Cancer Immunology Research

Filling the Tank: Keeping Antitumor T Cells Metabolically Fit for the Long Haul

Greg M. Delgoffe[1,2]

Abstract

Discoveries in tumor immunology and subsequent clinical advances in cancer immunotherapy have revealed that the immune system is not oblivious to tumor progression but heavily interacts with developing neoplasia and malignancy. A major factor preventing immune destruction is the establishment of a highly immunosuppressive tumor microenvironment (TME), which provides architecture to the tumor, supports indirect means of immunosuppression such as the recruitment of tolerogenic cells like regulatory T cells and myeloid-derived suppressor cells (MDSC), and represents a zone of metabolically dearth conditions. T-cell activation and consequent effector function are cellular states characterized by extreme metabolic demands, and activation in the context of insufficient metabolic substrates results in anergy or regulatory differentiation. Thus, T cells must endure both immunosuppression (co-inhibitory molecule ligation, regulatory T cells, and suppressive cytokines) but also a sort of metabolic suppression in the TME. Here I will review the general features of the TME, identify the metabolic demands of activated effector T cells, discuss the known metabolic checkpoints associated with intratumoral T cells, and propose strategies for generating superior antitumor T cells, whether *in vitro* for adoptive cell therapy or through *in vivo* reinvigoration of the existing immune response. *Cancer Immunol Res; 4(12); 1001–6. ©2016 AACR.*

Checkpoint Blockade and Adoptive Cell Therapies for Cancer Treatment

The immune system has the ability to remove specifically targeted cells with extreme precision, a feature that is very desirable for cancer therapy, as most traditional therapies fail to remove "every last cell." Although it was long thought that cancer represented a disease state that was "too" self and thus progressed outside of immune control, it is now clear that clinically apparent cancer is likely the product of a failure of surveillance and early elimination of neoplasia by the immune system (1).

For many years, researchers and clinicians sought to reinvigorate or supplement immunity to cancer, using vaccines or cytokine therapy, understanding and utilizing what was known of immune regulation and evasion at the time. Understanding the negative regulation of the immune response opened the door for blockade of these co-inhibitory checkpoints, the first of which was the blockade of the B7 ligand CTLA-4. A co-inhibitory molecule acting as an antagonist to CD28 signaling, CTLA-4 represents a molecule that, when blocked, enhances T-cell priming (2). Its blockade via the monoclonal antibody ipilimumab results in an unleashing of the immune response, resulting in durable antitumor responses in melanoma (3). Once researchers revealed that T-cell inhibition likely occurs locally in the tissues, as well as at the level of priming, it became possible to more directly enhance tumor-infiltrating T cells.

PD-1, a co-inhibitory checkpoint receptor upregulated upon activation and ligated in the tissues, now represents a key target in cancer immunotherapy (4). The PD-1 axis is a co-inhibitory interaction that occurs between T cells and tumors or those other cells that the tumor has conscripted (4). Tumor cells sense immune activation, in part via IFNγ and other microenvironmental factors, and upregulate PD-L1 as a means to inhibit immune destruction (5, 6). Blockade of PD-1 signaling through monoclonal antibodies like nivolumab and pembrolizumab has resulted in long-term durable responses in many patients, with less immune-related toxicities than ipilimumab treatment (7). However, it is important to note that, like their predecessors, this next wave of immunotherapies has similar problems: although a significant proportion of patients respond well to checkpoint blockade, the majority of patients will have little or no response (4). Combination therapies (8), optimal sequencing, and stratification based on various biomarkers have edged this proportion higher, but there are few predictors of those that will have an immunotherapeutic response (9), and even fewer alternatives for those patients who do not respond.

However, as our understanding of how the T-cell response is negatively regulated through co-inhibitory receptors opened the door for checkpoint blockade, new studies of additional mechanisms by which T cells are regulated through additional, potentially nonimmunologic mechanisms may shed new light on the extension of immunotherapies to additional patients.

We now appreciate that T cells and tumor cells do not interact in isolation, as antigen presenter and antigen detector. In fact, a very distinct constellation of cellular players characterize what is commonly known as the tumor microenvironment (TME; ref. 10). Suppressive regulatory T cells, myeloid-derived suppressor cells, and tumor-associated macrophages can generate an additional zone of immunoregulation that blankets the additional

[1]Department of Immunology, University of Pittsburgh, Pittsburgh, Pennsylvania. [2]Tumor Microenvironment Center, University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania.

Corresponding Author: Greg M. Delgoffe, University of Pittsburgh, 5117 Centre Ave, Hillman Cancer Center, Pittsburgh, PA 15232. Phone: 412-623-4658; Fax: 412-623-4640; E-mail: gdelgoffe@pitt.edu

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inhibitory ligations that T cells endure, often driven by the cancer cells themselves (11). Although these cells are currently being targeted through various modalities and utilized as biomarkers of response, behind the veil of these cells lies a distinct metabolic landscape, heavily impacted by the deregulated bioenergetics of the tumor and further exacerbated by altered angiogenesis and stromal deregulation (12).

Cancer cells divide continuously and unrestrainedly, fueling their proliferation through metabolic deregulation (13). Nucleotides and membranes must be generated and proteins must be duplicated, creating a major energetic demand on the cancer cell (13). Early biochemical work showed that tumor cells utilize glucose fermentation ("Warburg effect"), generating lactic acid rather than oxidizing glucose in the mitochondria, to generate biomass in the mitochondria required for cell growth (14). Tumor cells are, thus, hungry, and in the spatially restricted TME, act as a sink for nutrients and oxygen. Indeed, metabolic profiling has confirmed that tumors contain low concentrations of glucose, glutamine, and oxygen while having high amounts of the byproducts of tumor cell metabolism, including lactic acid, glutamate, and ketone bodies (15). Importantly, although deregulated metabolism is an "emerging hallmark" of cancer and certainly a common phenotype of tumor cells, the degree of this deregulation can be quite heterogeneous, from patient to patient and even within individuals (13, 16). Indeed, as cancers develop, individual cancer cells can adopt distinct metabolic patterns that can be associated or even causative in altered differentiation. Although more differentiated tumor cells tend to rely on glycolysis, cancer stem cells are heavily dependent on mitochondrial function for the maintenance of a stemness phenotype (13). At the individual level of cancer stem cells, numerous studies have shown great heterogeneity in cancer cells with stem properties, which can prioritize specific glycolytic or oxidative pathways as their primary metabolic phenotype, expertly reviewed elsewhere (17). Thus, the metabolic status of an individual's TME can be quite distinct, induced by the very nature of the tumor cell itself. Indeed, oncogenic mutations can promote distinct metabolic profiles in the tumor cell. For example, Myc can promote glycolysis and glutamine metabolism in tumor cells (18, 19), and Ras can promote glucose uptake and associated hexosamine and nucleotide synthesis (20, 21). Thus, tumor cell metabolic deregulation can be highly variable, dependent on many host- and tumor-derived factors. The changes induced by this metabolic deregulation are not trivial, and any other cells with high metabolic demands will effectively function at a loss unless bolstered to outcompete cancer cells for nutrients.

Race Cars and Semi-Trucks: Fueling the Effector and Memory Phase

Naïve T cells, quiescent for a lifetime, have barely detectable metabolic activity, likely as a means to preserve clonality throughout ontogeny (22). However, upon activation, T cells grow considerably for about 24 hours and then rapidly divide, performing cell cycles measured as quickly as 2 hours (23, 24). During this effector phase, T cells engage aerobic glycolysis, fermenting glucose much like cancer cells do, to preserve mitochondria for biosynthesis and limit oxidative damage (25). They also become very dependent on glutamine, engaging glutaminolysis pathways almost immediately upon T-cell receptor (TCR)–elicited *Myc* transcription (26). This "high octane" metabolism is not simply important for generating ATP, reducing intermediates, and nucleotides (through the pentose phosphate pathway) to support proliferation but actively contributes to the effector program of T cells through translational control of effector cytokines. Specifically, GAPDH, like many glycolytic enzymes, can "moonlight" as an RNA-binding protein during nonglycolytic periods (27). Glucose uptake and processing are also coupled to other effector T-cell responses, like calcium flux, and maintaining lineage stability (28, 29). Indeed glucose metabolism also can play roles in modulating epigenetic stability at the level of histones and DNA methylation (29). Thus, glucose (and its availability) can directly contribute to effector T-cell function, as well as provide energy to support their robust proliferation.

Mitochondria, too, play a vital role in T-cell activation. Although the fate of the majority of glucose lies outside of the mitochondria, activated T cells still utilize mitochondrial pathways for oxidative phosphorylation as well as many biosynthetic pathways (30–32). Importantly, mitochondria are also important for the final steps in the oxidation of other fuel sources, like amino acids and lipids, representing "diesel engines" that can burn "less refined" fuel. They are also critically important for calcium buffering and generating reactive oxygen species (33, 34). Mitochondria are dynamically regulated: quantitatively within the cell through mitochondrial biogenesis (35, 36), qualitatively through the development of "fissed" or "fused" mitochondrial networks (37) and spatially through cytoskeletal tethering into and out of the immunologic synapse (38, 39). As T cells progress to their memory phase, they further upregulate mitochondrial biogenesis to build spare respiratory capacity, a measurement of oxidative reserve used in stress situations (40). Memory T cells also engage in mitochondrial fusion, forming networks of mitochondria to effect higher oxidative phosphorylation function (37). In this way, memory cells are bioenergetically primed, able to respond with vigor to reencounter with an antigen but also able to re-enter the quiescent state to persist, potentially never seeing cognate antigen again. Thus, mitochondrial pathways are associated in T cells with longevity and persistence, as well as metabolic plasticity, effectively being able to oxidize a multitude of other fuel sources outside of glucose.

Metabolic Defects in Tumor-Infiltrating T Cells

The persistent nature of a tumor, a source of long-term antigen and inflammatory signals, has been likened to that of chronic viral infection, producing phenotypically similar exhausted T cells, which still perform but at limited efficacy (41). Thus, studies both in cancer and in experimental models of chronic viral infection have revealed much about the nature of T cells in this type of condition. Although *in vitro* studies have revealed much about the signaling of some of these T-cell subsets, it has been difficult to model the microenvironments associated with T-cell exhaustion with sufficient accuracy. Work identifying transcriptional signatures associated with exhausted T cells using the lymphocytic choriomeningitis virus system indeed revealed no "smoking gun" for the T-cell exhaustion phenotype (41). Rather, exhaustion, both in cancer and in chronic infection, represents a number of non-redundant deficiencies that seem to synergize to induce T-cell dysfunction. Indeed, exhausted T cells have a distinct metabolic transcriptional signature that is induced early, and in a distinct manner, from the effector responses of acute infections

(42). However, many of these changes are not transcriptionally induced but rather are limited by posttranslational or microenvironmental changes.

The metabolic defects of the TME are important to understand, as rapid proliferation, cytokine production, and cytolytic activity, important cellular functions for T cells, each have high energetic demands (43). Sustaining that effector response thus requires overcoming several metabolic barriers: sufficient metabolic substrates (glucose, glutamine, fatty acids, oxygen), cellular machinery to utilize these substrates (glycolytic enzymes, mitochondria, reducing intermediates), and nutrient sensors to mobilize this cellular machinery (mTOR, AMPK, Myc, ERRα; Fig. 1). Studies exploring the interface between these metabolic pathways and immunity have garnered much interest, both at the basic level and in various disease states, and this new field of "immunometabolism" has revealed that bioenergetics act as a sort of primordial mechanism for immunoregulation.

Studies of T-cell tolerance brought to light the impact of metabolites and nutrient sensing in T-cell function; this has been extensively reviewed elsewhere (44). Kinases like mTOR limit nutrients through nutrient sensing and other pathways during T-cell stimulation, which not only impacts immediate effector function but also can have long-term effects on the differentiation and future function of these cells (45). Although many of these studies were conducted *in vitro*, applying that rationale to the TME has revealed that in this physiologic environment in which nutrients are limiting, T cells fail by similar mechanisms. Glucose, a primary fuel for effector T cells, is limiting in the TME, consumed by tumor cells themselves (28, 46). This not only limits the immediate effector function of T cells but also limits their ability to take up glucose as well. Immunotherapies like PD-1 blockade may have a bystander effect of limiting tumor glucose consumption, thus tipping the balance in favor of antitumor immunity (46). Importantly, glucose metabolism also plays a critical role in promoting calcium signaling, which is critical for TCR-induced NFAT activation and subsequent effector function (28). Thus, the competition for glucose remains a major limiting factor for T-cell function in the tumor, but other metabolic substrates are limiting in the TME. Glutamine, arginine, tryptophan, and oxygen are all present at lowered concentrations in the TME, driven, at least in part, by tumor cell metabolism and competition. This limits T-cell metabolism and consequent effector function (19, 47–49). Improving T-cell metabolism for therapy need not be a heroic undertaking, however. Arginine supplementation of therapeutic T cells has been shown to improve their metabolism and can heighten antitumor responses in a model of adoptive cell therapy (50). This approach of directly "feeding" the T cells to promote their antitumor function may be of value in the future. It may also

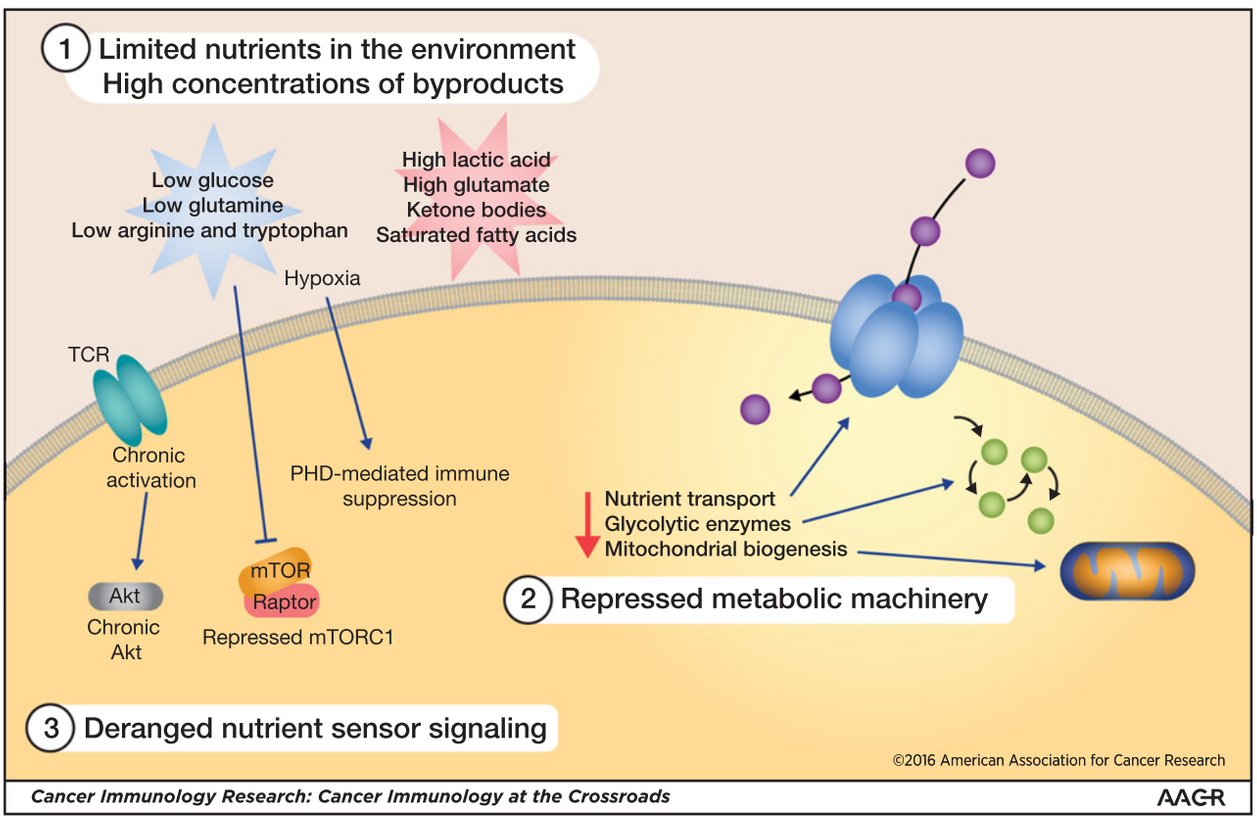

Figure 1.

Metabolic barriers to antitumor immunity. **1,** Altered tumor cell metabolism creates a microenvironment with low concentrations of primary metabolic substrates for T cells, including glucose, glutamine, and oxygen. Metabolites produced by tumor cells like lactic acid are in high concentrations and can be immunosuppressive. **2,** In addition, signals in the TME can repress the glycolytic machinery, as well as pathways involved in mitochondrial biogenesis. **3,** Finally, nutrient-sensing machinery can also be deregulated, resulting in a failure to mobilize these important pathways to promote sustained T-cell responses in the TME.

be attractive to utilize strategies to remodel the metabolism of the microenvironment itself, rather than providing T cells nutrients directly. These strategies could include, for example, identification and inhibition of a tumor-specific pathway that promotes the consumption of a T-cell primary fuel. Inhibiting oncogenic pathways that promote glycolysis might also lower tumor glucose consumption, providing fuel for T cells. Alternatively, utilizing inhibitors that are more broadly active but affect tumor cells disproportionately may be an attractive strategy. This may involve targeting metabolic drugs to tumors with antibodies, or, more simply, utilizing pharmacologic inhibitors which may be more actively taken up by tumor cells. Thus, it may be possible to lower a metabolic barrier through carefully designed strategies to inhibit tumor cell metabolism, creating an environment more permissive to T-cell function and consequent response to immunotherapy.

However, T cells are not only limited by the availability of nutrients in the TME. Rather, the metabolic machinery can also be repressed in tumor-infiltrating T cells. As mentioned above, T cells in the TME can repress the machinery required to process glucose, including glucose transporters and glycolytic enzymes (28, 46, 51). Our group has found that mitochondria, which require an active process termed "mitochondrial biogenesis" for their replication during cell division, are also limiting in tumor-infiltrating T cells (52). Tumor-infiltrating T cells have poor mitochondrial morphology, repressed mitochondrial function, and lower total mitochondrial mass (52). This process occurred independently of PD-1 blockade, suggesting that the mitochondrial repression occurs in parallel to PD-1–induced co-inhibition. Similar loss of oxidative metabolism is observed in exhausted T cells from chronic viral infection, including repression of mitochondrial biogenesis programs (42). Reprogramming of T cells to enforce mitochondrial biogenesis resulted in superior antitumor and antiviral T-cell function (42, 52). Thus, T cells lack the machinery to process available nutrients, another major barrier to effective antitumor immunity.

The nutrient-sensing machinery itself can be deranged in tumor-infiltrating T cells. Mitochondrial biogenesis is repressed through chronic Akt activation, which represses the activity of the PPARγ coactivator 1 (PGC1α) pathway, a transcriptional coactivator supporting this biogenesis pathway (53). In addition, several groups have observed repression of the mTORC1 pathway in exhausted T cells, which has major metabolic effects on tumor-infiltrating T cells, including the inhibition of glycolysis, mitochondrial activity, and translation, as well as the induction of autophagy (42, 52). The oxygen-sensing machinery plays a major role in immunosuppression in the TME (54). Whereas hypoxia can have both activating and suppressive functions in T cells (55), mitigating tumor hypoxia through respiratory supplemental oxygenation can improve immunotherapeutic responses in mice (48). Thus, T cells in TMEs must overcome a number of metabolic barriers to be able to fuel their antitumor function.

Retrofitting T Cells in Immunotherapy: Built for Speed or the Long Haul

Since the identification of tumor-infiltrating lymphocytes (TIL), researchers and clinicians have been trying to harness the power of T cells for therapy. In hematologic malignancies, mismatched hematopoietic cell transplants have long utilized the broad reactivity of T cells against alloantigens to target host tumors (graft-versus-leukemia), and the power of this response can be observed in the toxic and potentially lethal side effects of graft-versus-host disease (56). Specificity, in the form of chimeric antigen receptors or TCR-engineered T cells, has allowed the harnessing of this "serial-killer" response to generate durable immunity to several hematologic malignancies, targeting cell surface antigens like CD19. These re-engineered T cells have the capacity to kill tumor cells extraordinarily efficiently, clearing so much tumor that cytokine release must be mitigated therapeutically. However, this approach may not be directly applicable for immunotherapy of solid tumors. Responses to CAR- and TCR-engineered T cells in solid tumors have been underwhelming and in many cases accompanied by significant off-target toxicities (57).

Effector T cells can sense antigen upon presentation and receive many other signals in the form of costimulation/coinhibition and cytokines, but T cells are merely integrating signals: they know nothing about the persistence, scope, or nature of an antigen. Thus, during an effector phase, the hardwired response is to proliferate rapidly and kill quickly, with the "hope" that this is sufficient to eliminate infection. They do this at the expense of their continued longevity, favoring glycolysis over mitochondrial metabolism. Metabolic choices thus may determine the long-term potential of these cells, a supposition supported experimentally in models of TIL therapy (58, 59). This "shock-and-awe" response is likely sufficient to clear most acute infections, but in the case of a solid tumor, which smolders with continuing cell division, it may simply be not enough. Efforts to reinvigorate or redirect T cells to cancer have been focused on creating the "best" effector cells that will infiltrate the tumor and lyse cancer cells with reckless abandon. However, these effector cells (whether endogenously reinvigorated or adoptively transferred) are not engineered for the long haul; thus, clinical responses are infrequent or incomplete. In the solid-TME, nutrients are insufficient to support this type of response; glucose is limiting, acidosis makes regeneration of NAD^+ by lactate dehydrogenase (LDH) more difficult, and hypoxia prevents any residual mitochondrial activity (55). Clues from the CAR T-cell studies and the study of intratumoral T cells have taught us that memory-like T cells are superior antitumor T cells. Memory T cells are imbued with higher mitochondrial activity, more fused mitochondrial networks, and the ability to utilize additional substrates to fuel their secondary responses, all of which are desirable in long-lived T cells and in a nutrient-poor condition (30, 37, 40). Thus, promoting mitochondrial biogenesis to support memory states and decreasing hypoxia to allow for mitochondrial function are key factors in generating better long-lived T cells. Indeed, reprogramming T cells to favor mitochondrial biogenesis through enforced expression of PGC1α resulted in enhanced antitumor response, even with small numbers of cells (52). In other words, making a cell "metabolically" resemble a memory cell promotes memory-like states. This type of manipulation may be critical for the generation of long-term durable immune responses like those needed for the eradication of solid tumors.

Concluding Remarks

Recent clinical successes have brought cancer immunotherapy into the mainstream, providing hope to patients with advanced cancers and revealing new pathways by which we might bolster antitumor immunity to benefit a larger proportion of patients. Those successes were enabled through the

improved understanding of how immunity was regulated. It is clear that deregulated metabolism, a common feature of cancer cells, can also act as a negative regulator of immune cell function through the generation of an environment with a dearth of nutrients, coupled to the exploitation of the basic wiring of T-cell signaling. Modulating those intratumoral energetics, whether through reprogramming of T cells, selective targeting of tumor cell metabolism, or other undiscovered means, has the potential to further transform how the immune system is harnessed for cancer treatment.

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No potential conflicts of interest were disclosed.

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