Metabolic regulation of immune responses: therapeutic opportunities

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Immune cell metabolism is dynamically regulated in parallel with the substantial changes in cellular function that accompany immune cell activation. While these changes in metabolism are important for facilitating the increased energetic and biosynthetic demands of activated cells, immune cell metabolism also has direct roles in controlling the functions of immune cells and shaping the immune response. A theme is emerging wherein nutrients, metabolic enzymes, and metabolites can act as an extension of the established immune signal transduction pathways, thereby adding an extra layer of complexity to the regulation of immunity. This Review will outline the metabolic configurations adopted by different immune cell subsets, describe the emerging roles for metabolic enzymes and metabolites in the control of immune cell function, and discuss the therapeutic implications of this emerging immune regulatory axis.

Metabolic configurations match with immune function

Immune cell subsets have extremely diverse functions that can be associated with very different metabolic demands. For instance, while naive lymphocytes are relatively inert cells that require energy in the form of ATP but engage in minimal levels of cellular biosynthesis, activated lymphocytes have an extremely active metabolism that is required to facilitate robust growth, rapid cellular proliferation, and the production of large quantities of effector molecules. Unsurprisingly, the cellular metabolic configurations of these two cell types are very different; naive lymphocytes prioritize the production of ATP, while activated lymphocytes must ensure that cellular metabolic pathways are tuned to provide both energy and the molecules required for highly active biosynthetic pathways (1). Indeed, immune cell metabolism can be adjusted to a range of configurations to meet diverse cellular activities.

Aerobic glycolysis fuels cellular biosynthesis

To efficiently generate ATP, glucose is metabolized to pyruvate by glycolysis, and the pyruvate is further metabolized in the mitochondria to CO_2 and reducing equivalents (NADH and $FADH_2$) via the KREBS cycle, with the latter driving oxidative phosphorylation (OxPhos) for ATP synthesis (Figure 1). Under conditions of hypoxia, cells will metabolize glucose to lactate (anaerobic glycolysis), which is an inefficient way to make ATP (two molecules per glucose) but the only way to maintain energy homeostasis in the absence of oxygen. Following immune stimulation, effector lymphocytes engage in a form of glucose metabolism termed "aerobic glycolysis," in which glucose is metabolized to lactate in the presence of abundant oxygen. Additionally, these cells maintain high levels of OxPhos for ATP production. Aerobic glycolysis is a metabolic pathway that sacrifices efficiency in favor of speed (1, 2). In cells using aerobic glycolysis, glucose is used in greatly increased quantities as a key source of carbon molecules for biosynthesis as well as a fuel for ATP synthesis (Figure 1). Intermediates of glycolysis and the KREBS cycle can be converted to key biosynthetic precursors for the synthesis of protein, nucleic acids, and lipids; however, a large proportion of the glucose-derived carbon is secreted as waste in the form of lactate. While this appears to be an inefficient use of glucose-derived carbon, lactate secretion allows for greatly increased rates of glycolytic flux and, thus, increased rates of biosynthesis, as it regenerates the cofactor NAD^+, which is essential for the sixth step of the glycolytic pathway (Figure 1). During an immune response speed is of great importance, and the priority for activated lymphocytes is to achieve a maximal rate of clonal expansion, which is dependent on the rate of biomass synthesis. Therefore, when glucose is abundant, activated lymphocytes compromise metabolic efficiency and secrete large amounts of lactate in order to maximize the rate of cellular biosynthesis. In activated lymphocytes glutamine is also an important fuel that feeds the KREBS cycle to support both cellular biosynthesis and the production of ATP (Figure 1 and ref. 3).

Similarly to activated lymphocytes, LPS-activated DCs and M1 macrophages engage in aerobic glycolysis, but they also inactivate OxPhos, blocking mitochondrial ATP synthesis (4). This response allows these cells to repurpose the KREBS cycle enzymes and metabolites for inflammatory purposes (discussed below). The absence of mitochondrial ATP synthesis appears to contribute to the short life span of these cells, which is not surprising given the important role for mitochondrial energy metabolism in controlling apoptosis. Certainly, sustaining rates of OxPhos in DCs results in increased DC survival and prolonged DC-induced T cell responses (5). While these cells do not proliferate, glucose is still a key fuel for cellular biosynthesis and is required to meet the biosynthetic demands associated with the production of large quantities of cytokines and other effector molecules (6, 7).

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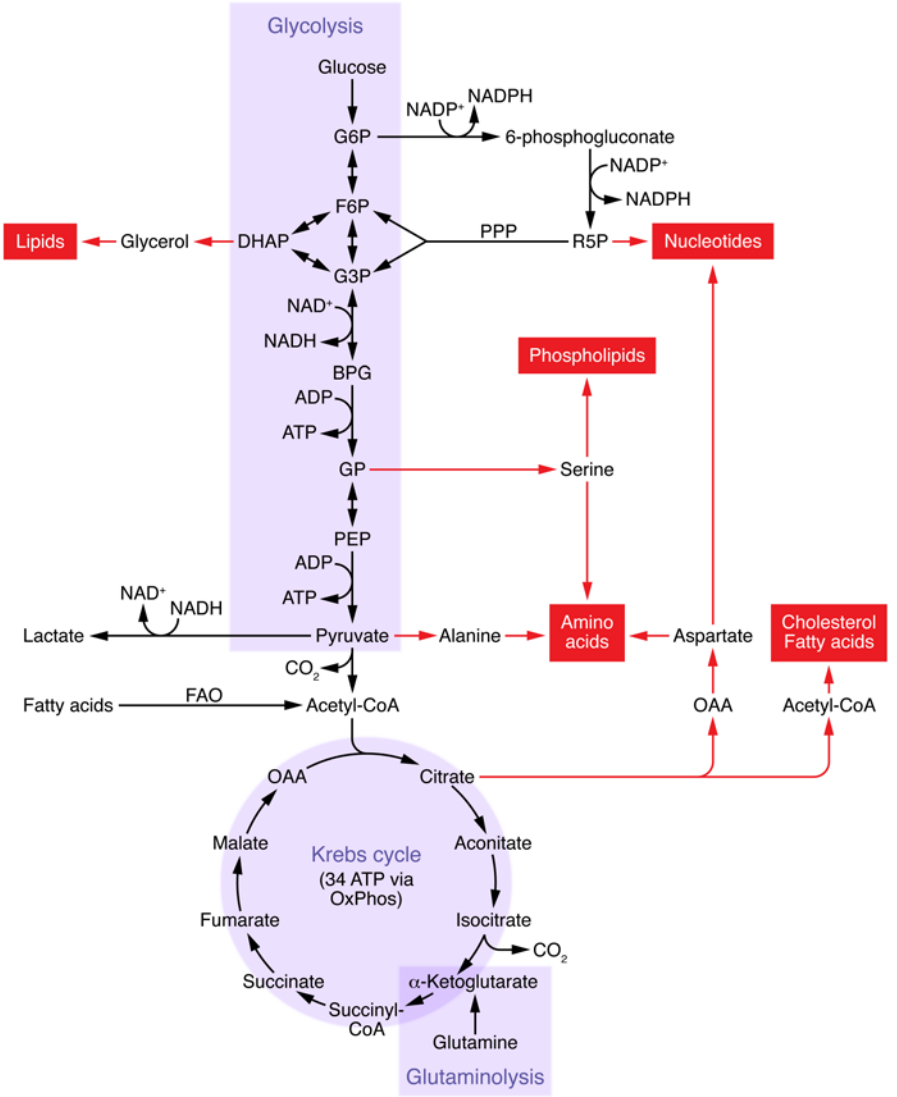

Figure 1. Glucose can be used for ATP synthesis and cellular biosynthesis. ATP is the key molecule that provides energy for cellular processes. Glucose can be metabolized via two integrated metabolic pathways, glycolysis and OxPhos, which efficiently convert glucose into ATP. Glycolysis converts glucose to pyruvate in the cytosol, generating two molecules of ATP. In the mitochondria, pyruvate is further metabolized to CO_2 by the KREBS cycle, which drives OxPhos and ATP synthase activity, generating up to 34 ATP per molecule of glucose. Cells can also metabolize alternative substrates, such as lipids and glutamine, which feed into the KREBS cycle to drive OxPhos and ATP synthesis. Aerobic glycolysis supports biosynthetic processes, as it allows the uptake of larger amounts of glucose and the maintenance of elevated glycolytic flux. Glycolytic intermediates are then diverted into other pathways for synthesis of biomolecules that support biosynthetic processes. For instance, glucose-6-phosphate (G6P) can feed into the pentose phosphate pathway (PPP), generating ribulose-5-phosphate (R5P) to support nucleotide synthesis. This pathway also generates NADPH, a cofactor that is essential for various biosynthetic processes, including lipid synthesis. Glucose can also be converted into cytoplasmic acetyl-CoA via citrate in the KREBS cycle for the production of cholesterol and fatty acids. Glycolytic intermediates are also converted into other biomolecules for protein and lipid synthesis. Glutamine feeds into the KREBS cycle and can also supply biomolecules for biosynthetic processes under certain conditions. F6P, fructose-6-phosphate; DHAP, dihydroxyacetone phosphate; G3P, glyceraldehyde 3-phosphate; BPG, 2,3-bisphosphoglycerate; GP, glycerate 3-phosphate; OAA, oxaloacetate.

OxPhos facilitates cellular longevity

Immune cells for which cell longevity is a key priority, such as memory T cells, adopt an oxidative metabolism that relies on OxPhos for energy production (7). In fact, oxidative metabolism is essential for the formation of long-lived memory T cells; promoting OxPhos enhances memory T cell formation, while inhibiting fatty acid oxidation–dependent (FAO-dependent) OxPhos represses memory T cell formation (8, 9). There are a number of studies that also support the notion that promoting OxPhos enhances cell survival and life span (10–12). Memory T cells use glucose and other fuels to synthesize an energy store in the form of triglycerides, which are then broken down by FAO, to fuel ATP synthesis (13, 14). In this way, these cells maintain both glycolysis and OxPhos primed and ready for immune activation, allowing memory T cells to initiate a metabolic response much quicker than naive T cells (15, 16).

While M1 macrophages form part of the first line of defence within hours to days of an immunological challenge, M2 macrophages are longer lived and have important roles within the resolution phase of the immune response and in tissue repair and remodeling (17). M2 macrophages adopt an oxidative metabolism that is similar to the metabolism of memory T cells, fueling OxPhos with fatty acids that have either been scavenged from the surrounding microenvironment or synthesized de novo from glucose (18). However, unlike memory T cells, M2 macrophages have significant biosynthetic requirements to support the synthesis of large quantities of effector molecules, including chemokines and the antiinflammatory cytokines IL-10 and TGF-β. Given that macrophages are professional scavengers of apoptotic debris and other biological molecules, including lipids, it is likely that M2 macrophages sustain cellular biosynthesis using biomolecules scavenged from the surrounding microenvironment (17, 18).

Targeting cellular metabolism to therapeutically alter immune responses

The realization that disrupting the balance of metabolic pathways can redirect the fate of immune cells reveals a number of therapeutic opportunities for a range of diseases. Below, potential strategies to therapeutically alter cellular metabolic pathways in immune cells will be discussed with respect to the treatment of tumors, infection, and autoimmunity.

Promoting glycolysis to maximize proinflammatory antitumor responses. The tumor microenvironment contains a range of conditions that are unfavorable for mounting an antitumor immune response. Preventing glycolytic metabolism in tumor-infiltrating effector lymphocytes by limiting glucose availability is one immune evasion strategy used by tumors (19–21). Disrupting glycolysis in effector T cells and NK cells inhibits the production of the proinflammatory cytokine IFN-γ and the expression of molecules important for cellular cytotoxicity (9, 16, 22–24). Low glucose levels also enforce oxidative metabolism on tumor-infiltrating macrophages, which inhibits proinflammatory functions and promotes an antiinflammatory macrophage phenotype (25, 26). Therapies that target the mechanisms through which tumors disrupt glycolytic metabolism in tumor-infiltrating leukocytes would be predicted to boost antitumor immune responses.

Tumor cells have very high rates of glucose uptake that create a glucose-deficient tumor microenvironment (20, 21, 27), and removing the fuel glucose directly inhibits glycolysis in infiltrating immune cells. Indeed, this may be an important reason why a range of adoptive transfer-based immunotherapeutic strategies that have good efficacy for hematological malignancies have limited success in the treatment of solid tumors (28, 29). The coordinated use of traditional anticancer therapies alongside immunotherapeutic strategies may provide added therapeutic value (30, 31). Chemotherapy and radiotherapy have been proposed to act as an adjuvant to immunotherapy by interfering with the mechanisms used by tumors to create an immunosuppressive environment in various ways (30, 31). Inducing cell death pathways in a proportion of tumor cells will reduce the demand for glucose and therefore increase glucose levels in the tumor microenvironment. Therefore, coordinated use of chemotherapy or radiotherapy may be an important mechanism to enhance immunotherapies by increasing glucose availability to facilitate glycolysis and robust antitumor lymphocyte responses.

Within the tumor microenvironment, tumor cells and myeloid suppressor cells often express ligands for inhibitory checkpoint receptors on effector T cells, such as programmed death ligand-1 (PD-L1), which binds to the inhibitory programmed cell death-1 (PD-1) T cell surface molecule. Interestingly, these interactions affect glycolytic dynamics within the tumor in multiple ways. First, ligation of the inhibitory receptors PD-1 or cytotoxic T lymphocyte antigen-4 (CTLA-4) alters signal transduction to directly inhibit T cell glycolysis (32). Additionally, PD-L1 signals to the tumor cells to increase glucose uptake and glycolysis, thereby depleting glucose from the tumor microenvironment (20). The checkpoint blockade antibodies anti–PD-1, anti–CTLA-4, or anti–PD-L1 block these interactions; relieve the direct inhibition of T cell glycolysis; and result in increased levels of glucose within the tumor microenvironment (20). Together, these events facilitate glycolytic metabolism in tumor-infiltrating T cells and optimal antitumor functions. There is also evidence in patients with multiple myeloma that NK cells express PD-1 and that anti–PD-1 antibody treatment enhances NK cell antitumor function (33–35). It is tempting to speculate that the effect of anti–PD-1 antibodies on NK cell function is because these antibodies are preventing PD-1–mediated direct inhibition of NK cell glycolysis; however, another possibility is that the increased NK cell function is due to increased glucose availability as a result of the effects on tumor cells, as discussed above. The direct impact that ligation of NK cell–expressed PD-1 has on NK cell glycolysis has yet to be investigated. If the competition for glucose in the tumor microenvironment is viewed as a "tug of war" between tumor cells and effector lymphocytes, then checkpoint blockade antibodies work to significantly shift the balance in favor of the immune cells. These antibodies have shown exciting antitumor activities against multiple tumor types and are currently approved by the US Food and Drug Administration for the treatment of metastatic melanoma and non–small-cell lung carcinoma (36–41). However, at this point it is not clear what proportion of the efficacy of these treatments is due to changes in metabolism.

The depletion of other nutrients may also indirectly impact glycolytic rates in immune cells by disrupting the activity of the metabolic regulator mTORC1 (22, 23). Tryptophan and arginine can be depleted in the tumor microenvironment by the action of the enzymes indoleamine-pyrrole 2,3-dioxygenase (IDO) and arginase-1, respectively. Multiple cells within tumors, including tumor cells and myeloid-derived suppressor cells, express IDO and arginase-1, and both the depletion of these amino acids and the resulting metabolites inhibit T cell and NK cell function (42–48). Importantly, activity of both IDO and arginase-1 has been shown to be sufficient to inhibit glycolysis in activated T cells, and tryptophan depletion can inhibit mTORC1 activity in T cells (46–48). Indeed, the effects of IDO on T cell responses mirror the effect of inhibiting mTORC1 or glycolysis in T cells, including inhibition of effector T cell responses and induction of Tregs (49–51). These data argue that nutrient-dependent regulation of mTORC1 activity, and thus glycolysis, in tumor-infiltrating lymphocytes may be important for the immunosuppressive functions of IDO. Inhibitors of IDO are at various stages in clinical trials and appear to have significant antineoplastic effects and the capacity to reactivate antitumor immune responses.

A two-pronged approach to increase glucose availability, by blocking PD-L1 or CTLA-4, and reestablish glycolytic signaling, by inhibiting IDO, may be particularly efficacious for the treatment of solid tumors. Indeed, an ongoing phase I/II clinical trial (ClinicalTrials.gov identifier NCT01604889) is testing the combination of an IDO inhibitor with the checkpoint blockade antibody ipilimumab (anti-CTLA4) in patients with metastatic melanoma. In general, immunotherapeutic approaches for solid cancers are likely to show increased efficacy if combined with strategies to create a more nutrient-replete tumor microenvironment. This notion is supported by numerous preclinical studies investigating combinations of strategies, though the importance of the tumor microenvironment and immune cell metabolism was largely unappreciated at the time. For instance, cancer vaccinations have been combined with checkpoint blockade antibodies, now known to enhance glucose availability and T cell glycolysis, to improve antitumor responses (52–55). Clinical trials have now been initiated to test the combination of a pancreatic cancer vaccine and an anti–PD-1 antibody, nivolumab (ClinicalTrials.gov identifier NCT02243371 and NCT02451982). In fact, multiple strategies that will affect immune metabolism within the tumor microenvironment have been investigated in the context of cancer vaccination (56). Patients treated with chimeric antigen receptor (CAR) T cell adoptive transfer–based immunotherapies for B cell

lymphoma receive a conditioning chemotherapy regimen prior to an infusion of anti-CD19 CAR T cells. It is tempting to speculate that this chemotherapy increases the efficacy of this treatment at least in part by effects on nutrient dynamics within the tumors. This is likely to occur through multiple mechanisms; the chemotherapeutic drugs will reduce the number of glucose-using tumor cells but have also been shown to decrease the expression of IDO, which negatively affects CAR T cell therapies (57). In preclinical studies the antitumor responses of CAR T cells were enhanced by the administration of checkpoint blockade antibodies (58). Therefore, the data clearly argue that effective therapeutics will encompass strategies designed to provide potent antitumor immune responses coupled with strategies that change the nature of the otherwise immunosuppressive tumor microenvironment, facilitating glycolysis in infiltrating antitumor effector lymphocytes.

The effect of nutrient-restricted conditions on tumor-infiltrating myeloid cells should also be considered. The tumor microenvironment is often populated with regulatory macrophages rather than proinflammatory macrophages, and this bias contributes significantly to the immunosuppressive conditions (59). Glucose metabolism is also closely linked to the proinflammatory versus antiinflammatory functions of macrophages. Elevated glycolysis and reduced OxPhos are important for the differentiation and function of inflammatory macrophages; sustaining OxPhos both inhibits proinflammatory outputs and promotes antiinflammatory functions (25, 26, 60–62). Therefore, glucose deprivation in the tumor microenvironment will promote a shift from proinflammatory to antiinflammatory macrophage function. Arginine depletion within the tumor microenvironment will also affect macrophage function. Arginine has been linked to the inhibition of OxPhos in activated DCs and macrophages as it is a substrate for NO synthase and is required for NO production (5, 63, 64). NO acts as an inhibitor of mitochondrial complex IV to block OxPhos; inhibition of NO production is predicted to promote regulatory macrophage differentiation and function (25, 60, 62). Indeed, arginine depletion has been shown to blunt antitumor T cell responses by inducing myeloid suppressor cells (46).

Promoting glycolysis to enhance immune response to infection. Competition for nutrients and oxygen also occurs at sites of infection, with implications for immune cell functions. For instance, infection with *Staphylococcus aureus*, a common human pathogen, can result in localized tissue hypoxia due to elevated levels of oxygen consumption by the invading bacteria (65). As glucose is a key fuel for these bacteria, the levels of glucose available to immune cells at the site of infection are likely to be low. Indeed, reduced glucose levels are reported in patients with bacterial meningitis (66). The first immune responders to bacterial infection, neutrophils, rely on glucose and glycolysis for key effector functions, including the formation of neutrophil extracellular traps (67–69). Viral infection may also lead to decreased glucose availability for infiltrating immune cells due to the infiltration of immune cells and the fact that virally infected cells increase glucose uptake to facilitate viral replication (70–75). Virally infected cells also increase the uptake of other nutrients, including the amino acid glutamine (74, 76), which is important for effector T cell responses (77). Interestingly, PD-1 is expressed on T cells and NK cells during various viral infections and is associated with dysfunctional or exhausted T cell and NK cell phenotypes (78–84). Therefore, many of the same conditions can exist at sites of viral and bacterial infection as seen in the tumor microenvironment. It will be of interest to establish whether glycolysis in infiltrating lymphocytes and neutrophils is similarly disrupted in these infectious microenvironments and whether this might be a target for novel therapeutic strategies.

Therapeutic manipulation of metabolism to inhibit effector T cell responses. Targeting metabolism may also be therapeutically beneficial for patients with autoimmune conditions or those receiving transplants by inhibiting proinflammatory immune cell functions. Direct inhibition of glycolysis, or disrupting the signaling pathways that support glycolysis, suppresses the differentiation of effector T cell subsets while promoting Treg generation in vitro (85, 86). Similarly, inhibition of glycolysis suppresses B cell antibody production in vitro, though a link between glycolysis and autoantibody production in vivo has yet to be investigated (87). However, while initial in vitro studies suggested that Tregs are nonglycolytic and rely instead on oxidative metabolism, there are now multiple examples in which this is not true. In mice, Foxp3$^-$ type 1 regulatory cells have high levels of glycolysis similar to effector T cells (88, 89). Furthermore, in humans, induced Tregs (iTregs) that express the Foxp3-E2 splice variant have high rates of glycolysis that are required for their potent immunosuppressive function (90). Indeed, inhibition of glycolysis prevents the formation of these Foxp3-E2 iTregs, and patients with multiple sclerosis or type 1 diabetes show decreased numbers of Foxp3-E2 iTregs (90). Therefore, while inhibition of glycolysis in the context of autoimmunity may inhibit the function of effector T and B cells, at present the impact upon Treg populations is not clear.

Oxidative metabolism is also important for effector T cell responses; inhibition of OxPhos or glutamine deprivation suppresses T cell clonal expansion and effector function (24, 91, 92). Indeed, inhibition of mitochondrial ATP synthesis was sufficient to inhibit alloreactive T cells in graft-versus-host disease models (93). A number of recent studies have tested the effect of simultaneously targeting glycolysis and mitochondrial metabolism on pathological T cell responses. Inhibition of glycolysis and OxPhos using 2-deoxyglucose and metformin, respectively, was shown to reduce disease severity in mouse models of lupus (94). Also, the combined inhibition of glycolysis, OxPhos, and glutaminolysis prevented graft rejection in fully mismatched skin and heart allograft transplantation models (95). While these two studies did not look at the impact of these drugs on effector T cells versus Tregs, the overall impact of metabolic inhibition in these disease models was the attenuation of autoreactive and alloreactive T cell responses (94, 95).

There may also be therapeutic value in targeting other aspects of T cell metabolism, such as de novo fatty acid synthesis. Th17 cells and Tregs have different requirements for fatty acid synthesis; Th17 cells rely on glycolysis to fuel de novo lipid synthesis to make biological membranes, whereas Tregs rely upon the uptake of exogenous fatty acids for this purpose (96). Inhibition of fatty acid synthesis restrains the formation of human and murine Th17 cells while promoting the differentiation of Tregs. This approach also ameliorates the pathogenesis of experimental autoimmune encephalomyelitis, a mouse model of multiple sclerosis (96). Additionally, aberrant lipid synthesis pathways

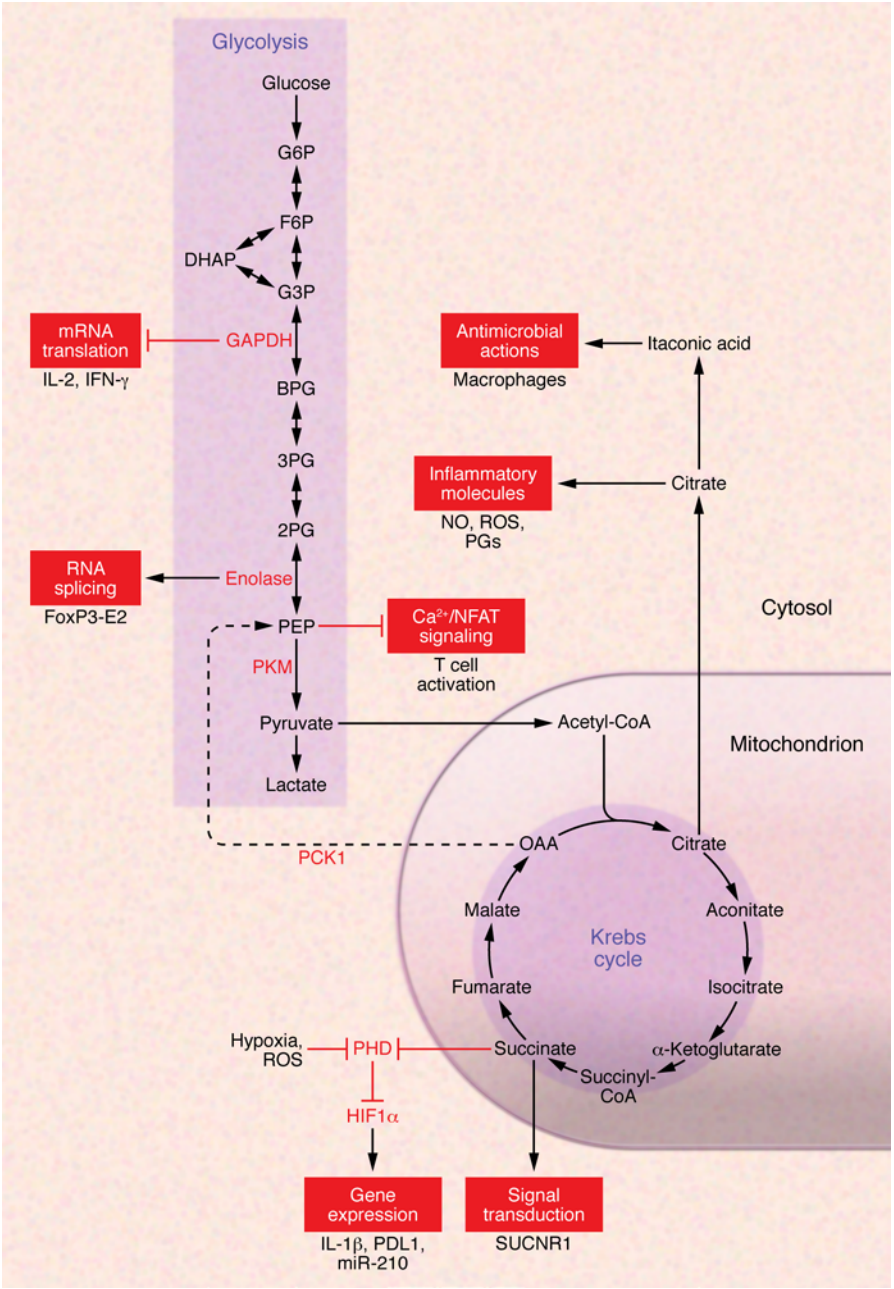

Figure 2. Metabolic regulation of immune cell function. GAPDH has mutually exclusive roles as a glycolytic enzyme and as an RNA-binding protein that represses protein translation. High rates of glycolysis engage GAPDH, leaving the translation of mRNAs, such as *IFNG* and *IL2* mRNA, unconstrained. When the rate of glycolysis is inhibited, a variant of the glycolytic enzyme enolase locates to the nucleus where it inhibits the formation of the Foxp3-E2 splice variant, which is expressed in human iTregs with potent immunosuppressive activities. The glycolytic metabolite PEP promotes Ca^{2+}/NFAT signaling and thereby T cell activation. Low glucose levels result in reduced PEP inhibiting NFAT and T cell activation. Strategies to increase PEP levels, such as inhibition of PKM or recombinant expression of PCK1, will restore normal T cell activation in low-glucose conditions. The Krebs cycle intermediate succinate contributes to the stabilization of HIF1α protein in inflammatory macrophages. HIF1α accumulates when the hydroxylases (PHDs) that promote HIF1α degradation are inhibited. Hypoxia and ROS also inhibit PHDs. Depending on the context, HIF1α can promote proinflammatory (IL-1β) or antiinflammatory (PD-L1, miR-210) functions in myeloid cells. Succinate also signals through ligating the cell surface receptor SUCNR1. Cytosolic citrate can also be metabolized to produce important inflammatory mediators such as ROS, NO, and prostaglandins (PGs). Citrate can also be metabolized to itaconic acid, which has important antimicrobial functions.

seem to contribute to defects in T cell function; CD4 T cells from lupus patients have elevated levels of glycosphingolipids. A glycosphingolipid synthesis inhibitor normalizes glycosphingolipid levels and restores function in CD4 T cells from these patients (97). Together, these studies suggest that there are opportunities to therapeutically modulate immune function though the manipulation of lipid metabolism.

Metabolic enzymes and metabolites: new players in immune signal transduction

An emerging theme is that cellular metabolism has an active and direct role in controlling immune responses, and harnessing metabolic control of immune cell function offers novel and exciting therapeutic possibilities. Below, we will discuss the emerging mechanisms by which metabolic enzymes and cellular metabolites can directly control the function of immune cells and how these new regulatory mechanisms might be harnessed for therapeutic purposes.

Metabolic enzymes control immune cell function. Metabolic enzymes can have important functions separate from their enzymatic activity, including regulation of transcription and translation. Numerous metabolic enzymes have been described to act as part of transcriptional regulation complexes, including the glycolytic enzymes GAPDH, hexokinase, and enolase (98). However, the significance of transcriptional regulation by these enzymes has not yet been elucidated in immune cells. GAPDH is also an RNA-binding protein that binds to *Ifng* and *Il2* mRNA in CD4 T cells to repress protein translation (Figure 2 and ref. 24). In highly glycolytic T cells GAPDH is engaged in glycolysis and the expression of IFN-γ and IL-2 is unconstrained. In fact, a large number of

metabolic enzymes can bind to mRNA, including those involved in glycolysis, the KREBS cycle, and fatty acid synthesis (99). However, the exact mRNA transcripts involved have not been identified and the significance for immune regulation has yet to be determined. With the ongoing developments in lymphocyte drug delivery systems, including the administration of small oligonucleotides (100, 101), targeting these enzyme/mRNA interactions is feasible and may be a novel strategy to modulate immune cell function. Disrupting GAPDH/*Ifng* mRNA interactions would be predicted to sustain IFN-γ production in T cells under conditions that limit the rate of glycolysis. Recent studies have revealed an important role for glycolysis and the glycolytic enzyme enolase in controlling the splicing of *FOXP3* in human Tregs (90). A translational variant of enolase, known as MPB-1, translocates to the nucleus when glycolysis is inhibited and represses the formation of the Foxp3-E2 splice variant that is expressed by iTregs with potent immunosuppressive function (90). It is tempting to speculate that therapeutic manipulations targeting nuclear enolase may be able to promote or inhibit the formation of these potent immunosuppressive Foxp3-E2–expressing iTregs.

Metabolites control immune signaling. Following the identification of signaling roles for the glycolytic intermediate phosphoenolpyruvate (PEP) and the KREBS cycle intermediate succinate, metabolites now need to be considered as potential immune signaling molecules (21, 26). A recent study demonstrated that PEP influences calcium signaling in activated T cells by regulating the reuptake of Ca^{2+} into the endoplasmic reticulum through the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA), thus affecting nuclear translocation of nuclear factor of activated T cells (NFAT) activity and the expression of a set of genes required for T cell activation. Low levels of glucose result in reduced levels of PEP, leading to increased Ca^{2+} uptake into the endoplasmic reticulum, the inhibition of NFAT activity, and reduced T cell effector function (21). These findings support a direct link between glycolysis and T cell signaling and the generation of functional T cell responses. This mode of metabolic regulation is also likely to be important in NK cells, in which calcium mobilization occurs downstream of multiple activating receptors and is important for NK functions, including the release of cytotoxic granules (102, 103). Another recent report has linked glucose sufficiency to the generation of polyfunctional T cell responses through the methyltransferase EZH2. Glucose is required for TCR-dependent inhibition of miR-101 and miR-26a, which facilitates the expression of the EZH2 (104). It seems likely that these observed defects in miR-101, miR-26a, and EZH2 are a result of reduced PEP levels and defective TCR signaling. It follows that under conditions in which glycolysis is constrained any intervention that elevates levels of PEP will facilitate Ca^{2+} signaling in lymphocytes and promote proinflammatory functions.

PEP levels are controlled by the balance of enolase-mediated PEP formation and the pyruvate kinase–mediated conversion of PEP to pyruvate. The pyruvate kinase isoform PKM2 is an important factor that promotes cellular growth in cells engaging in aerobic glycolysis, as this isoform is less active than the alternative splice variant PKM1. PKM2 expression reduces the rate of PEP conversion to pyruvate, resulting in a backlog of glycolytic intermediates that can then be channeled into biosynthetic pathways (105). PKM2 exists in either an active tetrameric form or a less active dimeric form. Pharmacological stabilization of the less active PKM2 dimer or direct inhibition of PKM1 and/or PKM2 catalytic activity would increase PEP levels and might be predicted to restore normal Ca^{2+} signaling under conditions of limiting glucose. However, PKM2 is a complicated enzyme with multiple described roles outside of glycolysis (105); therefore, a detailed study of PKM2 in lymphocytes is required before the therapeutic value of modifying PKM2 activity can be appreciated. Another strategy to increase PEP involves the recombinant expression of the gluconeogenic enzyme PEP carboxykinase 1 (PCK1), which is not normally expressed in immune cells. This enzyme converts oxaloacetate to PEP, allowing PEP to be generated from fuel sources other than glucose, such as glutamine and fatty acids (Figure 2). Ho and colleagues demonstrated that recombinant expression of PCK1 in tumor-infiltrating CD4 T cells restores PEP levels and normal TCR signaling (21). An exciting prospect would be the use of this strategy in adoptive transfer–based immunotherapies, such as CAR T cell therapies or allogeneic NK cell therapies, which, despite impressive responses in hematological malignancies, have not been effective in the treatment of solid tumors (29, 106). As these therapeutic approaches involve manipulation of isolated lymphocytes in the lab, it is entirely feasible to further engineer these cells to express recombinant PCK1. This would be predicted to bolster the antitumor responses within the low-glucose tumor microenvironment by allowing the effector lymphocytes to sustain PEP levels and activate Ca^{2+}-dependent signaling pathways.

Other metabolites are emerging as important immune signals, including the KREBS cycle intermediate succinate. Succinate levels are elevated in activated macrophages and succinate acts to promote the expression of the proinflammatory cytokine IL-1β through the stabilization of HIF1α (26). HIF1α protein is induced following the inhibition of the prolyl-hydroxylases that target HIF1α for degradation. HIF1α can also promote the expression of molecules that have immunosuppressive effects in myeloid cells, including miR-210 and PD-L1 (107, 108). In fact, depending on the context, HIF1α can promote proinflammatory or antiinflammatory functions of myeloid cells (26, 107–110). Succinate can also have proinflammatory effects through ligating the succinate receptor 1 (SUCNR1) to augment DC chemotaxis to enhance DC-induced T cell responses (111, 112). Indeed, there is evidence to suggest that succinate contributes to inflammatory disease (111). Citrate is also elevated in activated macrophages and can be metabolized to generate important inflammatory and antimicrobial molecules (Figure 2 and refs. 26, 111, 113). Understanding the exact mechanisms leading to increased KREBS cycle intermediates in myeloid cells will be an important step in identifying potential strategies to therapeutically decrease these proinflammatory metabolites.

Final comments

The rapid expansion in our understanding of the link between metabolism and immune cell function has revealed avenues for the development of immunomodulatory therapeutics. So, can immune cell metabolism be targeted efficaciously without causing unacceptable toxicity? Certainly there are already a number of drugs that have been used in patients with cancer that will affect immune cell metabolism (discussed above). In

addition, drugs that directly target glycolysis and OxPhos, such as 2-deoxyglucose and metformin, are well tolerated in humans and have been used for decades (114). While it is not clear how effective these drugs are at inhibiting metabolism in immune cells in vivo, these drugs in combination do effectively inhibit pathological T cell responses in mouse models, arguing that metabolic regulation of human T cell responses is feasible (94, 95). A significant amount of work remains to determine how other strategies targeting specific metabolic enzymes or metabolites might be implemented or indeed tolerated in humans. Local administration of therapies may be advantageous in conditions such as rheumatoid arthritis, though treating systemic inflammatory disease may be more challenging. In cancer immunotherapies that involve adoptive transfer methods there is a valuable opportunity to modulate metabolic pathways in the lab prior to administration to the patient, thus largely circumventing the issue of drug toxicity. The ongoing advances in cell type–specific drug targeting technologies may also become important factors in avoiding excessive toxicity (100, 101). In conclusion, metabolic pathways are an exciting regulatory axis for the control of immune responses that provide therapeutic opportunities for treating diseases from cancer to autoimmunity.

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