median of

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about 5% in normal tissues)[89,90]. The effect of hypoxia on T_{eff} cells is not straightforward. Complicating this area of study is the fact that HIF-1 transcriptional activity is upregulated in response to T cell activation in normoxic conditions[34], so it is challenging to understand the effect of hypoxia on further augmenting HIF-1 activity while also evaluating HIF-1-independent effects. Early in vitro studies of $CD8^+$ T_{eff} cell activation, differentiation and function showed that whereas proliferation and the expression of some cytokines were suppressed in hypoxia, the lytic capacity, activation markers and survival were improved[91]. Subsequent in vivo studies showed that $CD4^+$ and $CD8^+$ splenic T cells were more poorly activated after concanavalin A challenge in mice exposed to subatmospheric O_2 tension (8%) compared with mice exposed to ambient O_2 tension (20%)[92]. Other studies showed that in vitro hypoxic exposure causes intracellular accumulation of the metabolite (*S*)-2-hydroxyglutarate (*S*-2-HG), which profoundly alters $CD8^+$ T cell activation and differentiation, suppressing cytokine secretion and cytolytic capacity, but, interestingly, augmenting proliferation, long-term survival and antitumour response after in vivo transfer in mouse models[84]. Contrary to previous findings demonstrating the necessity of oxidative metabolism and oxidative metabolic capacity in forming long-lived memory $CD8^+$ T cells, glycolytic activity enforced through constitutive HIF-1α activity (achieved through conditional knockout of the HIF-1 regulator *Vhl*) actually favoured the formation of long-lived effector memory cells in mouse vaccine models[93]. Other work has demonstrated that hypoxia induced the expression of the ectonucleotidases CD39 and CD73 on various cells in the TME[94,95]. These enzymes break down ATP in the TME to adenosine. Adenosine is a ligand for the A2A and A2B purinergic receptors, which are expressed on a large range of immune cells, and is broadly immunosuppressive, inhibiting effector cell function and proliferation of T_{eff} cells[96-101]. Interestingly, supplemental oxygen enhanced the antitumour immune response of T cells in mice by downregulating the adenosine signalling pathway[102]. The effect of hypoxia on antitumour T cells is an evolving area of study. Further research will clearly benefit the field of immunotherapy, given both the prevalence of hypoxic regions in tumours as well as the profound effects hypoxia can have on the adaptive immune response.

Toxic metabolites.

In addition to adenosine, many other products generated from cancer cell metabolism influence infiltrating T cells (Figs 1 and 2). Elevated levels of extracellular lactate and H^+ in the TME can suppress T cell proliferation, survival, cytotoxicity and cytokine production in in vitro studies of mouse and human $CD8^+$ T cells[103,104]. The upregulation of the gene encoding the key T_{eff} cell transcription factor NFAT was impaired during in vitro activation of mouse $CD8^+$ T cells in the presence of high levels of lactate and H^+ compared with standard growth media[104]. In vivo mouse studies showed that mouse melanoma cells that have *Ldha* knocked down produced less lactate and were more responsive to immune-mediated tumour rejection than empty vector-transfected control melanoma cells[104]. MAP kinase signalling was also severely impaired in human effector $CD8^+$ T cells activated in the presence of elevated lactate and H^+ compared with control media[105].

The accumulation of specific amino acids within tumours can also suppress the T_{eff} cell response. Probably the most well studied in this regard are the effects of tryptophan

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metabolites, especially kynurenine, which is generated through the activity of IDO1 [106]. Functioning as an endogenous ligand for aryl hydrocarbon receptors on T cells, kynurenine caused upregulation of the PD1 co-inhibitory pathway on activated $CD8^+$ T cells in vitro compared with vehicle-treated control[107]. This upregulation of PD1 was also observed on tumour-infiltrating $CD8^+$ T cells in mouse models treated with exogenous kynurenine compared with vehicle-treated controls. Tumour-infiltrating $CD8^+$ T cells from kynurenine treated tumour-bearing mice produced less IFNγ and TNF.

Cancer cells have also been reported to suppress T cell activity through release of the oncometabolite (*R*)-2-hydroxyglutarate (*R*-2-HG). This metabolite can inhibit epigenetic dioxygenase enzymes, such as histone demethylases, leading to increased methylation and modified transcription. *R*-2-HG produced by isocitrate dehydrogenase (IDH)-mutant human glioma was taken up by T cells in in vitro studies. *R*-2-HG interfered with proliferation, T cell receptor signalling, NFAT activity and polyamine biosynthesis in activated human $CD4^+$ and $CD8^+$ T cells in vitro[108]. This was corroborated by the finding that *R*-2-HG released into the TME in IDH-mutant glioma-bearing mice inhibited complement-mediated antitumour response as well as T cell migration, proliferation and cytokine secretion[109]. These studies highlight the intricate interplay of cancer metabolites and immune function within the TME (Fig. 2).

High levels of necrosis lead to increased levels of potassium within the TME, which limits T cell effector function[110]. Mediated by reduced cytoplasmic levels of acetyl-CoA, this state induced epigenetic remodelling of activated T cells, inducing a dysfunctional state of T_{eff} cells within the TME[111]. However, this dysfunctional state was enriched with characteristics of T cell stemness. In accord with the induction of a stem-like state, ex vivo stimulation and expansion of T_{eff} cells in high potassium produced T cells with improved in vivo persistence, multipotency and capacity for tumour clearance[111]. The generation of T cell-suppressive metabolites through tumour necrosis and metabolic activity intrinsic to the TME forms an important mechanism of tumour immune evasion.

Metabolism and the innate effector response.

Because NK cells are particularly adept at cell killing during major histocompatibility complex class I (MHC-I) down-regulation, a common evasion strategy of cancer cells, they form a critical effector component of tire innate response. Metabolically, aerobic glycolysis and OXPHOS were upregulated after in vitro cytokine stimulation (IL-12 and IL-15) of NK cells[112]. Interestingly, SREBP transcription factors were required for these cytokine-induced metabolic changes during in vitro NK cell stimulation[113]. Pharmacologic inhibition of SREBP activity suppressed metabolic reprogramming, cytokine production and cytotoxicity in vitro and curtailed anti-tumour response in an adoptive NK cell mouse model. Interestingly, it has been reported that endogenous SREBP inhibitors, such as 27-hydroxycholesterol, can be increased within the TME and thus may be a mechanism of NK cell suppression[112,114-118]. Lung cancer progression in mice and tumour-associated transforming growth factor-β (TGFβ) are correlated with increased fructose-1,6-bisphosphatase (FBP1) expression in tumour-associated NK cells[119]. FBP1 is a key enzyme in gluconeogenesis, which, when activated, strongly suppressed glycolysis in NK cells,

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leading to dysfunction and diminished viability. Interestingly, pharmacologic inhibition of FBP1 was sufficient to re-establish glycolytic metabolism, as well as cytokine production and cytotoxicity in vitro, and improve antitumour response in adoptive cell therapy mouse models[119]. These studies showed that rescuing NK function through FBP1 inhibition was dependent on restoration of glucose metabolism, as blocking glucose metabolism with 2-deoxyglucose (2-DG) prevented the rescue caused by FBP1 inhibition. 2-DG by itself also led to NK cell dysfunction[119], implying that inhibition of glucose metabolism could have profound effects on NK cell antitumour response. Other metabolic derangements within the TME are likely to affect NK cell function as well. For instance, low arginine levels can impair NK cell proliferation and IFNγ production[120,121], and hypoxia can suppress cytolytic activity[122-124]. Human NK cell-activating receptors, such as NKp46 and NKp30, are suppressed in response to hypoxia or low arginine in in vitro studies[121,124]. High lactate levels and associated low pH, as in the TME, also suppressed NK cell cytotoxicity, cytokine production and NFAT signalling in in vitro studies[104,125,126]. Lastly, elevated adenosine levels within the TME can strongly suppress NK cell effector function and proliferation[127].

Other innate cells, macrophages and DCs also enact specific metabolic programmes upon activation. Although early in vitro work using activation schemes with specific cytokines classified macrophages into inflammatory (M1) or immunosuppressive (M2) phenotypes, there is poor evidence that these polarized phenotypes play distinct roles in vivo[128,129]. More recent work has uncovered a spectrum of macrophage phenotypes characterized by distinct transcriptional states[130]. That said, macrophages with inflammatory characteristics can play an important role in antitumour immunity[19], and in this regard it is instructive to examine what has been established regarding the metabolic programming of in vitro derived ‘M1’ macrophages. Glucose metabolism is a vital aspect of the inflammatory phenotype in macrophages. Upon activation, for example by a Toll-like receptor agonist, these cells showed increased expression of glycolytic genes, high levels of glucose uptake, increased lactate production and upregulated glutamine anaplerosis[131]. This metabolic reprogramming led to increased succinate levels, which increased expression of the inflammatory cytokine IL-1β by stabilizing HIF-1 (ReF.[132]). Inflammatory macrophages are also particularly reliant on the PPP for the generation of NADPH, with ^{13}C-glucose tracing studies confirming increased routing of glucose though this pathway upon activating inflammatory phenotypes in culture[133,134]. NADPH is necessary to produce high levels of ROS as part of an oxidative burst, a key effector mechanism for these cells[132,135,136]. Arginine is also a critical nutrient in the function of pro-inflammatory ‘M1’ macrophages as they express high levels of inducible nitric oxide synthase (iNOS) compared with alternatively activated or ‘M2’ polarized macrophages in in vitro studies[137]. iNOS requires arginine to generate cytotoxic nitric oxide, an important pro-inflammatory mediator of antitumour response[138,139].

Specific nutrient deficits within the TME, particularly glucose and arginine, can severely limit the metabolism and related elaboration of effector programmes in these cells. Glucose limitation not only suppresses glycolysis as a whole but can curtail PPP activity and TCA cycle function, thus limiting the generation of NADPH, ROS and succinate, all of which can severely limit M1 macrophage function. Supporting this idea, the secretion of pro-inflammatory cytokines by macrophages was significantly reduced by glycolysis inhibition with 2-DG[140].

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DCs are an important class of antigen-presenting cells involved in the antitumour response. Intratumoural DCs that are capable of antigen cross-presentation have specifically emerged as a vital component of this response[22]. Upon activation, DCs undergo maturation allowing antigen processing and presentation to T cells. This response was coupled to a metabolic switch from OXPHOS to aerobic glycolysis, mediated by HIF-1α in response to in vitro LPS activation[141], and by the PI3K–AKT pathway in response to Toll-like receptor stimulation in vitro[142]. This switch to glycolysis and away from OXPHOS during DC activation is critical for DC survival, production of stimulatory cytokines and activation of T cells[142]. Interestingly, pharmacologic activation of AMPK, which promotes mitochondrial biogenesis and oxidative respiration[143], was sufficient to block DC maturation in vitro[142]. Given this critical dependence on aerobic glycolysis, glucose competition in the TME may significantly suppress DC activation and viability and thus limit the ability of DCs to foster an effective and persistent T cell response.

The metabolism of cancer immune evasion

Metabolism of adaptive immune suppression.

Immunosuppressive T_{reg} cells preferentially rely on TCA cycle function and mitochondrial respiration[144,145]. Although initial studies demonstrating the dependence of T_{reg} cells on FAO did not account for off-target effects of etomoxir, other studies have shown that FAO does support OXPHOS in T_{reg} cells, although not as the sole pathway[82,146,147]. In contrast to T_{eff} cells, T_{reg} cells showed decreased glucose uptake and expressed lower levels of GLUT1 in vitro[144]. Interestingly, although glycolysis did not appear to play a crucial role in T_{reg} cell differentiation or a long-lived phenotype, our laboratory has reported that a subset of highly active T_{reg} cells, termed effector T_{reg} cells, relied on the upregulation of glycolysis for optimal function[148]. As such, T_{reg} cells appear to be metabolically flexible, which may allow them to thrive within relatively harsh and heterogeneous conditions, such as the TME. To this end, it has been reported that the T_{reg} cell-defining transcription factor, FOXP3, reprogrammes cellular metabolism through suppression of MYC favouring OXPHOS and NAD(H) oxidation[149]. In conditions of low glucose and high lactate, such as found in the TME, these adaptations allow for a metabolic advantage of these immunosuppressive cells, allowing T_{reg} cells to resist lactate-induced functional and proliferative suppression (unlike T_{eff} cells) in vitro[149]. Glucose or glutamine deprivation (leading to reduced intracellular αKG) in media during in vitro skewing experiments can alter CD4 differentiation and favour the development of T_{reg} cells[150,151].

Similar to T_{eff} cells, T_{reg} cell response to hypoxia is not entirely clear. Hypoxia has been shown to promote cytokine-mediated recruitment of T_{reg} cells into the tumour environment[152]. Other work has demonstrated that FOXP3 transcript is actually increased in response to HIF-1α induction[153-155]. Also, adoptively transferred T_{reg} cell-specific Hif1 knockout cells failed to migrate into brain tumours in mouse models compared with wild-type controls, an effect that was also observed in dichloroacetate-treated T_{reg} cells, in which glycolysis is inhibited compared with vehicle-treated control T_{reg} cells[156]. Interestingly, hypoxia-responsive adenosine signalling through the adenosine receptor A2A on T_{reg} cells induced proliferation and significantly stronger immunoregulatory activity in mixed

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lymphocyte culture experiments[157,158]. Conversely, several groups have reported that hypoxia-induced HIF-1α can destabilize T_{reg} cells, with reports demonstrating that hypoxia can promote T_H17 $CD4^+$ T cells through direct HIF-1α interactions with the cell subtype-defining transcription factors FOXP3 and RORγt, respectively[52,53,159].

The unique metabolism of amino acids within the TME can also have a profound effect on T_{reg} cells. IDO1 activity can strongly promote T_{reg}cell differentiation in vitro, an effect that appears to be secondary to both tryptophan deficiency as well as the generation of downstream metabolites, such as kynurenine[160,161]. Kynurenine has been found to induce the generation of FOXP3-expressing T_{reg} cells by functioning as an endogenous ligand for aryl hydrocarbon receptors on T cells[162]. Interestingly, many of the qualities of the TME that make it inhospitable for T_{eff} cells are either well tolerated by T_{reg} cells (elevated lactate and H^+) or can induce T_{reg} cell responses (for example, accumulation of adenosine, kynurenine and hypoxia).

Metabolism of innate immune suppression.

TAMs can adopt phenotypes that are highly immunosuppressive. Although there is a spectrum of TAM phenotypes as mentioned above, it is useful to examine the metabolic programming of what heretofore had been established as an 'M2' anti-inflammatory macrophage subset, characteristics of which are clearly evident within immunosuppressive TAMs. Like T_{reg} cells, M2 macrophages upregulate FAO and mitochondrial respiration[134,163]. Although early studies demonstrating that FAO is obligatory in M2 macrophages did not take into account the off-target effects of the CPT1A inhibitor etomoxir[164], forced induction of FAO and mitochondrial biogenesis by overexpression of *Pgc1α* primes macrophages for an immunosuppressive phenotype and strongly suppresses the production of pro-inflammatory cytokines[163].

M2 macrophages metabolize amino acids in a distinct manner from inflammatory macrophages, expressing high levels of arginase 1 (ARG1), which depletes arginine and generates polyamines that are important mediators of wound healing but also highly immunosuppressive[165-168].

Another group of tumour-associated immunosuppressive innate cells, MDSCs, appear to be highly metabolically active. Both aerobic glycolysis and OXPHOS were upregulated in tumour-associated MDSCs compared with MDSCs in the periphery[169]. In another study, granulocytic MDSCs from the spleens of tumour-bearing mice also showed increases in both aerobic glycolysis and OXPHOS compared with splenic neutrophils from the same mice. Interestingly, MD SC expansion in vitro and accumulation in the TME in mouse breast cancer models could be attenuated through blockade of glycolysis with 2-DG, likely by causing increased ROS levels in these cells[170].

Hypoxic regions within tumours have been associated with the accumulation of macrophages, where they aid tumour development through the production of angiogenesis factors, mitogenic factors and cytokines associated with tumour metastasis[171-173]. Furthermore, hypoxia can promote the generation of immunosuppressive macrophage phenotypes[174]. Adenosine, which can be generated as a result of hypoxia, can trigger

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signalling through A2A and A2B receptors on macrophages, both of which augment the differentiation and functional capabilities of immunosuppressive macrophages as well as attenuate the cytokine release of pro-inflammatory macrophages in vitro[96,175]. Elevated lactic acid in culture has been shown to promote the M2 phenotype, increasing ARG1 expression and polyamine-dependent immunosuppression[176]. High glycolytic rates in triple-negative breast cancer were shown to promote MDSCs, whereas restricting glycolysis in these cancer cells inhibited granulocyte colony-stimulating factor and granulocyte–macrophage colony-stimulating factor secretion from cancer cells and limited MDSC development[177]. Interestingly, hypoxia skewed MDSCs towards an immunosuppressive, M2-like TAM phenotype compared with normoxic MDSCs in vitro. This occurred via HIF-1α mechanisms, as *Hif1a* knockout MDSCs displayed increased tumour growth compared with wild-type MDSCs in mouse melanoma tumour models[178].

Exploiting differential metabolic plasticity

Whereas the activation, proliferation and function of T_{eff} cells can be attenuated through inhibiting numerous metabolic pathways, other attributes, such as long-term viability or effector function upon restimulation, may be enhanced. Although inhibiting glycolytic metabolism with 2-DG inhibited T_{eff} cell generation, it also conditioned T cells towards a long-lived, memory-like phenotype[50]. Interestingly, blocking glycolysis during ex vivo T cell activation and expansion, before reinfusion for tumour treatment, not only allowed for increased survival of antitumour T cells but also for improved cytokine production and cytotoxicity. Similar phenomena have been reported in $CD8^+$ T_{eff} cells in response to AKT inhibition, glutamine blockade, hypoxia, arginine supplementation and potassium supplementation[16,63,74,93, 111,179]. It may be possible to differentially affect cancer and the immune response. For example, acetate metabolism can rescue T cell function in glucose-restricted $CD8^+T_{eff}$ cells[87]. In addition, our group recently demonstrated the importance of this pathway in maintaining metabolic homeostasis in $CD8^+$ T cells undergoing glutamine blockade[16]. These findings could imply a generalizable therapeutic strategy, in that blocking the use of typical metabolic fuels, such as glucose or glutamine, may render some cancers metabolically compromised, but may leave antitumour T cells metabolically intact and functional given their ability to use alternative sources, such as acetate. Although specific metabolic interventions may be introduced pharmacologically as adjuncts to checkpoint blockade (TAbLe 2), these targets may be particularly applicable to CAR T cell therapy, wherein manipulation of metabolic pathways can be precisely defined through genetic means (box 2). Future studies delineating the degree of metabolic flexibility possible within a given immune cell subset and functional capacity are clearly warranted.

Checkpoint blockade and immunometabolism.

It is of great interest to define both the metabolic consequences of checkpoint therapy and the metabolic determinants of response. Checkpoint signalling has been shown to regulate metabolism in several studies. For example, PDL1 expression on cancer cells can drive Akt–mTOR activation and glycolysis in cancer cells, increasing glucose uptake and augmenting competition with T cells for glucose[14]. CD155-TIGIT signalling in T cells from human gastric cancer tissue dampened glucose uptake, lactate production and expression of

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glycolytic enzymes GLUT1 and HK2[180]. Conversely, agonism of the co-stimulatory pathway GITR broadly increased T cell metabolic activity and proliferation compared with isotype-treated control T cells[181]. Lastly, in vitro PD1 and CTLA4 signalling on activated human T cells suppressed metabolic pathways, such as aerobic glycolysis, that are associated with T cell activation[182]. To this end, the prospect of combining metabolic inhibitors (TAbLe 2) with checkpoint inhibitors holds promise to enhance the efficacy of checkpoint blockade. Targeting tumour metabolism by inhibiting glutamine metabolism in mouse models inhibited tumour growth and conditioned the TME to be more hospitable for antitumour effector cells[16]. Also, metabolically reprogramming T cells to make them more robust, long-lasting memory cells might improve their response to checkpoint inhibitors. This has been heralded by recent clinical trials combining the anti-folate pemetrexed with anti-PDL1 immune checkpoint blockade[183]. In addition to having direct antitumour effects, pemetrexed treatment enhanced the metabolic fitness and effector function of antitumour $CD8^+$ T cells, as well as induced immunologic cell death of cancer cells to trigger the immune response.

Conclusion and perspective

Although much of the foundation of immunometabolism has been informed by observations of cancer metabolism, it is clear that there are distinct differences between cancer and immunologic metabolic reprogramming. These differences provide opportunities to target metabolism as a means of enhancing the efficacy of immunotherapy (Fig. 3). Such an approach can be achieved through numerous different strategies. These include targeting tumourmetabolic programmes to inhibit growth and alter the TME, targeting the metabolism of suppressive immune cells to inhibit their function and targeting effector cell metabolism to enhance tumour killing. Likewise, ex vivo pharmacologic or genetic reprogramming of T cell metabolic pathways prior to adoptive cellular therapy offers an opportunity to dramatically engineer enhanced features, which may include longevity or enhanced effector function (bOX 2).

Future work should begin to focus on the metabolic interdependence of immune cells and cancer cells within the TME. In addition to nutrient depletion and the generation of metabolites that can suppress the immune response at certain concentrations, cancer cells can engage in metabolic crosstalk with other cells within the TME, wherein metabolic programmes can be induced and co-opted to benefit malignant progression. It has been reported that pancreatic stellate cells can provide alanine to cancer cells and, thus, fuel proliferation[184], and bone marrow stromal cells have been reported to provide cysteine to promote survival of chronic lymphocytic leukaemia cells[185]. In another report, ammonia from cancer cell glutamine metabolism diffused through the TME and triggered autophagy in cancer-associated fibroblasts, which in turn provided protein breakdown products, such as glutamine itself, to further support cancer cell metabolism[186]. It will be important to understand whether and by what mechanism immune-evading cancers may be co-opting the metabolic machinery of immune cells and benefitting from their remarkable metabolic flexibility.

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Oxidative phosphorylation (OXPHOS).

A highly efficient form of cellular respiration synthesizing ATP from the phosphorylation of ADP using electrochemical potential energy generated by the transfer of electrons from NADH or $FADH_2$ to oxygen through a series of mitochondrial electron carriers.

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Immune checkpoint pathways

Pathways mediated by cell surface proteins on immune cells, such as PD1 or CTLA4, that serve to suppress the immune response, which can be activated by ligands within the tumour microenvironment or draining lymph nodes.

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Chimeric antigen receptor T cells (CAR T cells).

T cells harvested from a patien's blood and genetically modified to express a special receptor that can recognize and respond to specific, predefined molecular targets on tumour cells.

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Hexosamine biosynthesis pathway (HbP).

A branch of glycolysis that generates building blocks used for glycosylation of proteins and lipids.

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Pentose phosphate pathway (PPP).

A metabolic branch of glycolysis generating NADPH, used for fatty acid synthesis and redox homeostasis, and 5-carbon sugars used in nucleotide synthesis.

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Nuclear factor of activated T cells (NFAT).

A calcium-dependent transcription factor activated in response to T cell receptor stimulation. Cooperation with the AP-1 transcription factor results in a productive immune response and transcription of pro-inflammatory cytokines, such as iL-2 and interferon-γ.

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Cataplerosis

The loss of metabolic intermediates in a metabolic pathway (particularly the tricarboxylic acid cycle) owing to consumption or degradation.

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Anaplerosis

The process of replenishing intermediates of the tricarboxylic acid cycle to support biosynthesis.

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De novo lipid synthesis

The cellular biosynthesis of fatty acids, triglycerides, cholesterol and other lipids from carbohydrates or other non-lipid precursors.

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2-Oxoglutarate-dependent dioxygenases (2OgDD).

A family of enzymes that catalyse the hydroxylation of macromolecules, often as a prerequisite to demethylation, reliant on α-ketoglutarate, Fe^{2+}, ascorbate and oxygen as cofactors.

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Major histocompatibility complex (MHC).

MHC class i (MHC-i) is expressed on all nucleated cells, a molecular complex presenting intracellular peptide epitopes for $CD8^+$ T cell receptor recognition. Also expressed on antigen-presenting cells, allowing initial antigen-specific activation of cytotoxic $CD8^+$ T cells. MHC-ii is highly expressed on antigen-presenting cells for presenting antigenic epitopes for $CD4^+$ T cell receptor recognition and activation.

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Antigen cross-presentation

The ability of antigen-presenting cells to process extracellular antigens and present them to $CD8^+$ T cells through major histocompatibility complex class 1 presentation.

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Box 1 |

Metabolism of T cell exhaustion

$CD8^+$ T cells in the tumour microenvironment (TME) can adopt a state of functional exhaustion wherein they are poorly proliferative and unable to generate sufficient cytotoxicity against target cancer cells. A similar cell subset exists during chronic viral infections, such as lymphocytic choriomeningitis virus (LCMV) clone 13 in mice or hepatitis C virus in humans[243]. There are numerous metabolic features that are emerging as characteristic of this set of immune cells, termed $CD8^+$ exhausted T cells. Some of the metabolic characteristics appear to be a consequence of co-inhibitory signalling, such as PD1, which is highly characteristic of $CD8^+$ exhausted T cells. In a chronic LCMV mouse model, PD1 signalling inhibited the expression of peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1α), which disrupted mitochondrial and effector function and led to significantly less oxidative capacity compared with T cells responding to an acute LCMV infection[244]. Overexpression of PGC1α in adoptively transferred T cells improved mitochondrial function and restored T cell function. T cells infiltrating tumours showed similar mitochondrial dysfunction and loss of oxidative capacity secondary to inhibited PGC1α activity[57]. Interestingly, antitumour T cells also regained function through overexpression of PGC1α, implying that the activity of a metabolic programme can, in and of itself, overcome functional T cell exhaustion. PD1 signalling also suppressed mTOR complex 1 (mTORC1) signalling and glycolytic activity in infiltrating $CD8^+$ T cells in a progressive mouse tumour model[14]. Given the dependence of T cell function (and loss of function) on metabolic programming, more studies are needed to assess the determinants of metabolic dysfunction and associated T cell exhaustion within the TME.

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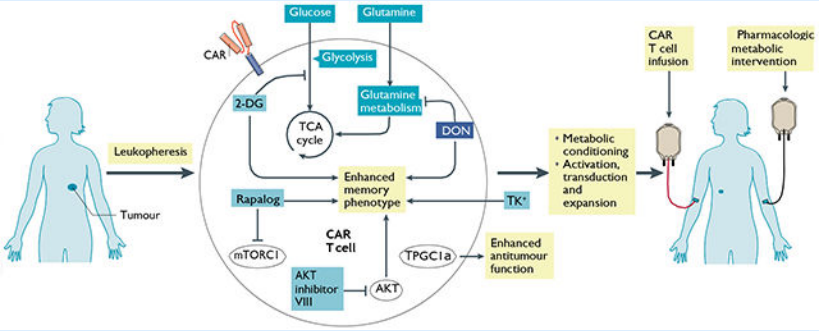
Box 2 |

Adoptive T cell therapies enable highly flexible approaches to metabolic therapy through ex vivo culture conditioning or genetic targeting of metabolic processes

T cells in such regimens can be genetically modified to express chimeric antigen receptors (CARs) that recognize known tumour surface antigens and trigger T cell receptor signalling in the absence of major histocompatibility complex presentation. T cells can be metabolically conditioned through the use of chemical inhibitors or genetic editing during ex vivo expansion, through metabolically engineered media or through pharmacologic treatment after T cell reinfusion. Many of the interventions discussed, including inhibiting glycolysis, glutamine metabolism with 6-diazo-5-oxo-l-norleucine (DON), AKT–mTOR signalling and potassium supplementation, have been used in ex vivo T cell expansion and led to improved T cell persistence and antitumour response[50,74,111,179] (see the figure). Recent studies demonstrated that forced expression of peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1α), which promotes mitochondrial biogenesis, in adoptively transferred $CD8^+$ T cells resulted in superior intratumoural metabolic and effector function[57]. Several groups have also reported the importance of the co-stimulatory receptor 4-1BB in positively conditioning mitochondrial health and biogenesis for robust antitumour immunity[245,246]. These findings have been validated in the CAR T cell field, wherein the addition of the 4-1BB receptor module has enhanced T cell persistence and increased therapeutic efficacy[247-251]. 2-DG, 2-deoxyglucose; TCA, tricarboxylic acid.



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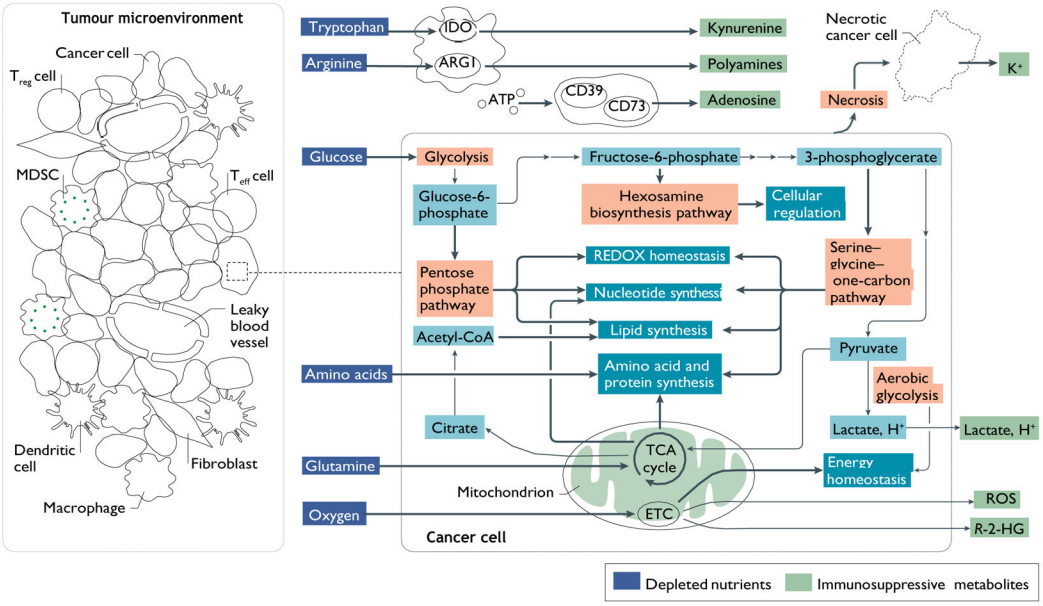

Fig. 1 |. Cancer cell metabolism and derangements in the TME.

Mitochondrial oxidation of nutrients, including glucose, amino acids and fatty acids, through the tricarboxylic acid (TCA) cycle and the electron transport chain (ETC) is a highly efficient means of producing energy for quiescent, differentiated cells. However, during periods of increased proliferation, such as after immune activation or malignant transformation, cells upregulate an alternative pathway for glucose metabolism, called aerobic glycolysis. Although less efficient in generating ATP, aerobic glycolysis allows for more rapid metabolism of glucose, efficient disposal of excess carbon and regeneration of NAD^+ while preserving mitochondrial enzymatic activity for anabolic processes[242]. Glycolytic intermediates are channelled through other essential pathways, such as the pentose phosphate pathway, the one-carbon pathway and the hexosamine biosynthesis pathway. These pathways support cellular processes that are critical for highly proliferative cells, such as synthesis of fatty acids and nucleic acids. Pathways for the metabolism of glutamine are also upregulated in the setting of increased proliferation[72]. In addition to supplying the TCA cycle with carbon skeletons that maintain intermediates for biosynthesis of amino acids, nucleic acids and fatty acids (a process known as anaplerosis), glutamine is the primary source of nitrogen used for amino acid and nucleic acid synthesis. These cells also upregulate a broad range of amino acid transporters and maintain tightly controlled redox balance, primarily through NADPH synthesis. Many cells within the tumour microenvironment (TME) express ectoenzymes, such as indoleamine 2,3-dioxygenase (IDO), arginase 1 (ARG1) and CD73, which deplete nutrients, as well as increase immunosuppressive metabolites, such as kynurenine and adenosine. Along with a deranged microvasculature, these metabolic adaptations can have profound effects on the metabolic make-up of the TME, leading to depletion of vital nutrients, hypoxia, acidosis and the generation of immune-toxic metabolites as shown. MDSC, myeloid-derived suppressor cell; *R*-2-HG, (*R*)-2-hydroxyglutarate; ROS, reactive oxygen species; T_{eff}, effector T; T_{reg}, regulatory T.

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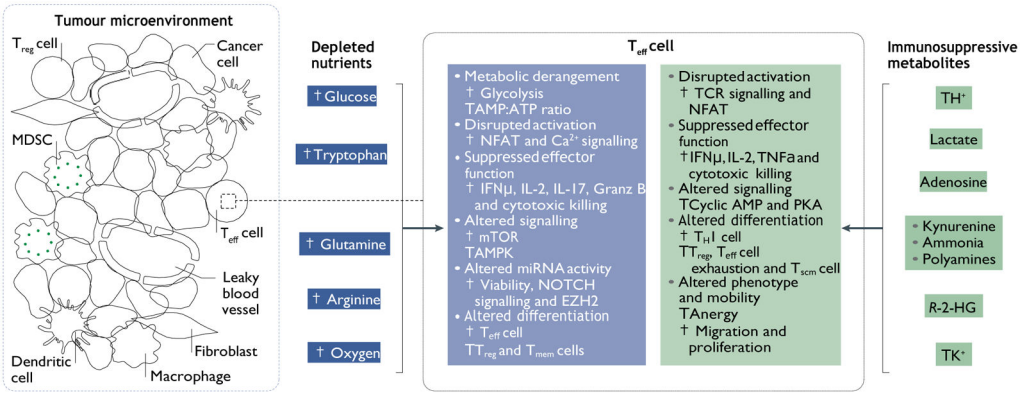

Fig. 2 |. Metabolic derangements in the TME inhibit T cell function.

The metabolic milieu of the tumour microenvironment (TME) is a reflection of cancer metabolic programmes. Nutrient deprivation, hypoxia and toxic metabolites are conditions within the TME that confront and influence T cell metabolism and function. The consequences of TME conditions on immune cell responses can be predicted based on a growing literature of preclinical, translational and clinical studies. AMPK, AMP kinase; EZH2, enhancer of zeste homologue 2; Granz B, granzyme B; IFNγ, interferon-γ; MDSC, myeloid-derived suppressor cell; miRNA, microRNA; NFAT, nuclear factor of activated T cells; PKA, protein kinase A; *R*-2-HG, (*R*)-2-hydroxyglutarate; TCR, T cell receptor; T_{eff}, effector T; T_H1, T helper 1; T_{mem}, memory T;T_{reg}, regulatory T;T_{scm}, stem cell memory T; TNF, tumour necrosis factor.

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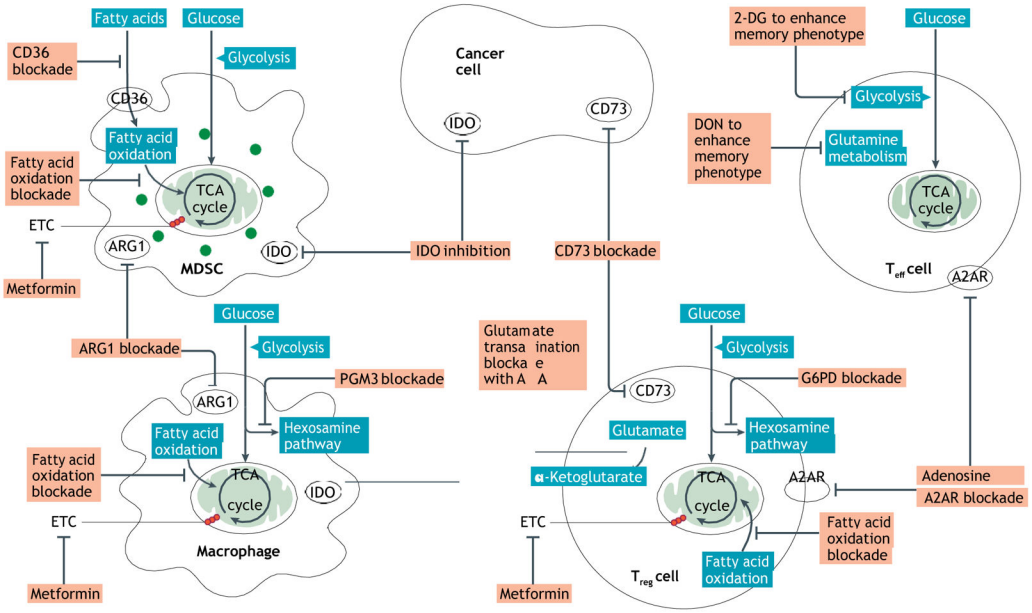

Fig. 3 |. Potential metabolic targets for enhancing immune response in cancer.

Using small molecules, monoclonal antibodies and genetic editing, metabolic processes can be targeted to either disable cancer and suppressive immune cell metabolism or, conversely, engage and support effector cell metabolism. Metabolic processes in suppressive immune populations and cancer cells can be targeted to directly decrease viability, as well as to disable metabolic pathways that deplete nutrients (for example, arginase 1 (ARG1) and indoleamine 2,3-dioxygenase (IDO)), lead to toxic metabolites (for example, lactate and CD73) or induce metabolic control of effector cell populations (for example, mutant IDH1 generation of the oncometabolite (R)-2-hydroxyglutarate (*R*-2-HG)). Metabolic interventions may also be able to induce beneficial changes in effector populations, such as increasing longevity and antigen-specific immunologic memory. A2AR, adenosine receptor subtype A2A; AOA, amino-oxyacetic acid; 2-DG, 2-deoxyglucose; DON, 6-diazo-5-oxo-l-norleucine; ETC, electron transport chain; G6PD, glucose-6-phosphate dehydrogenase; MDSC, myeloid-derived suppressor cell; PGM3, phosphoglucomutase; TCA, tricarboxylic acid; T_{eff}, effector T; T_{reg}, regulatory T.

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Table 1 |

Functional and metabolic phenotypes of immune cells within the TME

Cell type	Function	Metabolic phenotype
Immune activation or inflammatory		
NK cell	MHC-independent cytotoxicity: Perforin, granzymes FASL, TRAIL IFNγ, TNF	Glycolysis and OXPHOS
Inflammatory TAM	MHC-independent cytotoxicity: TNF, IL-1β Oxidative burst Antigen presentation	Glycolysis and PPP
DC	DAMP processing T_{eff} cell activation Antigen presentation	Glycolysis
T_{eff}cell	Antigen-specific cytotoxicity: Perforin, granzymes FASL IFNγ, TNF	Highly glycolytic and OXPHOS Amino acid metabolism (arginine, tryptophan, serine, leucine, glutamine, cysteine) PPP
T_{mem} Cell	Maintain long-lived response	OXPHOS
Immunosuppression		
MDSC	IL-10, TGFβ Amino acid depletion Polyamines, kynurenine	Glycolysis and OXPHOS
Immunosuppressive TAM	IL-10 Amino acid depletion Polyamines, kynurenine VEGF	OXPHOS, HBP
T_{reg}cell	IL-2 sequestration: Dampen APC co-stimulation IL-10, TGFβ Adenosine	OXPHOS

APC, antigen-presenting cell; DAMP, damage-associated molecular pattern; DC, dendritic cell; FASL, fas ligand; HBP, hexosamine biosynthesis pathway; IFNγ, interferon-γ; MDSC, myeloid-derived suppressor cell; MHC, major histocompatibility complex; NK, natural killer; OXPHOS, oxidative phosphorylation; PPP, pentose phosphate pathway; TAM, tumour-associated macrophage; TGFβ, transforming growth factor-β; T_{eff}, effector T; TME, tumour microenvironment; T_{mem}, memory T; TNF, tumour necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; T_{reg}, regulatory T; VEGF, vascular endothelial growth factor.

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Table 2 |

Metabolic inhibitors with potential for clinical translation

Pathway	Target	Representative drugs	Representative phase II/III clinical trials	Refs
Glycolysis	GLUT1	WZB117	NA	187
	HK	2-DG	NA	50
		3-Bromopyruvate	NA	188
	PFKFB3	3-(3-Pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3-PO), PFK158	NA	189
	GAPDH	Dimethyl fumarate (DMF)	Phase II[a]	190,191
		Heptelidic acid	NA	192
	PDHK1	Dichloroacetate (DCA)	Phase II	193,194
	LDHA	NCI-737, NCI-006	NA	195
FAO	CPT1A	Etomoxir	NA	196
		Perhexidine	NA	197
	CD36	ABT-511	Phase II	198,199
FAS	ACC1	Firsocostat	NA	200
Cholesterol esterification	ACAT	Pactimibe	NA	201
Electron transport and/or mitochondrial function	AMPK	Metformin	Phase II[a]	202-205
Glutaminolysis[b]	GLS	BPTES	NA	206
		CB-839	Phase II[a]	207,208
Glutamine metabolism	Glutamine requiring enzymes	DON	NA	16
		JHU083	NA	209
One-carbon metabolism	SHMT2	RZ-2994	NA	44
One-carbon metabolism	TS, DHFR, GARFT	Pemetrexed	Phase II[a] and phase III[a]	192,210,211
		Methotrexate	Phase II[a]	212-214
	TS	5-Fluorouracil	Phase III[a]	215-217
Reduction of ROS levels	Antioxidant	NAC	Phase II	218,219
Increase of ROS levels	GSH	Menadione	NA	220
Pentose phosphate pathway	G6PD	Polydatin	NA	221
Hexosamine biosynthetic pathway	PGM3	FR054	NA	222

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Pathway	Target	Representative drugs	Representative phase II/III clinical trials	Refs
Arginine pathway	ARG1	CB-1158	Phase II[a]	223-225
Arginine pathway	Pegylated arginine deiminase	ADI-PEG20	NA	226
IDO inhibitors	IDO	Epacadostat	Phase II[a]	227-229
		Indoximod	Phase II[a]	227,230,231
		Navoximod	NA	227
R-2-HG synthesis	Mutant IDH1	FT-2102	Phase II[a]	232-234
Adenosine pathway	CD73	Oleclumab	Phase II[a]	98,235-237
		BMS-986179	Phase II[a]	98,235,238
		NZV930, CPI-006	NA	98,235
	Adenosine receptor A2A	PBF-509	Phase II[a]	98,239
		CPI-444	Phase II[a]	98,240
		AZD4635	Phase II[a]	98,241

ACAT, acetyl-CoA acetyltransferase; ACC1, acetyl-CoA carboxylase; AMPK, AMP kinase; ARG1, arginase; CPT1A, carnitine palmitoyl transferase 1; 2-DG, 2-deoxyglucose; DHFR, dihydrofolate reductase; DON, 6-diazo-5-oxo-l-norleucine; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GARFT, glycinamide ribonucleotide formyltransferase; GLS, glutaminase; GLUT1, glucose transporter, type 1; G6PD, glucose-6-phosphate dehydrogenase; GSH, glutathione; HK, hexokinase; IDH1, isocitrate dehydrogenase; IDO, indoleamine 2,3-dioxygenase; LDHA, lactate dehydrogenase A; NA, not applicable; NAC, *N*-acetyl cysteine; PDHK1, pyruvate dehydrogenase kinase; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase; PGM3, phosphoglucomutase; *R*-2-HG, (*R*)-2-hydroxyglutarate; ROS, reactive oxygen species; SHMT2, serine hydroxymethyltransferase, mitochondrial; TS, thymidylate synthase.

[a]Denotes clinical trial in combination with established immunotherapy agents.

[b]The enzymatic catabolism of glutamine to generate tricarboxylic acid cycle intermediates.