Review

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Targeting T cell metabolism for therapy

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In the past several years a wealth of evidence has emerged illustrating how metabolism supports many aspects of T cell biology, as well as how metabolic changes drive T cell differentiation and fate. We outline developing principles in the regulation of T cell metabolism, and discuss how these processes are affected in settings of inflammation and cancer. In this context we discuss how metabolic pathways might be manipulated for the treatment of human disease, including how metabolism may be targeted to prevent T cell dysfunction in inhospitable microenvironments, to generate more effective adoptive cellular immunotherapies in cancer, and to direct T cell differentiation and function towards non-pathogenic phenotypes in settings of autoimmunity.

Introduction

During the course of an immune response, naïve T cells recognize foreign antigen (Ag) in the form of peptide complexed to MHC molecules and, with proper co-stimulation, become activated, rapidly proliferate, and produce a variety of effector molecules that lead to control of a pathogen. T cell activation, clonal expansion, and the acquisition of effector functions are energetically demanding processes that are accompanied by and dependent upon marked changes in nutrient uptake and cellular metabolism [1,2]. Once the Ag burden is diminished, the majority of Ag-specific effector T cells die, leaving behind only a small number of stable memory T cells that persist and can rapidly respond to future Ag challenge. Memory T cells must also reprogram cellular metabolic pathways to support their development, longevity, and 'rapid recall' ability [3,4]. Thus, proper metabolic programming in T cells is required for a productive immune response.

The processes of cellular activation, differentiation, and extensive proliferation that take place during a T cell response are unusual for cells in a healthy adult organism, where most cells have differentiated to a terminal phenotype [5]. This aspect of T cell biology, combined with the modern tools for assaying these cells and highly tractable *in vivo* systems, make them uniquely suitable for studying how metabolic pathways support vigorous changes in cellular activity. In addition, and perhaps more importantly from a human health standpoint, each of these metabolic changes that occur as part of the normal development of a T cell are intimately linked to cell fate and function, and, as such, represent points for clinical intervention. Because many infections, cancers, and autoimmune diseases might be controlled, or at least mitigated, by eliciting a desired response from T cells, novel approaches to therapeutically target these cells have clinical potential. Many comprehensive and up-to-date reviews on T cell metabolism are available [1,2,6–9]. We focus on recent advances in the mechanisms that link metabolic changes with T cell fate and function and consider novel approaches in which T cells might be manipulated by blocking, or potentiating, metabolic pathways.

The basics of T cell metabolism

Naive T cells have a metabolically quiescent phenotype and generate energy by breaking down glucose, fatty acids,

Glossary

Adoptive cellular immunotherapy (ACI): a T cell based immunotherapy whereby T cells are taken from a patient and stimulated and/or genetically manipulated *in vitro*. Following population expansion, the cells are transferred back into a patient.
Aerobic glycolysis: the metabolism of glucose into lactate, in the presence of oxygen.
Bi-specific antibodies: engineered proteins composed of two independently targeted antigen (Ag)-binding regions (Fab) within an antibody, or two separate antibodies targeted against distinct epitopes that are connected by a linker protein.
Chimeric antigen receptor (CAR) T cells: patient T cells that have been isolated and then engineered to express a cell surface receptor(s) that recognizes a specific Ag or protein.
Electron transport chain (ETC): components in the mitochondrial membrane that couple the transfer of electrons via redox reactions to the movement of protons across the membrane, creating an electrochemical gradient that can power ATP production.
Fatty acid oxidation (FAO): β-oxidation of fatty acids in the mitochondria; this process generates acetyl CoA, which feeds into the TCA cycle and contributes to energy production.
Glutaminolysis: a series of reactions that converts glutamine into α-ketoglutarate, which is used as an intermediate within the TCA cycle.
Oxidative phosphorylation (OXPHOS): a mitochondrion-based process whereby metabolic substrates are oxidized in the TCA cycle to produce ATP via the ETC.
Reductive carboxylation: an alternative pathway of glutamine metabolism whereby glutamine-derived α-ketoglutarate is converted to citrate. This process often occurs in cells with defective mitochondria or under conditions of hypoxia because it allows the cells to still derive citrate for anabolic processes, while minimizing oxidative metabolism.
Solute carrier family (SLC): a collection of membrane solute transporter proteins consisting of over 300 known members.
Spare respiratory capacity (SRC): the reserve capacity of mitochondria to produce energy over and above normal levels of energy output.
Tricarboxylic acid (TCA) cycle: a series of chemical reactions that use metabolic substrates to derive reducing agents NADH and FADH that fuel the ETC, as well as providing metabolic precursors for amino acid and fatty acid synthesis.
Tumor-infiltrating lymphocytes (TILs): the term refers to lymphocytes found within a tumor.

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and amino acids to fuel oxidative phosphorylation (OXPHOS; see Glossary) [10–12]. The transition from a resting naïve T cell into activated and highly proliferative effector T cells requires substantial metabolic reprogramming. While mitochondrial OXPHOS and reactive oxygen species (ROS) production increase, and are crucial for T cell activation and the development of effector T cells, rapid induction of aerobic glycolysis also occurs during this time [4,13,14]. Aerobic glycolysis involves the mitochondrion-independent metabolism of glucose into pyruvate and its subsequent conversion into lactate. ATP can be generated through this pathway, in what is believed to be a rapid but inefficient fashion. Specifically, only two molecules of ATP are gained per molecule of glucose via aerobic glycolysis, whereas OXPHOS generates up to 36 ATP molecules per molecule of glucose [5]. Aerobic glycolysis may, however, afford a metabolic advantage to effector cells not only by allowing the rapid production of ATP in glucose-replete environments but also by supplying metabolic intermediates for the synthesis of lipids, protein, carbohydrates, and nucleic acids, as well as by providing a means for maintaining redox balance [5,15–17]. In addition, it has been found that although T cells can use OXPHOS or aerobic glycolysis interchangeably depending on their environment, engagement of aerobic glycolysis is needed for the acquisition of full effector functions [18–20]. Glutamine metabolism is also required for proper effector T cell development, and utilization of this amino acid is augmented following activation [21,22]. Glutamine can be used as a carbon source for the tricarboxylic acid (TCA) cycle in the form of α-ketoglutarate through the process of glutaminolysis, or can contribute to the citrate pool via reductive carboxylation [23,24]. Deletion of glutamine or glucose transporters impairs T cell activation and function [15,22,25,26].

Metabolic reprogramming in activated T cells is driven by several signaling pathways and transcription factors. A key regulator of T cell metabolism is mechanistic target of rapamycin (mTOR), which functions as two distinct complexes, mTORC1 and mTORC2, that differ in their regulation and downstream targets [27]. mTOR integrates signaling pathways associated with nutrient levels, energy status, cell stress responses, T cell receptor and growth factor signaling, and can induce multiple pathways associated with cell growth, proliferation, and metabolism [28–30].

The metabolic transition towards increased glycolysis and glutaminolysis is associated with mTOR induction as well as the expression of the transcription factors Myc and hypoxia inducible factor 1α (HIF-1α) [21,31]. HIF-1α is a transcription factor that, when induced by hypoxia or mTORC1 activity, leads to increased glucose uptake and diverts glucose away from OXPHOS towards aerobic glycolysis [31–33]. Myc also promotes aerobic glycolysis and glutaminolysis through enzyme expression, and enhances anabolic processes such as lipid, amino acid, and nucleic acid synthesis [21]. Collectively these factors enforce metabolic phenotypes in effector T cells appropriate for their function, and alterations in these pathways can be used to manipulate effector T cell differentiation.

AMP-activated protein kinase (AMPK) is another key metabolic regulator in T cells that acts as a metabolic stress sensor, becoming activated when the ratio of AMP to ATP increases. Despite being transiently activated upon T cell activation, it can work in opposition to mTOR-mediated anabolism by promoting catabolic pathways and energy conservation during metabolic stress [34,35]. AMPK is important for the development of memory T cells [36], and more recently is was shown that it is also crucial for effector T cell development and metabolic flexibility in response to changing nutrient environments [37]. These studies highlight the importance of this kinase in T cells during nutrient stress, and also help to sediment the idea that T cells are under metabolic constraints *in vivo*.

In contrast to effector T cells, memory T cells do not engage aerobic glycolysis highly, and instead preferentially rely on OXPHOS, a process that is fueled, at least in part, by the catabolism of intracellular fatty acids in the mitochondria of these cells [3,38]. Memory T cells also maintain substantial spare respiratory capacity (SRC) and have increased mitochondrial mass, both of which confer a metabolic advantage for survival and recall following Ag challenge [3,4]. Augmenting catabolic pathways in activated $CD8^+$ T cells with rapamycin or the AMPK activator metformin reduces the differentiation of $CD8^+$ effector T cells, and instead enhances $CD8^+$ memory T cell development [39]. Attenuation of aerobic glycolysis or the enhancement of OXPHOS in activated $CD4^+$ T cells also alters their phenotype dramatically. For example, in $CD4^+$ T cells the inhibition of mTOR by rapamycin, or the inhibition of glycolysis by the hexokinase inhibitor 2-deoxyglucose (2-DG), blocks the differentiation of T helper 17 (Th17) cells while promoting regulatory T (Treg) cell development [14,40,41]. Likewise, AMPK activation, which enhances fatty acid oxidation (FAO) and energy conservation by antagonizing anabolic pathways, also alters this balance in favor of Treg cells [14,42].

In settings such as chronic infection and cancer, T cells can become anergic, or exhausted, losing the capacity to properly respond to stimulation. This hyporesponsiveness may be in large part due to an inability of the cells to optimally utilize appropriate metabolic pathways [2]. Gene expression analysis of exhausted T cells indicates that several genes involved in energy metabolism are transcriptionally downregulated [43], and inhibiting leucine or glucose metabolism during T cell activation can lead to an anergic phenotype [44]. Furthermore, ligation of inhibitory receptors that are highly expressed on exhausted T cells, such as cytotoxic T lymphocyte-associated protein 4 (CTLA-4) or programmed cell death protein-1 (PD-1), inhibits the upregulation of glucose and glutamine metabolism following T cell receptor (TCR) engagement and co-stimulation [45,46]. Expression of these inhibitory receptors may restrain T cells from correctly remodeling their metabolism, and hence dampen their function. Targeting exhausted T cells with the aim of enhancing glycolysis may be a way to reactivate these cells. Consistent with this idea, T cells lacking the von Hippel–Lindau (VHL) tumor suppressor, a negative regulator of HIF-1α, had enhanced glycolysis and were resistant to exhaustion following persistent viral infection [31].

Metabolites within cells can also act as signaling molecules that influence diverse, and sometimes non-metabolic, processes [7,18,47,48], thus the availability of particular metabolites can vastly affect both cellular metabolism and cell signaling. For example, the key metabolic intermediate acetyl-CoA is not only oxidized in the tricarboxylic acid (TCA) cycle for energy production, but it is also needed for the acetylation of histones [47] and other proteins, including transcription factors and metabolic enzymes [49]. Levels of histone acetylation correlate to the activity of ATP citrate lyase (ACL, which converts citrate into acetyl-CoA and oxaloacetate), and the availability of glucose (a major source of acetyl CoA) can alter histone acetylation in an ACL-dependent process [50]. The TCA cycle intermediate succinate also acts as an inflammatory signal in macrophages by inducing IL-1β through HIF-1α stabilization [48]. The accumulation of fumarate, due to fumarate hydratase deficiency, leads to several changes within cancer cells, including hypermethylation and HIF-1α stabilization [51]. Leucine transport into the cell is also required for T cell metabolic reprogramming [25]. Leucine can activate mTOR via leucyl-tRNA synthetase [52], and thus low concentrations of intracellular leucine can impair mTOR activation. Accordingly, it was shown that expression of cytosolic branched-chain aminotransferase (BCATc), which transaminates leucine, regulates mTOR activity following T cell activation in a process that limits mTOR overactivation [53]. Interestingly, it was found that BCATc is increased in anergic cells. This raises the intriguing possibility that leucine depletion by BCATc could contribute to T cell anergy through suppression of mTOR activity, and that inhibition of BCATc may be an interesting target within this context. Metabolites can also directly act as endogenous ligands for nuclear receptors, such as the liver X receptor (LXR) or the aryl hydrocarbon receptor (AhR), both of which have been shown to regulate T cell differentiation and proliferation [54,55]. It is clear that more work needs to be done to understand how signaling from metabolites influences cell function.

Enhancing T cell function in tissue microenvironments

T cells are influenced by nutrients and other supportive signals, such as those provided by growth factor cytokines, that are available in their environment. We speculate that lymphoid organs are nutrient-replete, but that other sites infiltrated by T cells may be much less nutrient-rich. Manipulating the metabolism of tissues in which they reside, or substrates within a tissue, may provide a therapeutic approach to enhance T cell function. An example of this is provided by a consideration of a commonly expressed melanoma oncogenic mutation BRAF V600E, which generates a tumor that has a strongly immunosuppressive microenvironment [56]. This mutation induces constitutive activation of the MEK–MAPK pathway, leading to enhanced tumor cell proliferation, suppression of OXPHOS, and a highly glycolytic phenotype [57,58]. Because glucose and glutamine are crucial for T cell differentiation and function, and depletion of glucose impairs cytolytic activity as well as interferon-γ (IFN-γ) production [2,18,19], it is likely that the highly-glycolytic phenotype of BRAF V600E melanoma contributes to the immunosuppressive environment it imposes. This would be consistent with findings that effective therapeutic treatment using small-molecule inhibitors of BRAF restricts glycolysis and glutaminolysis in BRAF V600E tumors, and that these inhibitors can reverse some of the immunosuppressive features within the tumor microenvironment [56–60]. Further supporting this concept, it was shown that T cells isolated from tumors had increased IFN-γ and CD40L expression after BRAF V600E inhibition, and blockade of IFN-γ or CD40L compromised the tumor-suppressive effects of BRAF V600E inhibition [56]. These data indicate that inhibition of BRAF V600E operates, at least in part, through an immune cell dependent mechanism, and suggest that directly altering tumor metabolism allows anti-tumor T cells to work more effectively. The immunosuppressive metabolic environment induced by the BRAF V600E mutation could be further enhanced by tumor expression of inhibitory ligands for PD-1 and CTLA-4 which, when bound to their cognate receptors on T cells, limit T cell-intrinsic glutaminolysis, glucose uptake, and glycolysis [45,46]. It has also been shown that inhibition of such interactions (i.e., checkpoint-blockade therapy) enhances tumor immune therapy [61]. Preliminary observations from our laboratory also indicate that checkpoint-blockade therapy alters the metabolic balance between tumors and their infiltrating T cells. We postulate that immunosuppression in the tumor microenvironment is at least in part driven by the inability of T cells to acquire the nutrients to support their metabolism. Establishing that this is an important mechanism of immunosuppression may lead to new ways to manipulate the tumor microenvironment to better suit the metabolic needs of infiltrating T cells [62].

While it is relatively easy to envisage how T cells in a solid tumor could be at a competitive disadvantage for nutrients, and that this would negatively effect their function, this paradigm can additionally be extended to other, perhaps less obvious, settings. For example, gut microbiota produce several metabolites that can interact with host tissues and the immune system, and these can have profound effects on T cell development and function [63,64]. The bacterial production of short-chain fatty acids such as butyrate within the gut has been shown to alter the balance of Th17 and Treg cells as well as altering T cell mTOR signaling [65–68]. It is also likely that microbe-derived amino acids and other fatty acids could regulate T cell responses [64]. Recently, it has been shown that *de novo* fatty acid synthesis controls the fate between Treg cells and Th17 cells [69]. Specifically, when acetyl-coA carboxylase, an important enzyme in fatty acid synthesis, is blocked either genetically or by the bacterial metabolite Soraphen A, Th17 cells fail to develop, and instead naïve T cells polarize to a Treg cell fate. Altering metabolic pathways to bias T cell differentiation away from Th17 cell development could be exploited in diseases such as multiple sclerosis or Crohn's disease where Th17-mediated pathology has been implicated [70]. Perhaps other metabolites similar to Soraphen A are produced from gut bacteria that modulate cell metabolism and influence immune responses, possibly regulating autoimmune susceptibility in humans. Understanding how commensal organisms influence the intestinal microenvironment, and how this

environment then dictates metabolic pathway engagement by intestinal T cells, and thus their differentiation and function, is an important subject for future study.

Metabolism and adoptive cellular immunotherapy

Substrate availability in the tissue microenvironment has a major impact on T cell function *in vivo*, and clearly the environment will also have an impact on T cells that are cultured *in vitro*. There has been intense interest in developing adoptive cellular immunotherapy (ACI) for cancer and chronic viral infections, whereby naturally occurring or engineered T cells are stimulated and expanded *in vitro*, then transferred to the patient [71]. Although some success has been achieved using this approach, many ACI strategies have failed or have had less than optimal therapeutic outcomes [72,73]. While a substantial amount of research has focused on optimizing T cell activation and the use of appropriate adjuvants for ACI, relatively little research has been directed at manipulating metabolic pathways which could potentially enhance therapeutic efficacy. Altering T cell metabolism can positively affect cell function and longevity [18,39,74], and perhaps placing more consideration on metabolic parameters when designing and implementing ACI would lead to better patient outcomes.

In the context of cancer research, two of the most-common forms of ACI involve either the *in vitro* expansion of tumor-infiltrating lymphocytes (TILs) isolated from resected tumors or the engineering of chimeric Ag receptor T cells (CARs) derived from peripheral T cell populations of the patient [75]. In both methods, large populations of Ag-specific T cells are reintroduced into the patient. It has been observed that therapeutic efficacy is enhanced when transferred T cells maintain both replicative capacity and the ability to persist for long periods [76], and restraining differentiation to a terminal phenotype *in vitro* can improve the efficacy of *in vivo* treatment [74,77].

Altering culture conditions for ACI to take into account metabolism is one way in which T cells could be restrained from terminal differentiation while being optimized for persistence *in vivo*. For example, although some culture media contain levels of glucose that approximate blood glucose concentrations (around 5.5 mM), many commonly used media (including media used for ACI) have glucose concentrations that range from 10–35 mM, which is substantially higher than normal physiological levels. This could potentially program proliferating T cells to become overly dependent on glycolysis [78], which would be expected to result in impaired function and survival when T cells for ACI are transferred back into patients and thus exposed to lower physiological glucose levels. It has been shown that augmentation of glycolysis in $CD8^+$ T cells limits long-term survival [74]. T cells expanded *in vitro* are often larger in size compared to *in vivo* proliferating T cells, which may be a function of increased glucose availability [28,79,80]. Exaggerated glycolysis or cell size can negatively impact T cell survival *in vivo* [74,81]. It has been demonstrated that, independently of proliferation, increases in aerobic glycolysis in hepatocytes correlate to increased cell size and, conversely, cell size is inversely proportional to mitochondrial gene expression [82]. Likewise, in T cells, constitutive activation of Akt, which enhances glycolysis [83], or increased cell surface expression of the glucose transporter Glut 1, result in increased basal T cell size [20]; limiting glycolysis using low dose 2-deoxyglucose (2-DG, which inhibits hexokinase and thus glycolysis) in cultured T cells can reduce cell size and also increase longevity, without impairing proliferative capacity [74]. Collectively, these data suggest that limiting glycolysis and cell size, either through direct modulation of metabolism or through careful consideration of culture conditions, could improve ACI. Furthermore, cell size and/or high glycolic rates could potentially be used as proxy indicators of poor *in vivo* survival and function of *in vitro* activated T cells. Monitoring these factors while optimizing *in vitro* metabolic conditions may provide an efficient and effective read-out of T cell fitness.

Another way to enhance the replicative capacity and long-term persistence of ACI cells may be to promote OXPHOS or mitochondrial biogenesis. A recent study has demonstrated that inhibition of Akt in *in vitro* expanded TILs resulted in an altered metabolic profile with increased rates of OXPHOS and FAO, and these cells exhibited enhanced *in vivo* persistence and improved anti-tumor immunity [84]. Consistent with this idea, *in vitro* activation of T cells in the presence of cytokines that signal via receptors containing the common γ chain, such as interleukin-15 (IL-15) or IL-7, allows substantial population expansion and improved *in vivo* T cell survival and anti-tumor efficacy [77,81,85]. These beneficial effects are likely to be related, at least in part, to metabolic changes induced by these cytokines; IL-15 reduces glycolysis while enhancing OXPHOS and SRC in activated $CD8^+$ T cells, as well as increasing mitochondrial mass [3,38]. In other types of tissue, cell longevity is often associated with a reliance on mitochondrial metabolism [86–88]. Unpublished observations from our laboratory indicate that memory T cells, in addition to gaining more mitochondrial mass, have mitochondria that are morphologically distinct and appear to be networked, as compared to those in effector T cells. Mitochondria are dynamic organelles that constantly fuse and divide, and these fission and fusion events can regulate metabolism, longevity, and cell fitness [89–91]. There also might be potential for enhancing T cell mitochondrial function though the use of Szeto–Schiller (SS) peptides, which target cardiolipin within mitochondria and optimize the efficiency of the electron transport chain (ETC) [92]. One of these peptides, SS-31 (or Bendavia), which is currently in Phase II clinical trials as a treatment for ischemic reperfusion injury, could conceivably be used to enhance T cell mitochondrial health and integrity [93]. Pharmacologically modulating mitochondria to enhance their function [94,95], for example by targeting fission/fusion events or enhancing ETC efficiency, may provide an effective way to improve the fitness and longevity of ACI T cells.

Optimizing the culture conditions for T cells used in ACI could also be combined with strategies that modify the tumor microenvironment to make it more metabolically favorable for T cells. Tumors are not composed of malignant cells alone, but also contain stromal and epithelial cells, as well as other immune cells, which often make up a substantial proportion of the total tumor mass [96]. The

presence of these various cell types, in addition to the tumor cells, can result in an unfavorable metabolic environment for effector T cells. For example, the tumor microenvironment can alter T cell metabolism through the depletion of amino acids such as arginine and tryptophan [97,98], the competitive consumption of other nutrients [18,99], and the production of metabolites such as lactate [100]. A recent study showed that tumor-derived lactate drives the expression of arginase 1 in tumor-associated macrophages, driving polarization into an M2 phenotype [101]. M2 macrophages can suppress T cell function, and therefore may support tumor growth [102]. It seems probable that not only tumor cells but also infiltrating immune cells contribute to the overall suppressive nature of the tumor microenvironment, and could thus collectively contribute to impaired T cell function within the tumor. In fact, the production of metabolites such as lactate or kynurenine may also favor the development of immunosuppressive cell populations including myeloid-derived suppressor cells and Treg cells [55,103].

There is substantial interest in modifying tumor metabolism, and multiple metabolic pathways can be exploited in this regard [104]. However, because activated T cells and cancer cells often share similar metabolic traits [5], targeting tumor cell metabolism has the potential to also negatively impact upon infiltrating effector T cells. Some therapeutic targets are clearly tumor-specific [104], such as the aforementioned BRAF V600E mutation in melanoma; however, many therapeutic strategies in clinical trials target metabolic pathways that are active not only in tumor cells but also in effector T cells, such as aerobic glycolysis [105]. Therefore, it is likely that such treatments could have a beneficial effect in terms of inhibition of metabolic pathways in neoplastic cells, but could also detrimentally effect tumor-infiltrating T cell populations, which could in turn limit the effectiveness of the therapy.

One way to avoid this problem is through the development of therapies that target metabolic pathways in a tumor-specific manner. Examples of this approach are the experimental compounds AGI-5198 and AGI6780, which specifically inhibit the mutant forms of the enzymes isocitrate dehydrogenase (IDH) 1 and 2, respectively, and which have shown anti-cancer potential against glioma and leukemia cell lines [106,107]. Alternatively, antineoplastic therapies could be beneficially used before ACI treatments. Using this sequential treatment approach, initial administration of a therapeutic could be used to reduce tumor size and alter metabolism of the tumor, then T cells could be adoptively transferred after tumor metabolism has been altered. An example of this could be to use the Glut 1 inhibitor WZB117 [108] or the alkalizing agent 3-bromopyruvate that is transported into cells through MCT-1 [109], before ACI. Treatment should be sustained for a sufficient amount of time to reduce the tumor mass and to alter the metabolism of the remaining tumor cells, then drug treatment could cease and ACI T cells could be transferred into the patient. This method would theoretically provide a tumor microenvironment that is more metabolically favorable (i.e., nutrient-replete) for the infiltrating ACI T cells, and allow them to optimally function. It is possible that drug companies have developed compounds that modulate tumor cell metabolism for the specific purpose of killing tumor cells, but it is unlikely that these types of compounds have been tested for their potential ability to concomitantly augment the efficacy of TILs through the creation of more nutrient-rich tumor microenvironment. Considering how potential anti-cancer compounds could positively or negatively impact TIL metabolism may lead to improved treatment options.

Targeting metabolism therapeutically

Metabolic reprogramming is necessary to support T cell activation and function. Therefore, modulating the metabolism of T cells may be a way to target T cell function therapeutically (Figure 1). Depending on the setting, T cells can have distinct metabolic phenotypes. With this in mind, treatments with broad metabolic effects could potentially be used to target specific subsets of T cells. For example, because alloreactive T cells from graft versus host disease (GvHD) appear to depend on OXPHOS, targeting mitochondrial ATP production could be used to specifically inhibit these cells [110]. Further, because metformin has been recently shown to inhibit complex 1 of the ETC, it is possible that metformin might be useful in GvHD, particularly given that alloreactive T cells exhibit hyperpolarized mitochondria and metformin is predicated to concentrate in the mitochondria as a function of membrane potential [110,111]. Pharmacological agents that inhibit the oxidation of long-chain fatty acids in the mitochondria, such as the carnitine palmitoyl transferase (CPT)-1a inhibitors etomoxir or perhexiline, could likewise be used to selectively target GvHD T cells, which have higher rates of FAO compared to other effector T cell populations [112,113].

Compounds such as rapamycin can also have differing effects on T cells depending on the context for example, although traditionally thought of as an immunosuppressant, when administered after acute viral or bacterial infection, this compound can promote memory $CD8^+$ T cell formation [39,114,115]. Because mitochondrial health and respiration is fundamental to effective memory T cell development, it is conceivable that enhanced memory T cell development could also be achieved by the aforementioned SS peptides which promote efficient ETC function [3,92]. Although these examples provide possible therapeutic options that could be administered systemically, a limitation in the systemic administration of many drugs that could modify T cell metabolic processes is the potential for off-target side effects as a result of alterations in metabolic pathways within other tissues. Therefore, future therapeutics may need to be directed more specifically to particular T cell types or subsets.

One potential way to target T cell populations might be through transporter-facilitated drug uptake, whereby a metabolite is conjugated to a drug to enhance delivery into a target cell population [116]. An example of this approach is glufosfamide, a cancer chemotherapeutic that reached Phase III trails [117], and which is a conjugate of D-glucose and the antineoplastic drug ifosfamide. This construct utilizes the D-glucose portion of the compound as a way to gain access to cells via glucose transporters, resulting in

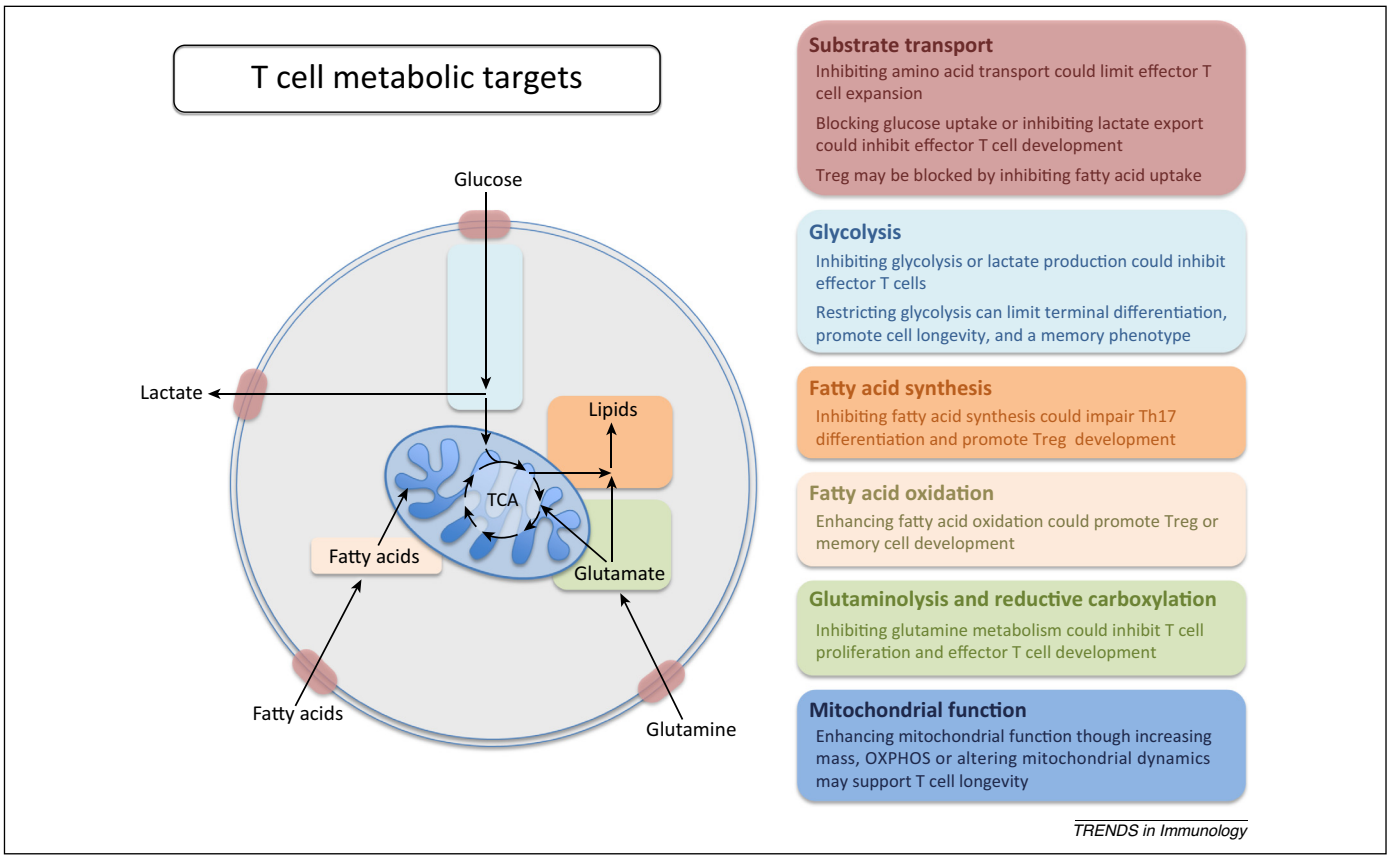

Figure 1. Metabolic targets in T cells. Abbreviations: OXPHOS, oxidative phosphorylation; TCA, tricarboxylic acid cycle; T reg, regulatory T cell.

the preferential accumulation of ifosfamide in cells that have high glucose uptake [118]. A similar construct with direct relevance to metabolism is an experimental compound consisting of a conjugate of D-glucose and a *N*-hydroxyindole-based lactate dehydrogenase inhibitor; this compound accumulates at higher concentrations in glycolytic cells and results in the targeted inhibition of aerobic glycolysis within these cells by blocking lactate production [119]. Although these systems have used glucose to facilitate drug transport, it is conceivable that other metabolites, such as amino acids, could be conjugated to drugs in a similar manner, allowing semi-selective targeting of T cell populations.

Nanotechnology is now being used to target Ags or immune-modulatory compounds to specific cell types [120]. This involves encapsulating drugs in biodegradable nanospheres that are then conjugated to antibodies, a technique that is already being explored for the delivery of cytokines and other therapeutics [121]. Because this method allows the controlled and sustained release of a compound in a cell specific manner, it could be an extremely useful tool for delivering metabolism-modifying compounds to T cells.

An example of this type of drug encapsulation and delivery system is the use of biodegradable poly(lactide-co-glyceride) (PLGA) nanoparticles, which can be manipulated in several ways to optimize biocompatibility/biodegradability to specific applications, and allows controlled release of drug ranging from days to months [122]. The use of PLGA nanoparticles conjugated to anti-CD4 antibodies has proven to be an effective delivery system for leukemia inhibitory factor, a cytokine used to oppose Th17 cell differentiation and enhance Treg cell development in a mouse model of allograft rejection [123]. It is conceivable that this technology could be used to manipulate the metabolic microenvironment of T cells. For example, encapsulation of the glycolysis inhibitors 2-DG [14] or dichloroacetate [124], the L-type amino acid transport inhibitor JPH203 [125], or lactate transporter inhibitors like AR-C141990 [126], could be targeted to T cells as a way to block metabolic pathways in these cells, and this could be useful in settings where T cells are hyperactive, as in autoimmunity. The *de novo* fatty acid synthesis inhibitor Soraphen A [69] could be targeted in a similar way to CD4 T cells to modify the Treg to Th17 cell balance in autoimmune conditions. Finally, the AMPK agonist AICAR or 6-diazo-5-oxy-L-norleucine (DON), an inhibitor of glutaminase (enzyme that converts glutamine to glutamate), could be targeted to effector T cells to limit their inflammatory responses [21,127–129].

Another potential approach to specifically target T cell metabolism for therapy would be to use antibodies to block nutrient transporters. The SLC family of membrane transporters includes over 300 genes that code for solute carrier proteins (SLCs) [130]. Proteins in the SLC family transport various molecules across the membranes surrounding the cell and its component parts [130]. There is often competition for nutrients between cells in a given microenvironment: for example, as is the case between tumor cells and T cells. During cancer progression, tumor

cells can outcompete T cells for nutrients in a solid tumor [18,99]. This differential usage of nutrients, as well as the fact that many transporter families have multiple isoforms with different substrate specificities, transport kinetics, and expression levels between different cell populations, suggests that targeting distinct transport proteins with antibodies might render only one cell type susceptible to therapy, while leaving the others unperturbed.

In terms of gaining a higher level of specificity, bispecific antibodies could be applied to this approach. Bispecific antibodies are engineered proteins composed of two independently targeted Fab regions within an antibody, or two separate antibodies, targeted against distinct epitopes, connected by a linker protein [131,132]. These proteins have been primarily explored as cancer therapies where the bi-specific antibody is used to simultaneously bind both a tumor cell and cytotoxic T cell [133,134], or in the case of tri-functional antibodies, the protein can additionally bind a cell with a Fc receptor, such as a macrophage or dendritic cell [135]. The result of this association is to bring these cells into close proximity such that the T cells can kill the tumor cells in a targeted fashion [131,132]. In the context of T cell metabolism, the use of a strong-binding antibody against a cell surface marker, such as CD8 on T cells, could be used in conjunction with a weak-binding antagonistic antibody against a specific substrate transporter. This system could allow the preferential binding to the target cell population by the dominant antibody, followed by specific inhibition of cell surface transporter by the auxiliary antibody. The advantage of this approach would be that the lower-affinity antibody against the nutrient transport protein would only bind if held in direct proximity by the high-affinity antibody, resulting in nutrient transporter inhibition only on cells that express CD8.

It is fairly obvious how blocking Glut1 on T cells would dampen their ability to use glucose, and thus inhibit their activation and function. However, there are many other transporters which could be targeted to alter T cell metabolism. For example, blocking amino acid transporters such as Slc1a5, Slc7a5, and Slc3a2, which are required for effector T cell metabolic reprogramming and differentiation following stimulation [25,125,136], could effectively inhibit T cell effector function or activation *in vivo*. This approach could be particularly useful in autoimmune disease, where dampening effector T cell function would be advantageous. Recent data suggest that Treg cells acquire exogenous fatty acids, while Th17 cells synthesize fatty acids intracellularly [69]. Perhaps targeting fatty acid transporters on T cells would be a way to inhibit Treg cell development. A strategy such as this might be useful in a tumor setting where infiltration with Treg cells is associated with poor prognosis.

Concluding remarks

Cells in the immune system undergo dynamic changes in metabolism during an immune response. In the past several years a wealth of exciting data has emerged illustrating how metabolism supports many aspects of T cell biology, as well as how metabolic changes drive T cell differentiation and fate. Because cellular metabolism is linked to immune cell function, understanding more about metabolic pathways in T cells will likely illuminate new ways to exploit these pathways to harness immunity *in vivo*. Approaching this task with an appreciation for the role of metabolism in dictating immune cell function could likely result in revolutionary new treatments, as well as prove to be a fruitful area of exciting future research.

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