Cancer Immunology Research

Metabolic Consequences of T-cell Costimulation in Anticancer Immunity

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Abstract

T-cell functional behavior and performance are closely regulated by nutrient availability and the control of metabolism within the T cell. T cells have distinct energetic and anabolic needs when nascently activated, actively proliferating, in naïveté, or in a resting, memory state. As a consequence, bioenergetics are key for T cells to mount adequate immune responses in health and disease. Solid tumors are particularly hostile metabolic environments, characterized by low glucose concentration, hypoxia, and low pH. These metabolic conditions in the tumor are known to hinder antitumor immune responses of T cells by limiting nutrient availability and energetic efficiency. In such immunosuppressive environments, artificial modulation of glycolysis, mitochondrial respiratory capabilities, and fatty acid β-oxidation are known to enhance antitumor performance. Reportedly, costimulatory molecules, such as CD28 and CD137, are important regulators of metabolic routes in T cells. In this sense, different costimulatory signals and cytokines induce diverse metabolic changes that critically involve mitochondrial mass and function. For instance, the efficacy of chimeric antigen receptors (CAR) encompassing costimulatory domains, agonist antibodies to costimulatory receptors, and checkpoint inhibitors depends on the associated metabolic events in immune cells. Here, we review the metabolic changes that costimulatory receptors can promote in T cells and the potential consequences for cancer immunotherapy. Our focus is mostly on discoveries regarding the physiology and pharmacology of IL15, CD28, PD-1, and CD137 (4-1BB).

Mitochondrial Functions in T Cells

Mitochondria act as central regulators of T-cell function and also regulate aerobic metabolism in these cells. In fact, mitochondria are "hubs" for a number of signaling pathways and functional processes in T cells, and are the epicenter of some of the most relevant processes for T-cell metabolism by directly regulating the catabolic/anabolic equilibrium and the speed/efficiency of energy requirements. Resting, naïve T cells rely on mitochondrial respiration and fatty acid oxidation (FAO) to survive in their quiescent state, minimizing the presence of toxic metabolites that could damage cell performance. Upon activation, T cells upregulate glycolysis (1) but continue to use aerobic respiration to generate the ATP that is needed for proliferation and T-cell activity. Concomitantly, one-carbon metabolic pathways are induced to produce the building blocks that will be needed for the rapid anabolic processes demanded by fast proliferation (2). Acetyl CoA is produced as a product of the Krebs cycle or FAO and plays an important role in acetylation reactions that promote epigenetic changes during T-cell activation (3). This is found to be especially relevant in the control of IFNγ production. Alpha-ketoglutarate, produced by the Krebs cycle, remains an essential component of demethylation reactions, including demethylation of DNA genomic sequences (4). In contrast, memory T cells will predominantly acquire a metabolism based on FAO and mitochondrial respiration (5), as metabolic needs are minimal at this stage.

During T-cell activation, mitochondrial respiration also results in the production of reactive oxygen species (ROS) that stimulate NFAT, a key transcription factor responsible for early events of T-cell activation such as IL2 transcription (6). ROS can also directly inhibit oxygen-dependent histone demethylases, which are critical for epigenetic remodeling and reprogramming (7).

Mitochondria are also key players in the apoptosis cascade regulating cytochrome C and apoptotic protease activating factor-1 (APAF) release, which are initial mitochondrial mediators of the caspase-3/7 apoptotic pathway, by multiple mechanisms (8). Costimulation usually upregulates antiapoptotic proteins such as Bcl-2 and Bcl-XL (8), which inhibit the mitochondrial routes of cell death. It is likely that mitochondrial functional parameters controlled by T-cell signaling routes may also regulate these mitochondrial functions in apoptosis (9).

Mitochondria have also been found to participate in T-cell migration and adhesion. Mitochondria can become polarized to particular T-cell areas during cell migration and cell–cell contact, where they provide local ATP to sustain actin dynamics and vesicular trafficking (10). Mitochondrial repositioning is found in T cells undergoing migration (11) and the underlying immune synapses (12) with antigen-presenting cells (APC). It has been reported that alteration of mitochondrial fission dynamics

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impairs appropriate polarization and, therefore, assembly of T-cell receptor clustering at the immune synapse and the migration toward chemokines. Polarization of mitochondria to the T-cell–APC contact site is also required for effective calcium influx and appropriate T-cell activation (13). Mitochondria are, hence, central players in a number of biological processes exerted by T cells, and, thus, their functional regulation by costimulation signals can profoundly shape T-cell biology.

T-cell Bioenergetics in Cancer Immunotherapy

Solid tumors are heterogeneous masses composed of many different cell types and soluble mediators, and during carcinogenesis, tumor cells can escape antitumor immune responses. Although T cells do play a pivotal role in the control of tumor growth, cancer cells have developed a number of mechanisms to impede T-cell function within the tumor microenvironment (TME). To perform effector functions, T cells need to energetically adapt to sustain all the cellular processes needed for target cell recognition, killing, cytokine production, and proliferation. A number of features of the TME, which are related to T-cell and malignant cell metabolism, can limit T-cell performance. The hyperactive metabolism of tumor cells can deprive T cells of the nutrients needed for their function. Chang and colleagues showed that enhancing glycolysis in a sarcoma model by educating tumor cells *in vitro* to high glucose concentrations increases glucose consumption by these tumor cells. This increased glucose uptake by transformed cells limits T-cell function by depriving them of glucose as an energetic source (14). In another study, $CD4^+$ Th1 responses were dependent on glucose concentrations within the TME of *Braf*/*Pten* genetically induced melanomas (15).

HIF1α is the main transcription factor activated by hypoxic conditions and controls T-cell responses in hypoxic environments such as tumors. Its activation activates glycolysis in an oxygen-independent mechanism to obtain energy. In line with previously described results, T cells that lack HIF1α in a conditional knockout model fail to control tumors due, in part, to their inability to activate glycolysis (16). Tumor-infiltrating lymphocytes (TIL) were shown to enhance fatty acid catabolism in the TME as a consequence of low glucose and oxygen concentrations (17). Some metabolites, such as lactate produced by tumor cells as a consequence of their metabolism, may dampen T-cell function when accumulated within tumors (18).

Observations on T-cell metabolic performance *in vivo* by genetic ablation or *ex vivo* metabolic profiling reveal important differences between metabolic performance in physiologic/pathologic conditions *in vivo* and in cell cultures. *In vivo*, and most particularly in tumors, T cells are exposed to lower concentrations of nutrients, different relative concentrations of energy sources, toxic metabolites, lower/changing concentrations of oxygen, and possibly changes in pH that are not mimicked in the cell cultures. Thus, effector functions studied in cell culture containing supraphysiologic concentrations of oxygen and nutrients may not reflect what is occurring in the TME. These particular limitations in tumor-related studies have been extensively reviewed elsewhere (19).

Because metabolic insufficiency is a relatively common defect in tumor-infiltrating T cells, approaches to enhance or recover the metabolic fitness of T cells have been attempted to maximize their antitumor function. For instance, promoting mitochondrial functions with drugs that directly promote mitochondrial activity or biogenesis (20) shows promising effects in combination with anti–PD-1 administration in preclinical mouse models. Immunotherapy treatments that rely on the use of adoptive T-cell transfer [expanded TILs, transgenic T-cell receptors (TCR), or chimeric antigen receptor (CAR) T cells] also benefit from the selection of metabolically fit cells or transgenic modifications to enhance T-cell bioenergetics. Extensive evidence supports the fact that enhanced mitochondrial respiration and FAO can increase the persistence of adoptively transferred T cells, favoring better tumor control in preclinical models (21).

Regulation of T-cell Metabolism by Costimulatory Molecules

To modulate TCR signaling and avoid anergy or T-cell overactivation, T cells express in their membrane costimulatory and coinhibitory receptors. These are membrane receptors that belong to either the TNFR or the immunoglobulin superfamily, and the integration of costimulatory/coinhibitory signals controls T-cell survival, proliferation, and functional differentiation (8). Emerging data collectively show that signaling from these molecules modulates metabolic routes in T cells, highlighting new mechanisms by which interventions on these costimulatory/coinhibitory molecules may promote better antitumor performance of T cells, which can be exploited in immunotherapy.

Metabolic control of T cells by the CD28 family

CD28 is expressed constitutively on naïve T cells. CD28 ligation enhances TCR signaling but drives unique signaling events (22). The early mechanisms that drive unique signaling by CD28 have been difficult to identify, but the functional effects provided by CD28 costimulation are well-known (IL2 production, Bcl-XL upregulation, alternative splicing of some genes, stabilization of some RNAs, etc.; ref. 22). CD28 can be exploited in cancer immunotherapy by means of its inclusion in the cytoplasmic signaling domains in CARs. A deep understanding of the main downstream functional effects of CD28 signaling is, therefore, particularly relevant for the choice of a specific CAR intracellular sequence.

Emerging data have shown that CD28 costimulation plays a pivotal role in the control of T-cell metabolism. CD28 costimulation upregulates GLUT1, the main transmembrane channel involved in glucose import into cells (23), and hexokinase, involved in the first regulated step of glycolysis, the phosphorylation of glucose into glucose-6-phosphate (24), in T cells. Both proteins regulate substrate availability within the T cell, and as a consequence, their upregulation activates glycolysis (25). CD28 also activates mTOR, a signaling kinase that is a central player in cell metabolism, and its regulation importantly affects a variety metabolic routes such as protein, lipid, and sugar catabolism/anabolism. CD28 also provides transcriptional upregulation of metabolic machinery, like nutrient transporters, in an mTOR-dependent manner (26). Findings have shown that CD28 also controls mitochondrial performance in $CD8^+$ T cells. CD28 cosignaling rapidly upregulates Cpt1a in T cells, shuttling fatty acids for oxidation to the mitochondria. Concomitantly, CD28 stimulation leads to enhanced cristae tightening in these organelles (27). Morphology and tightening of mitochondria cristae are directly related to respiratory function. Mitochondria can form extensive networks generating large and tubular units in the cells.

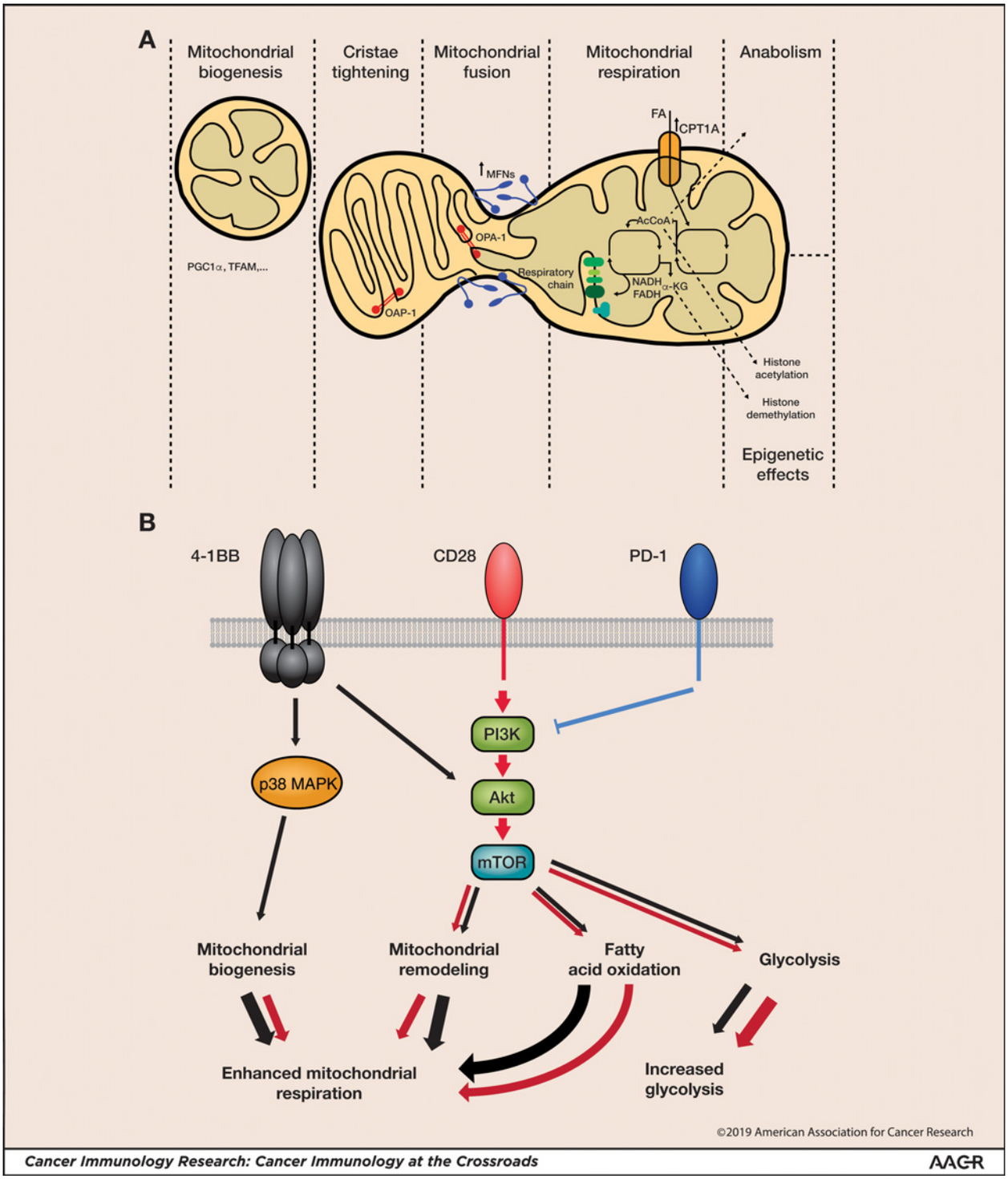

Figure 1.
Metabolic effects induced by costimulation of T cells. **A,** Different metabolic routes, implicated signaling pathways, and final metabolic outputs regulated by costimulatory receptors in T cells. Dotted arrows, export of the tricarboxylic acid cycle (TCA) intermediates in extramitochondrial functions. Solid arrows, metabolic routes of molecules within mitochondria. Red circles with lines, OPA-1 molecules. Blue lines with ovals, mitofusions (MFN; according to their proposed shape). Green-scale shapes, components of the respiratory chain. Orange shape, the CPT1A transporter. AcCoA, acetyl-CoA; α-KG, α-ketoglutarate; FA, fatty acid. **B,** Biological processes of mitochondria related to metabolism that can be controlled by costimulatory molecules and T-cell signaling. (*Continued on the following page.*)

Mitochondria can also be found in small, round independent units. Small and separated mitochondria are less effective in energy production and more prone to mitophagy, whereas larger mitochondrial networks exhibit efficient energy production and rarely undergo mitophagy (28). Mitochondria can very dynamically change between these two extreme morphologies in a process that is regulated by the mitochondrial fusion (MFN-1 and MFN-2) and fission (DRP-1 and FIS-1) proteins (Fig. 1A). Dynamic reorganization of the cristae membrane within the mitochondria is also very important in the control of respiratory efficiency. OPA-1 (optic atrophy one) is the main protein responsible for controlling fusion and reorganization of cristae within the mitochondria. Specific functions and regulation of this key protein in mitochondrial dynamics have been reviewed in-depth and involve posttranscriptional proteolytic cleavage of OPA-1 (29). Effective cristae tightening mediated by OPA-1 favors the formation of respiratory chain protein supercomplexes and enhances the efficiency of its respiratory functions (30). This temporal mitochondrial remodeling during T-cell priming has surprisingly relevant consequences for subsequent memory T-cell differentiation by mechanisms that are yet unclear.

ICOS is a costimulatory molecule of the CD28 family whose expression is induced following antigen activation. ICOS has been identified to directly modulate metabolism. For instance, during T-follicular helper (Tfh) differentiation, ICOS ligation promotes glucose uptake and metabolism via mTOR activation (31). Although ICOS activation has been used and proposed as a target in cancer immunotherapy, both by agonist pharmacologic activation (32) and by inclusion of its cytoplasmic tail in CARs (33), very little information is available on how its ligation promotes T-cell metabolism in the context of cancer immunotherapy.

The coinhibitory receptor PD-1 has also been linked to T-cell metabolism. Bengsch and colleagues have shown that hyporesponsive cells in chronic lymphocytic choriomeningitis virus (LCMV) infection rapidly lose glycolytic capacity and mitochondrial function. In their hands, virus-specific $CD8^+$ T cells that lacked PD-1 did not experience such metabolic dysfunctions, thus suggesting a role of PD-1 in regulating bioenergetic flux in T cells (34). Similarly, Scharping and colleagues have shown that TILs rapidly lose functional mitochondrial mass and metabolic capacity upon activation within tumors. In their experiments, PD-1 blockade slightly, but not significantly, enhanced mitochondrial mass in TILs, suggesting that other additional factors present in the TME are responsible for the crippling mitochondrial atrophy in T cells (35).

Metabolic control of T cells by the TNFR family

The TNFR family of costimulatory molecules is composed of more than 25 receptors. Most of the transmembrane receptors of this superfamily expressed on T cells are considered to be costimulatory receptors. Members include CD137, OX40, CD27, and GITR, all of which are being pursued as targets in cancer immunotherapy. Among all these receptors, CD137 (4-1BB) has been the most explored molecule in relation to metabolic control of T cells. CD137 is a molecule of great interest in tumor immunology because of the many different strategies that induce its activation.

CD137 activation on T cells promotes, like CD28, glucose metabolism by upregulating GLUT1 on the T-cell surface (36). When comparing the cytoplasmic tails of CD137 and CD28 used in CAR-T cells, CD137's cytoplasmic tail promoted less efficient glycolysis than CD28-containing CAR-T cells (37). In contrast, CARs containing the cytoplasmic tail of CD137 were more efficient in promoting mitochondrial respiration. Activation of CD137 by agonistic antibodies is also able to promote enhanced respiratory capacity, even at very early time points after stimulation and more efficiently than CD28 (38, 39). We have shown in two simultaneous independent studies that CD137 enhances mitochondrial biogenesis and dynamics by controlling PGC1α (a protein that promotes mitochondrial biogenesis) and OPA-1 expression and function. In line with these observations, the CD137 cytoplasmic tail inclusion in CAR-T cells was able to promote mitochondrial biogenesis (37–39). This is a conserved mechanism that can be demonstrated in both human and mouse $CD8^+$ T cells using agonist antibodies or CD137L as a natural ligand (39).

Previous studies have shown that CD137 activation enhances FAO. FAO is needed for mitochondrial antiapoptotic functions induced by CD137 and most likely is important for the enhanced mitochondrial respiratory functions of $CD8^+$ T cells receiving CD137 costimulation (36). Importantly, mitochondrial function enhancement, promoted by CD137 ligation, also occurs *in vivo* in tumor-infiltrating $CD8^+$ T cells. Treatment with anti-CD137 agonists reinvigorates T cells metabolically to efficiently act against cancer, and interfering with this metabolic enhancement impedes their immunotherapeutic effects. Interestingly, sequenced immunotherapy treatment with anti-CD137 allowed $CD8^+$ T cells to become responsive to PD-1 blockade in B16 melanoma. Although the involved signaling routes are not completely well understood, our data suggest that both signaling through Akt in conjunction with TCR signaling and TCR-independent signaling through p38 MAPK are needed for the overall enhancement in mitochondrial function of $CD8^+$ T cells (Fig. 1B). Therefore, CD137 costimulation induces mitochondrial biogenesis and individual mitochondria enlargement and promotes their respiratory functions, allowing cytotoxic T lymphocytes to better perform in the TME and to persist in adoptive transfer experiments. We believe that CD137 ligation starts a metabolic program in $CD8^+$ T cells that is distinct from CD28 agonism in some processes, while similar in others (Fig. 1B). In this sense, both costimulatory receptors boost glycolysis and mitochondrial respiration during the effector phase. However, CD137 seems to be a more effective mitochondrial stimulator. This could be due to mitochondrial fusion and cristae remodeling mediated by OPA-1 and MFN2, which in conjunction with enhanced biogenesis upregulated by PGC1α and TFAM (main transcription factor involved in mitochondrial DNA replication) and augmented FAO resulting from CPT1a upregulation, sustains enhanced mitochondrial function (Fig. 1A).

OX40 is another member of TNFR costimulatory receptors that is being exploited in cancer immunotherapy using agonist

(*Continued.*) The main molecular players for each process are highlighted. Engagement of 4-1BB (CD137; black arrows) leads to p38 MAPK activation, as well as activation of Akt signaling. Engagement of CD28 (red arrows) also results in PI3K/AKt/mTOR activation. These interactions lead to mitochondrial biogenesis and remodeling and FAO or glycolysis. PD-1 interaction with ligands (blue blunted line) can inhibit this signaling. These metabolic steps can lead to enhanced mitochondrial respiration and further increased glycolytic function. 4-1BB activation leads to comparatively more mitochondrial activity (represented by larger, weighted black arrows in the mitochondrial functions). CD28 activation, however, leads to a more pronounced glycolytic phenotype (represented by the larger, weighted red arrow).

antibodies. OX40 receptor has a similar biology to CD137, and its activation is likely to promote metabolic effects on T cells. However, so far, no solid experimental information on this is available. It has been shown in human immunodeficiency virus–positive patients that OX40 expression correlates with high Glut1 expression on $CD4^+$ T cells, although no functional relationship has been documented (40). Similarly, tumor-infiltrating regulatory T cells (Treg) expressing high OX40 are found to be enriched in genes involved in glycolysis and fatty acid synthesis (41). Interestingly, treatment with agonist OX40 antibody increases the lipid content in tumor-infiltrating Tregs, although no other mechanistic evidence of OX40's role in this observation was provided.

GITR, another member of the TNFR costimulatory family, has also been shown to upregulate a number of metabolic factors in $CD8^+$ T cells. Similar to CD137, GITR activation with agonist mAbs enhances glycolysis and mitochondrial respiration and promotes FAO and lipid uptake. *In vivo*, GITR agonism with mAbs promotes metabolic fitness in tumor-reactive T lymphocytes located in tumor-draining lymph nodes or mouse tumors (42).

Metabolic control of T cells by the IL15/IL2 axis

Cytokines have also been shown to regulate T-cell metabolism and, therefore, are key players in shaping T-cell bioenergetics important for tumor immunotherapy. IL2 is a cytokine that drives T cells toward an effector-like phenotype that is metabolically characterized by enhanced glycolysis. However, IL15 more effectively upregulates T-cell mitochondrial respiration (5). IL15 promotes mitochondrial biogenesis to sustain oxidative phosphorylation and keep T-cell energy production efficient with low toxicity for long periods of time, as is needed in memory T cells. To this end, IL15 promotes FAO, a metabolic pathway also related to long-lasting memory T cells (5). IL15 regulates mitochondrial dynamics to promote a more interconnected network of fused mitochondria that enhances the energetic efficiency of mitochondrial respiration (43). As observed for CD137, IL15 also upregulates OPA-1. In the case of IL15, a study directly showed enhanced cristae tightening using transmission electron microscopy (43). Therefore, IL15 gives rise to a metabolic signature able to sustain the long-lived phenotype of memory cells.

IL7 is a homeostatic cytokine needed for T-cell survival and is also important in memory T-cell homeostasis. With regard to T-cell metabolism, IL7 promotes glycerol uptake by aquaporin-9 in a way that supports continuous triacylglyceride generation to fuel FAO as needed for mitochondrial respiration in memory T cells (44). In keeping with these findings, mice with conditionally ablated IL7R in T cells showed diminished glycolytic capacity and atrophy of this memory T-lymphocyte population, hence linking IL7 homeostatic functions to glucose metabolism (45).

The Road Ahead for Metabolic Enhancement of Cancer Immunotherapy

All strategies that aim to take direct advantage of T cells to induce tumor rejection in patients require thoughtful consideration of their energetic costs. We and others have shown that CD137 signaling promotes increases in $CD8^+$ T-cell mitochondrial mass and function, and that this metabolic reprogramming is a key mechanism of action behind its immunotherapeutic potential. This is a costimulatory molecule of particular interest because of the excellent clinical results with CARs encompassing the CD137 cytoplasmic tail (46). Metabolomics of T cells is likely to be a rising field to identify molecular biomarkers for response to immunotherapy, such as the measurement of T-cell fitness.

The road ahead needs to further clarify the differential modulation that costimulatory receptors and cytokines induce in the metabolic routes of T cells. Of much importance, well-known drugs in endocrinology that have potent metabolic reprogramming functions, such as metformin or bezafibrate, have been found to invigorate T-cell mitochondrial functions and synergize with PD-1 blockade (47, 48). Taking advantage of efficient costimulation and regulation of bioenergetics will substantially benefit the performance of T cells in adoptive transfer strategies (CAR-T cells, expanded T cells transduced with specific TCRs, or TIL cultures), as well as antibody-based immunotherapy acting on costimulation or coinhibitory receptors.

Disclosure of Potential Conflicts of Interest

I. Melero reports receiving commercial research grants from Bristol-Myers Squibb, Roche, Alligator, and Bioncotech, has received honoraria from the speakers bureau of MSD, has ownership interest (including patents) in Tusk, and is a consultant/advisory board member for Bristol-Myers Squibb, Roche, Bayer, EMD Serono, Numab, Genmab, Bioncotech, and MSD. G.M. Delgoffe is the Founder of and a scientific consultant for TTMS, Inc., is a consultant for Pieris Pharmaceuticals, reports receiving other commercial research support from Pfizer, Bluebirdbio, and TCR2 Therapeutics, and has ownership interest (including patents) in TTMS, Inc. No potential conflicts of interest were disclosed by the other authors.

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