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Tumor Microenvironment Metabolism as a Primordial Checkpoint in Antitumor T Cell Immunity

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KEY POINTS

- Tumor cells become metabolically deregulated to support their unrestrained proliferation.
- The type and degree of metabolic deregulation can be variable between patients and cancer types.
- T cells have considerable metabolic needs for activation and persistence.
- The metabolic landscape of the tumor microenvironment is detrimental to immune function.
- Different subsets of T cells have distinct metabolic requirements.
- Alterations of T cell and tumor cell metabolism can modulate immune activity and enhance immunotherapeutic response.

INTRODUCTION

It is now clear that the immune system is not oblivious to the initiation and progression of cancer and, in fact, can stimulate T cells with very high affinity for tumor-associated antigens. These T cells are capable of being re-invigorated through exogenous manipulations, such as blockade of co-inhibitory "checkpoint" molecules (like programmed death 1/PD-1), cytokine administration, oncolytic viruses, and vaccination, resulting in durable antitumor immunity and regression. However, the fact remains that the majority of patients that receive immunotherapies do not respond or receive little benefit.

The heterogeneity of patient responses and current lack of true predictive biomarkers, while frustrating, suggest that the resistance to immunotherapies like PD-1 blockade may not be due to alterations in treatment efficacy. These resistances may be due to patient-specific variabilities like single nucleotide polymorphisms (SNPs) in immune, inflammatory, or chemotactic genes or environmental-specific factors like obesity, age, or, more likely, tumor-specific variabilities.

The ability of tumor cells to continuously mutate and evolve in a Darwinian fashion underlies many of their more insidious traits: metastasis, altered differentiation of stromal tissue, and, indeed, immune evasion. As tumor cells evolve they can produce antigen-loss variants, become defective in antigen presentation, upregulate ligands for co-inhibitory receptors, secrete immunosuppressive cytokines like TGF-β and IL-10, induce T cell death, and recruit regulatory populations like regulatory T cells and myeloid-derived suppressor cells.[1] Many of these evolved traits are even further enhanced by contact with the immune system, for instance, the upregulation of PD-L1 in response to interferons.[2] However, none of these potential immune escape mechanisms fully explain the heterogeneity of patient responses, suggesting that other, more nonimmunologic mechanisms may be at play.

DEREGULATED METABOLISM AS A KEY HALLMARK OF TUMOR CELLS

Otto Heinrich Warburg was a German biochemist who made a number of seminal discoveries regarding carbohydrate metabolism in malignancy.[3] The one with which he is perhaps most well known was the demonstration that tumor cells fermented a heightened level of imported glucose into lactic acid rather than oxidize it in the mitochondria.[3] Lactic acid production in mammalian cells is generally a feedback effect, induced when oxygen is limited in the environment, but tumor cells were discovered to do this even in the presence of oxygen. This phenomenon was thus termed "aerobic glycolysis" or the "Warburg effect" and has been the subject of much study for several decades.[4] Respiration, the process by which pyruvate is converted into acetyl-CoA, driving the TCA cycle to produce reducing intermediates for oxidative

phosphorylation (OXPHOS)-mediated production of ATP, is commonly considered to be much more bioenergetically favorable as more ATP is produced per molecule of pyruvate. This has left many wondering why tumor cells would adapt this seemingly unfavorable metabolic phenotype. However, cytosolic fermentation of lactate through lactate dehydrogenase (LDH) has many considerable advantages, especially in a glucose-rich environment or, in the case of a tumor, a cell that outcompetes others for glucose.[4] First, LDH-mediated conversion of pyruvate to lactate results requires the donation of a proton from nicotinamide adenine dinucleotide hydrogen (NADH) stores, thus regenerating NAD^+ in the cytosol. Second, while, indeed, aerobic glycolysis does produce far less ATP per molecule of glucose than OXPHOS, kinetic studies have revealed that the aerobic glycolysis reaction takes place almost 100 times faster than that of TCA coupled to OXPHOS.[5] Third, upregulation of aerobic glycolysis machinery might give the cell an initial competitive advantage if oxygen eventually does become limited, a common occurrence in the tumor microenvironment.[6] Finally, and perhaps most importantly, restricting ATP production to the cytosol allows mitochondrial function to be diverted into a more anabolic state, in which TCA cycle intermediates can be used for the production of biomass like amino acids, lipids, and nucleotides, rather than oxidized for ATP generation.[7] This prevents ROS-mediated mitochondrial damage during periods of intense proliferation, among many other sorts of oxidative damage.[8] Taken together, deregulated carbohydrate metabolism is considered to be a major and common phenotype of cancer cells.

However, this is not to say that mitochondrial activity is *suppressed* in tumor cells. While many studies have focused on the bioenergetic fate of glucose, tumor cells, of course, also upregulate multiple other metabolic pathways to support their unrestrained proliferation. Amino acid uptake is increased; cancer cells upregulate several amino acid transporters and become highly dependent on glutaminolysis.[9,10] Tumor cells also become much more dependent on exogenous fatty acid uptake, as a significant proportion of their lipid metabolism is devoted to generation of new membranes.[11] This process is so highly upregulated that the cell represses activity of several desaturase enyzmes, rendering the cancer cell dependent on unsaturated fatty acids and causing a build-up of saturated fats in tumor cells and their microenvironment.[11]

Taken together, a wide variety of studies suggests that a major component of the phenotype of cancer is metabolic deregulation (Figure 46.1). While this has, of course, important implications for the cancer cells themselves, this metabolic state contributes to the generation of a local area of relatively dearth metabolic conditions and the formation of the tumor microenvironment.

METABOLIC FEATURES OF THE TUMOR MICROENVIRONMENT

As mentioned previously, the altered metabolism of tumor cells benefits the tumor in many ways. Intratumoral metabolic heterogeneity ensures that at least some part of the tumor will be successful and find a fuel source that it can use and deplete, while hypoxic regions can protect cancer stem cells and prevent terminal differentiation.[12,13] However, the local depletion of nutrients can have a wide-reaching effect on the microenvironment, including alterations in stromal cell metabolism, altered angiogenesis, and inhibition of tumor-infiltrating leukocyte function.

Cancer cells upregulate high levels of glucose transporters, especially GLUTs 1 and 3, as well as maintain these transmembrane proteins' trafficking to the cell surface.[13] Most cancer cells also upregulate several key rate-limiting enzymes in the glycolytic pathway, including several isoforms of hexokinase, phosphofructokinase, phosphoglycerate mutase, and pyruvate kinase M2.[12] Cancer cells also utilize glucose metabolites for nucleotide synthesis through the pentose phosphate pathway, as well as utilize glucose-derived carbon for the generation of fatty acids used in membrane synthesis.[4] It is this persistent hunger for glucose that enables the use of the FDG tracer for PET imaging. Thus, among all available fuel sources, glucose remains tumor cells' primary one and as such is present in extraordinarily low concentrations in the tumor microenvironment.

That dependence (and preference) for glucose and subsequent aerobic glycolysis also engender the tumor microenvironment with another metabolic feature. As pyruvate is converted into lactate, NADH is converted to NAD^+, which generates a proton. This proton is used to shuttle lactate across the plasma membrane through the monocarboxylate transporter (MCT), secreting the lactate and proton and acidifying the extracellular space.[14] Thus, the tumor microenvironment is markedly acidic. Apart from high levels of lactate ion (nearly 50 mM at tumor cores), studies utilizing pH biosensors or even more direct measurements (pH probes inserted into tumors) reveal, indeed, that the pH of the interstitial space in tumors can be as low as 6.5, endangering a considerable amount of extracellular chemistry and preventing uptake of molecules that are coupled to pH gradients.[15,16]

While tumor cells do perform glycolytic metabolism preferentially, a common myth is that glycolysis occurs at the expense of the mitochondria. However, it is likely more accurate that a heightened proportion of glucose gets fermented to lactate (55%–60%, by most measurements), and in fact, it is quite appropriate to say that tumor cells remain extraordinarily oxidative.[4] Indeed, tumor cells have high mitochondrial mass and perform significant levels of oxidative phosphorylation. Thus,

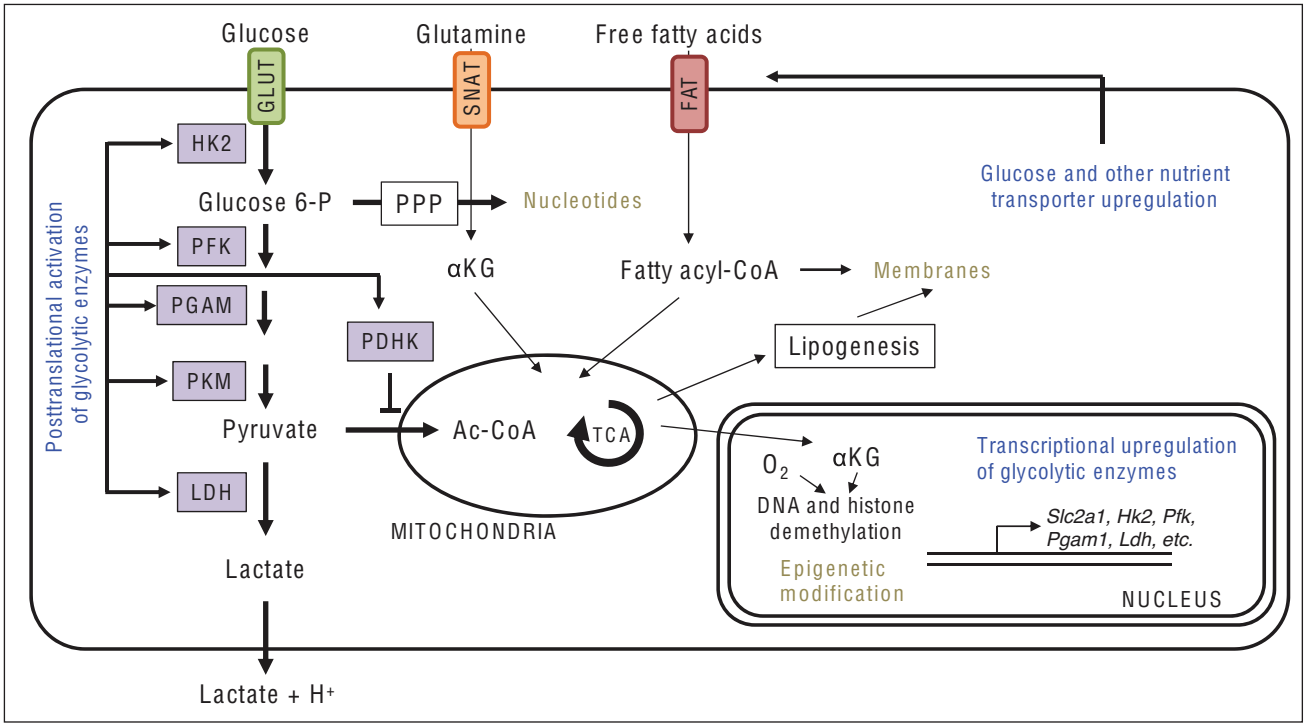

Figure 46.1 Deregulated metabolism as a common phenotype of cancer cells. Cancer cells develop several metabolic adaptations to support their unrestrained proliferation. Aerobic glycolysis is promoted through the transcriptional deregulation of glucose and nutrient transporters and several key glycolytic enzymes as noted. In addition, oncogenic signaling can promote post-translational activation of glycolytic enzymes as well. Tumor cells meet their metabolic needs by utilizing the pentose phosphate pathway (PPP) to generate nucleotides, generating membranes through lipogenesis, and making epigenetic changes like DNA and histone demethylation.

Ac-CoA, acetyl-CoA; aKG, alpha ketogluratate; FAT, fatty acid transporter; HK, hexokinase; LDH, lactate dehydrogenase; PDHK, pyruvate dehydrogenase kinase; PFK, phosphofructokinase; PGAM, phosphoglycerate mutase; PKM, pyruvate kinase M; SNAT, sodium-coupled neutral amino acid transporter; TCA, tricarboxylic acid cycle.

it is becoming clearer that oxygen, too, is an essential metabolite that is outcompeted by tumor cells. That, coupled to deregulated and tortuous angiogenesis, induced through aberrant VEGF signaling, results in areas of extreme hypoxia (1%–2% O_2),[17] far lower than typical hypoxia seen in other inflamed tissues, or in regions that have local hypoxia-like kidneys or bone marrow.

Amino acids represent another pool of essential metabolites that have altered levels in the tumor microenvironment. Glutamine, essential for tumor cell metabolism, is heavily depleted in the tumor microenvironment, whereas glutamate is observed at higher levels in the tumor.[18] Tryptophan and arginine are also depleted actively by both tumor cells and certain suppressive myeloid cell populations through indoleamine 2,3-dioxygenase (IDO) and arginase activity, respectively.[19] Importantly, not only do these suppressive enzymes deplete these critical amino acids from the environment, but the reaction products (tryptophan catabolites like kynurenine and arginine metabolites ornithine and urea) can be heavily immunosuppressive on their own.

T CELL ACTIVATION AND METABOLISM

Prior to recognition of their cognate antigen, naïve T cells must persist for a lifetime in a state of relative quiescence, really only dividing homeostatically when stroma-derived IL-7 signals build-up in the secondary lymphoid organs. These cells are small, having very little cytoplasm, extraordinarily condensed chromatin, and having no discernible function other than simply surviving. However, once a naïve T cell's TCR recognizes its antigen in the context of co-stimulation, a number of very important changes take place. Calcium and lipid-based second messengers activate nuclear factor of activated T cells (NFAT) and AP-1 to initiate transcription of activation-induced genes.[20] The cell rapidly enters a growth phase, synthesizing new membranes, organelles, and nucleotides to prepare for cell division.[7] Chromatin remodeling is initiated, allowing for rapid transcription and DNA replication.[21] And, after a period of around 24 hours, the cell begins undergoing extremely rapid proliferation, averaging cell cycles of around 4 to 6 hours.[22] After a number of divisions, the cell also begins secreting cytokines and, in the

case of $CD8^+$ T cells, forming cytotoxic granules that will be used to induce cell death in target cells.

This rapid shift in cellular functionality, from extreme quiescence to extreme activity, is not without cost. Synthesis of membranes requires new fatty acid synthesis. DNA replication requires nucleotide synthesis. Chromatin modification requires post-translational histone and DNA modifications by acetyl groups and other short-carbon chains. Cytokine and granule genes must be transcribed and translated. Cellular motility requires dynamic actin reorganization. Central to all of these processes is metabolism. As such, the bioenergetic demands of an activated, effector T cell are extraordinarily high.[7,22,23]

It was noted, before the cloning of the T cell receptor or MHC restriction, that phytohemaglutanin-stimulated lymphocytes changed the way they metabolized sugars. Even in cell culture with abundant oxygen, the lymphocytes would ferment glucose into lactic acid rather than oxidize it in the mitochondria.[24] While the importance of these pathways in cellular fate and function would not be fully recognized for another 30 years, this initial discovery, that T lymphocytes also performed Warburg metabolism upon activation, paved the way for an entire field of "immunometabolism" research.

METABOLIC REGULATION OF T CELL EFFECTOR FUNCTION AND FATE

Not long after the discovery of T cell glycolysis, several studies utilized the newly developed chromium release assay to measure metabolic control of T cell function, which revealed that while glucose was important for T cell proliferation, it was largely dispensable for T cell cytolysis.[25] However, the exploration of metabolism as a mediator of immune cell activity sat relatively dormant until advances in genetic and flow cytometric analysis would be able to answer some of these questions. Activated T cells upregulate glucose transporters, ensure surface trafficking of said transporters, and upregulate much of the glycolytic machinery, much of this through Akt activation.[26–30] Recent studies utilizing extracellular flux analysis have revealed just how important glycolysis is and that T cells begin diverting glucose to lactic acid production very rapidly upon activation.[31–33] The so-called switch to glycolysis is a multi-step process, orchestrated by molecules like Myc, HIF1a, Akt, mTOR, and the pyruvate carrier inhibitor PDHK1.[8]

Glycolysis in T cells has been shown to be important for many important T cell functions, not merely proliferation. T cells require glycolysis for calcium flux, effector T cell expansion, glycosylation of several signaling intermediates, and the avoidance of tolerogenic programs like anergy.[22,34] The notion that the "moonlighting" functions of many glycolytic enzymes as RNA binding proteins, known for many years in the cancer field, has important roles in the elaboration of effector cytokines has brought this metabolic pathway to the front and center of much of the focus in T cell biology.[35] Indeed, the dehydrogenase enzymes GAPDH and LDH have been shown to bind the 3′ UTR of cytokine mRNA and inhibit translation when metabolically inactive.[33,35] In this way, glycolysis enables the translation and synthesis of cytokine upon T cell activation.

Importantly, as in all cells that perform Warburg metabolism, it is important to remember that glycolysis does not proceed at the expense of mitochondrial metabolism, and, in fact, T cells upregulate OXPHOS pathways after activation as well. More recently, mitochondria have also been studied as not only energy producers but key nodes in cellular fate and function in lymphocytes. Mitochondrial metabolism is sufficient to maintain the survival of quiescent cells, a key point for naïve T cells, which prefer these pathways for their minimal activity and occasional homeostatic division.[31] As these naïve cells receive a homeostatic signal, specifically IL-7 stimulation, they upregulate the glucose transporter GLUT1 as a means to fuel that relatively minor expansion.[36,37] However, after an effector response, T cells enter a memory phase, during which they contract back into quiescence, but are prepared, both quantitatively and qualitatively, to respond again with vigor.[31] Interestingly, T cells shift their metabolic preferences during this memory phase, back from aerobic glycolysis to more OXPHOS-mediated events.[7] Importantly, during the memory transition, T cells also upregulate mitochondrial capacity, such that memory T cells have more and "better" mitochondria.[31] This is thought to bioenergetically "prime" them for reactivation such that they are ready to enter the effector phase upon re-exposure to antigen. This also confers longevity and stemness to this memory T cell. Thus, both mitochondrial and nonmitochondrial energy production are inherently important to all phases of the T cell immune response.

NUTRIENT SENSING IN CONTROL OF T CELL FATE AND FUNCTION

Every somatic cell has some form of nutrient-sensing mechanism. This is important for almost all cellular activity: a cell does not want to translate protein, replicate DNA, make membrane, and divide if there are not sufficient nutrients in the environment to do so. However, with a few notable exceptions, most somatic cells can afford to be lost; a fibroblast will be replaced by its neighbor, and a neutrophil has billions of brethren waiting to be deployed. However, even at the naïve state, a T cell represents the product of a number of life or death decisions that have generated a functional T

cell receptor that is specific for non-self peptide with self MHC. A lot of energy has gone into making that clone, and the immune system does not want to lose it due to some perturbations in nutrient availability. Thus, during evolution, T cells have conscripted the nutrient-sensing machinery to make more than simply growth and death decisions and instead have utilized nutrient sensors to dictate complex fate decisions.[38]

In addition to the energetic studies of lymphocytes in the 1970s, the discovery of the macrolide antibiotic rapamycin and its pharmacologic target mTOR had a major impact in the field of immunometabolism.[39,40] Mechanistically, rapamycin binding to FKBP12 promotes the dissociation of mTOR and raptor, one of its adaptor proteins. Biochemical analysis of mTOR in rapamycin-treated cells also revealed the existence of a distinct second complex.[41] Thus, mTOR signaling can occur through two protein complexes, mTORC1 and mTORC2. mTOR acts as a nutrient sensor in most cells, tying together signals from a diverse array of extracellular and intracellular signaling pathways, including energy charge, insulin, cytokines, lipid intermediates, and activation signals. mTOR's level of activation then dictates, through downstream substrates, whether cells will translate protein, initiate ribosome biogenesis, engage lipolysis pathways, or activate the autophagic mechanisms of the cell.[38]

Although a poor antifungal agent, it was soon noted after its discovery that rapamycin was a potent immunosuppressive molecule.[42] However, unlike other potent immunosuppressant molecules like cyclosporine A/FK506, the effects of rapamycin were not acute: they did not result in inhibition of T cell activation, but rather promoted a long-term state of tolerance.[43] Thus, rapamycin and its derivatives are now commonly used to promote graft tolerance and have been shown to promote long-term bone marrow chimerism.[44–46]

Inhibition of mTOR by rapamycin during activation results in anergy, a hyporesponsive state induced when T cells see antigen in the absence of co-stimulatory context.[47] This led many to believe that mTOR may function as a signal integrator for co-stimulation. Genetic deletion of mTOR in T cells, however, revealed that T cells require mTOR activation as a third signal to escape from quiescence and acquire an effector phenotype. $CD4^+$ T cells stimulated in the presence of high doses of rapamycin or when mTOR has been deleted acquire a regulatory phenotype, becoming potently suppressive and expressing Foxp3. Thus, mTOR plays a role in acquiring an effector phenotype.[48]

Interestingly, though, is that mTOR inhibition, like most pathways involved in metabolism, is not merely a switch, and since its discovery has been shown to have a complex role in immune cell fate and function. In 2008, Ahmed and colleagues described a role for mTOR in the effector versus memory response of $CD8^+$ T cells.[49] Interestingly, when mice were treated with very low doses of rapamycin during acute infection, they generated a superior memory response. This is consistent with the idea that at these low doses, mTORC1 is targeted, while mTORC2 is spared.[50] However, mTOR must be dynamically regulated to achieve effector fates, as genetic evidence has shown that, indeed, while mTORC1 deletion in $CD8^+$ T cells results in a poor effector response and enhanced memory differentiation, those memory cells require mTOR to re-engage a recall response.[51]

Deletion of the specific mTOR complexes indeed has shown that mTORC1 and mTORC2 have dynamic regulation of $CD4^+$ T cell fate, such that mTORC2 is dispensable for Th1 and Th17 differentiation.[50] mTORC1, required for inflammatory Th1 and Th17 cells, is dispensable for generation of type 2 immunity.[50] Only through inhibition of both complexes does regulatory T cell differentiation occur.[50]

While mTOR is a dominant nutrient-sensing kinase in T cells, other nutrient sensors play important roles in T cell fate and function. Myc, a transcription factor associated with metabolic reprogramming and glycolysis, is dynamically regulated upon T cell activation and licenses glycolysis and glutaminolysis to occur.[52] It coordinates with mTOR and HIF1α to reprogram T cells for that short-lived effector metabolism associated with rapid proliferation.[52] AMPK, a sensor for energy charge (AMP/ATP balance) in cells, acts to negatively regulate the mTOR machinery as well as program mitochondrial biogenesis and oxidative metabolism.[53] AMPK-deficient T cells make poor memory and regulatory cells, suggesting AMPK acts as a balance to mTOR.[53] Taken together, the wealth of immunologic data on these critical kinases suggests that nutrient sensing not only acutely controls activation and metabolism in immunity but can have long-term effects on T cell function.

T CELL HYPORESPONSIVE PHENOTYPES AND THEIR METABOLIC LINKS

There are many ways in which T cells can be rendered hyporesponsive, probably more than we can adequately identify and measure. In many cases, T cell hyporesponsiveness is a desired trait; a T cell that has escaped central tolerance responds to some self-antigen in the periphery. As it avoids deletion, it still may be a useful clone if a pathogen shares that epitope, but the body does not want to risk autoimmune damage, so the T cell has cell-intrinsic programming to self-regulate: this is referred to as anergy.[22]

Clonal anergy was originally described by Jenkins and Schwartz as a means by which T cells might be rendered inert by self-peptide.[54] TCR ligation occurring in the absence of co-stimulation (canonically CD28 signaling)

results in a transcriptional program driven, in part, by NFAT in the absence of AP-1.[55] This program activates negative regulators of T cell signaling, represses metabolic machinery, and inhibits IL-2 translation.[56]

Another form of T cell dysfunction is senescence, which can occur from chronic signaling as well as in aging. Senescent T cells lose their reactivity to the TCR, downregulate co-stimulatory molecules, and have short telomeres.[57] Importantly, these T cells do not necessarily fail to function but rather lose sensitivity to the TCR and can secrete low-level cytokines in a more continuous fashion.

Probably the most "pathologic" of these hyporesponsive phenotypes is one driven not by lack of signaling but through persistent inflammatory signaling. Originally described in chronic viral infection,[58] T cell exhaustion results in a failure to secrete cytokines, proliferate effectively, or lyse target cells.[59] Exhaustion has been extensively studied in the mouse in the lymphochloriomeningitis virus model, but it has become increasingly apparent that the persistent activation associated with cancer also promotes an exhausted phenotype.[60] These studies in T cell exhaustion revealed that as T cells become chronically stimulated, they upregulate co-inhibitory checkpoint molecules like PD-1, LAG-3, and TIM-3, which act both as markers of chronic activation and also inhibitors of T cell activation.[59] Blockade of these molecules or their ligands can reinvigorate T cells in cancer and chronic viral infection.[61,62] Importantly, though, these inhibitory receptors do not outright *cause* T cell exhaustion; T cells deficient in PD-1, for example, still develop an exhausted phenotype.[63] Rather, there are basic processes that underlie T cell exhaustion and PD-1, and other co-inhibitory molecules may simply enforce the phenotype.

Interestingly, these phenotypes of T cell hyporesponsiveness, while having alternative initiating events, have similar metabolic characteristics.[22] Anergic T cells, despite being previously activated, fail to upregulate the metabolic machinery associated with effector T cells—glucose, iron, and amino acid transporters—and demonstrate lower glycolytic output.[64] Senescent T cells have low-level glycolysis continuously, consistent with their lack of TCR reactivity and their constant low-level cytokine production.[57,65] This may be induced by mitochondrial dysfunction, as *Tfam* deficiency can promote T cell senescence in mouse models.[66] The metabolic underpinnings of T cell exhaustion have been most heavily studied in recent years. Several groups have shown exhausted T cells have impaired glucose metabolism and oxidative function, and repressed mitochondrial activity and capacity.[67–69] Notably, more recent data suggest that mitochondrial dysfunction, induced through a number of pathologic signals, likely causes the exhausted T cell phenotype.[70,71] Thus, these data strongly suggest that metabolism plays a key and central role in T cell function and dysfunction. To truly harness the immune response to cancer, we must identify and mitigate these metabolic checkpoints to allow for unrestrained immunity in the tumor microenvironment.

IMPLICATIONS FOR EFFECTIVE ANTITUMOR IMMUNITY AND IMPROVEMENTS IN IMMUNOTHERAPY

Having understood that the metabolism plays a key and central role in T cell fate, function, and dysfunction, how do these pathways intersect when T cells infiltrate the tumor microenvironment and attempt to carry out an antitumor immune response?

A major driver of this type of "metabolic exhaustion" is competition. T cell metabolic uptake and downstream function, while highly upregulated, are not *de*regulated.[72] Tumor cells are larger, express higher levels of most metabolite transporters, and thus, in most competitive assays, will actively sequester most usable carbon sources. Thus, the energetic potential to carry out an immune response represents another, more primordial type of checkpoint that T cells must overcome in order to effectively carry out an immune response. This has been shown in a number of ways, as those interested in T cell metabolism began applying that study to the tumor microenvironment. First, tumors resistant to immunotherapy tend to take up more glucose, while sensitive tumor models are more metabolically quiescent.[73] T cells that infiltrate tumors cannot compete for glucose, and this loss of glycolytic function can inhibit calcium signaling and subsequent effector function.[74] Extracellular flux analysis has enabled these analyses directly from patient samples, identifying that oxidative metabolism and subsequent generation of hypoxia play a critical role in resistance to anti-PD1 immunotherapy.[75] Additionally, anti-PDL1 treatment of responding tumors can also act to alter the glycolytic function of tumor cells, which suggests that PD-1 blockade works, in part, by altering metabolic competition.[73]

However, in addition to metabolic competition in situ, the very nature of T cell dysfunction in tumor responses may also metabolically cripple the T cell. A T cell has no context when it is responding to antigen; it is merely integrating signals from the environment.[76] As such, it has no sense of the duration, scope, or persistence of activation signals. This is thought to be a major driver of T cell exhaustion in chronic viral infections, and similar phenotypes can be found in cancer cells, especially those of high affinity for tumor antigens.[77–79] As activation drives glycolysis, and the cessation of activation signals promotes mitochondrial biogenesis and activation, an antitumor response, by its persistent nature, actively represses

mitochondrial function, which could allow for metabolic plasticity in the tumor microenvironment and upregulates the machinery required to use glycolysis, requiring a fuel which is in the lowest supply.[73] It has been shown that this is indeed the case; tumor-specific T cells in the tumor microenvironment actively repress mitochondrial biogenesis and show decreases in mitochondrial activity and mass, creating a dependence on glycolysis.[68] This is dependent on chronic Akt signaling, which drives down the expression of the mitochondrial biogenesis factor PGC1α.[68] Indeed, it is persistent signaling that alters the metabolic plasticity of T cells, which creates metabolic vulnerabilities and the generation of dysfunctional mitochondria that produce ROS.[69–71] Antioxidant approaches can alleviate T cell exhaustion and promote responses to immunotherapy. Similar results have been found in chronic viral infection, suggesting that there are at least two metabolic checkpoints to overcome: competition in the microenvironment as well as T cell-intrinsic metabolic insufficiency (Figure 46.2).[67]

Not all T cells are functionally crippled in the tumor microenvironment, most notably Foxp3-expressing regulatory T cells (T_{reg} cells). T_{reg} cells are extremely active in cancer, being highly overrepresented in tumors but also being highly proliferative. Thus, T_{reg} cells may possess metabolic proclivities that allow them to thrive within tumors. Indeed, T_{reg} cells eschew glucose metabolism in favor of other sources of carbon, rendering them insensitive to the metabolic insufficiencies in the tumor microenvironment.[80,81] T_{reg} cells have been shown to rely both on fatty acid sources as well as metabolic byproducts like lactic acid, which allow them to thrive in the tumor microenvironment.[82,83] In this way, tumors evade immune destruction not only by starving antitumor immunity but also by feeding suppressor populations.

How can we overcome these metabolic checkpoints to improve cancer therapy? Do more precise ways exist to hinder the metabolism of tumor cells or bolster the metabolism of T cells in a specific manner? Can you tip the energetic balance in favor of antitumor immunity?

As one of the major drivers of metabolic inhibition in the tumor microenvironment is competition, one could envision a scenario in which tumor cell metabolism is targeted. Previous clinical attempts at this have not been successful, as many of these metabolic inhibitors also affect other cells: stromal cells, vasculature, and immune cells. Thus, these therapies can sometimes end up being a zero-sum game. However, advances in understanding the pharmacodynamics of certain inhibitors as well as specific tumor cell targeting mechanisms may reinvigorate some of these strategies. First, as the tumor cell outcompetes other cells for nearly every other substrate, any drug that requires transport rather than passive diffusion is likely to affect the tumor cell first as well as more potently. For instance, it has been demonstrated that the mitochondrial complex I inhibitor metformin can synergize and enable checkpoint blockade immunotherapy in murine models.[84] Second, direct targeting to the tumor cell may be a strategy for delivering metabolic inhibition: this could be done through antibody targeting strategies, tumor-specific moieties, or even through more complex approaches like oncolytic, tumor-targeting viruses. Indeed, oncolytic viruses, as they infect tumor cells, can be engineered to deliver genetic cargo (the FDA-approved T-VEC, for instance, also encodes GM-CSF). Indeed, this genetic cargo can be metabolic

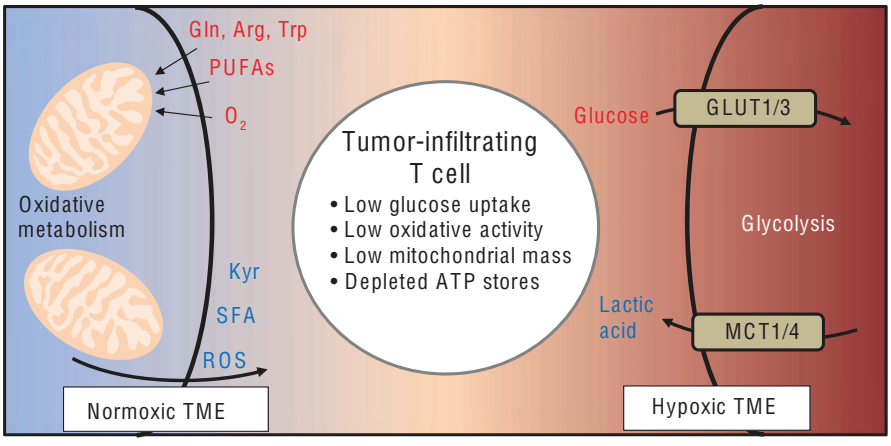

Figure 46.2 The tumor microenvironment imposes metabolic checkpoints on tumor-infiltrating T cells. Whether individual areas of a tumor are normoxic (left) or hypoxic (right), the deregulated metabolism of the tumor cells and alterations to surrounding stroma create metabolic competition for the T cell. This can have long-term inhibitory effects on T cell fate, as well as immediately inhibit T cell function.

ATP, adenosine triphosphate; Gln, Arg, Trp, glutamine, arginine, tryptophan; GLUT, glucose transporter; Kyr, kynurenine; MCT, monocarboxylate transporter; PUFAs, polyunsaturated fatty acids; ROS, reactive oxygen species; SFA, saturated fatty acids; TME, tumor microenvironment.

in nature, and encoding the gene for leptin in an oncolytic virus can dramatically reprogram the metabolism of tumor-infiltrating T cells.[85]

Of course, another way to alleviate these metabolic checkpoints would be to metabolically reprogram the T cell itself. This might not only repair cell-intrinsic defects but also arm the T cell to be more metabolically fit in the nutrient-poor microenvironment. Bolstering mitochondrial metabolism through PGC1α-mediated metabolic reprogramming results in superior antitumor function,[68,86] similar to studies using PCK1-mediated reprogramming done by the Kaech group.[74] Chimeric antigen receptor T cells, which are virally redirected to the tumor site, seem like the first and most obvious application of this type of amplification, although drugs designed at bolstering mitochondrial metabolism, in general, might synergize well with other types of immunotherapy in vivo. Further, we have learned that engaging lost co-stimulatory pathways, like the TNFR family member 4-1BB, can promote mitochondrial biogenesis and enable T cell responses.[87,88] Importantly, understanding the defects in these tumor-infiltrating T cells may allow us to also harvest and culture them more effectively ex vivo, resulting in a superior T cell productive for adoptive TIL therapy.

CONCLUSION

The intersection of metabolism and bioenergetics with immunity has garnered much recent interest. It is now clear from work done in metabolic pathway regulation that T cells have extraordinary metabolic needs and utilize nutrient sensors to divert and shape effective immunity for the host. However, these links are not trivial nor academic in nature: T cell function can be inhibited or improved through modulation of metabolism. As T cells enter the tumor microenvironment, chronic activation and inflammation drive them to engage an unsustainable immune response: There is simply not enough fuel in the environment to feed their function, at least with how they are programmed at baseline. Strategies to remodel the environment or bioenergetically arm the T cell have the potential to not only improve existing immunotherapies but to evolve into new therapies for the treatment of cancer.

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Only key references appear in the print edition. The full reference list appears in the digital product on Springer Publishing Connect: connect.springerpub.com/content/book/978-0-8261-3743-2/part/part03/chapter/ch46

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