REVIEW

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Immunometabolism of regulatory T cells

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The bidirectional interaction between the immune system and whole-body metabolism has been well recognized for many years. Via effects on adipocytes and hepatocytes, immune cells can modulate whole-body metabolism (in metabolic syndromes such as type 2 diabetes and obesity) and, reciprocally, host nutrition and commensal-microbiota-derived metabolites modulate immunological homeostasis. Studies demonstrating the metabolic similarities of proliferating immune cells and cancer cells have helped give birth to the new field of immunometabolism, which focuses on how the cell-intrinsic metabolic properties of lymphocytes and macrophages can themselves dictate the fate and function of the cells and eventually shape an immune response. We focus on this aspect here, particularly as it relates to regulatory T cells.

Resting T cells are relatively inert from a metabolic standpoint and require little energy generation or expenditure to 'keep the engine idling'. Upon activation, their energy needs increase substantially and, as will be described below, various substrates, including glucose, amino acids (especially glutamine) and fatty acids, are used to meet this demand. Most of the initial studies of T cells focused on naive T cells and effector T cells (T_{eff} cells)–memory T cells (T_{mem} cells), which have both shared metabolic features and distinct metabolic features. Subsequently, increasing attention has been focused on regulatory T cells (T_{reg} cells), with the recognition that these cells have their own signaling and metabolic 'preferences' that can drive and dictate their function and stability.

The best-characterized subset of T_{reg} cells is defined by expression of the co-receptor CD4, the cytokine receptor CD25 and the transcription factor Foxp3 (encoded by an X-linked gene). The importance of T_{reg} cells is exemplified by patients with the immunodeficiency syndrome IPEX ('immunodysregulation polyendocrinopathy enteropathy X-linked') and mice of the scurfy strain, each of which lack functional Foxp3 and suffer from severe systemic autoimmunity. T_{reg} cells can originate in the thymus, as well as extrathymically in the periphery as a consequence of the induction of Foxp3 expression following the activation of naive T cells[1]. In this Review, we will use 'tT_{reg} cells' for thymus-derived T_{reg} cells, 'pT_{reg} cells' for peripherally induced T_{reg} cells, and 'iT_{reg} cells' for *in-vitro*-generated T_{reg} cells[2]. Although tT_{reg} cells and pT_{reg} cells share many key features, such as their reliance on Foxp3 expression and dependence on interleukin 2 (IL-2) for their suppressive function and maintenance, they differ in the repertoires of their T cell antigen receptors (TCRs) and in the epigenetic marking of control elements in the *Foxp3* locus[3–7]. Most importantly, of course, they differ in whether Foxp3 is expressed constitutively (tT_{reg} cells) or whether its expression is induced following antigen-mediated activation (pT_{reg} cells). Given these distinctions, it is likely that tT_{reg} cells and pT_{reg} cells will not be found to be metabolically identical, and these differences might arise from specific developmental programming and/or context-dependent external cues.

In this Review we aim to provide a comprehensive understanding of the metabolic properties of both subsets of T_{reg} cells (i.e., thymus derived and extra-thymically induced) and how these can modulate and be reciprocally influenced by the immune response.

T cell bioenergetics and features of T_{reg} cell metabolism

Upon being activated, resting naive T cells that differentiate toward the T_{eff} cell lineage shift from catabolic energy metabolism to an anabolic state. This is driven predominantly by the glycolytic-lipogenic pathway and is associated with glutamine oxidation that fuels mitochondrial oxidative phosphorylation through the tricarboxylic acid (TCA) cycle. This use of aerobic glycolysis, similar to the metabolism in many cancer cells, is called the 'Warburg effect' and is orchestrated via the mTOR-dependent nutrient-sensing pathway activated downstream of signaling via the kinases PI(3)K and Akt[8–10]. As an immune response resolves, cells that persist and/or transit into the memory pool (as demonstrated by $CD8^+$ T cells) revert to a catabolic state and rely mainly on lipid oxidation regulated by signaling via the AMP-activated kinase AMPK and promoted by increased mitochondrial biogenesis, both of which are associated with cellular longevity and the ability of T cells to rapidly respond to reinfection[10–12].

Glycolysis-driven *de novo* fatty-acid synthesis is a critical determinant of the fate of the T_H1, T_H2 and T_H17 subsets of helper T cells[13–15]. Consistent with that, T_{eff} cell differentiation can be inhibited by various means, including inhibition of HIF-1α ('hypoxia-inducible factor 1α'), the transcription factor required for glycolysis; blockade of PDHK ('pyruvate dehydrogenase kinase'), the TCA enzyme that indirectly promotes glycolysis by blocking pyruvate dehydrogenase (PDH); or blockade of ACC1 ('acetyl-CoA carboxylase 1'), the key enzyme that drives fatty-acid synthesis. This has been demonstrated not only genetically but also pharmacologically, via treatment with 2-deoxy-glucose (2-DG), dicholoroacetate or soraphen, which block each of those three processes,

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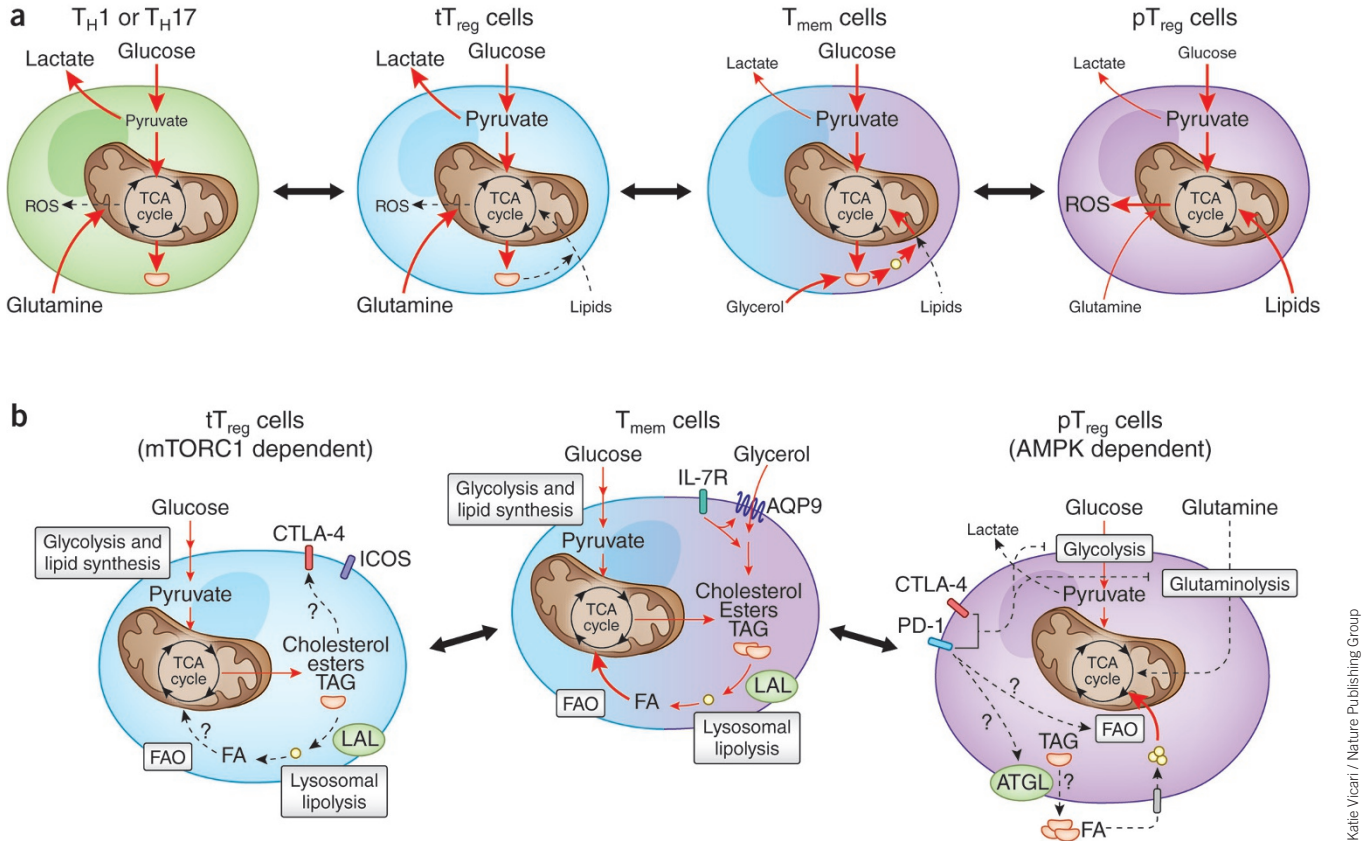

Figure 1 Proposed model for the metabolic signatures of various T_{reg} cell subsets. (**a**) Activated CD4$^+$ T cells that differentiate into the T_{eff} cell lineage (green) (T_H1 or T_H17 cells) are dependent mainly on carbon substrates such as glucose and glutamine for their anabolic metabolism. In contrast to that, pT_{reg} cells that potentially mirror activated T cells that have differentiated into the iT_{reg} cell lineage *in vitro* (purple) can rely on exogenous lipids and glucose-derived pyruvate that they can oxidize in the TCA cycle. Owing to their substantial dependence on FAO, iT_{reg} cells generate increased amounts of ROS but are resistant to ROS-mediated damage, as they might be armed with antioxidant molecules to maintain their integrity. However, the metabolic properties of tT_{reg} cells (blue) seem to resemble those of activated T_{eff} cells to a greater degree than those of their pT_{reg} cell counterparts in that they might be more dependent on glucose and glutamine than on fatty acids. (**b**) T_{mem} cells depend on glycolysis-driven lipogenesis and IL-7 receptor (IL-7R)-mediated expression of AQP9 for uptake of glycerol to generate cholesterol esters and triacylglycerols that can be hydrolyzed by LAL to mobilize free fatty acids (FA) to fuel FAO. Both tT_{reg} cells and pT_{reg} cells mirror certain metabolic properties of T_{mem} cells, in that they seem to rely on glucose-derived lipogenesis and FAO, respectively. Furthermore, the activation of co-inhibitory receptors such as CTLA-4 and PD-1 (which inhibit glycolysis while promoting FAO in activated T cells) might potentially have a role in influencing FAO in pT_{reg} cells. In particular, activation of PD-1 has been shown to upregulate the enzyme ATGL ('adipose triglyceride lipase') that hydrolyzes intracellular triacylglycerol (TAG) into glycerol-3-phosphate and fatty acids for their utilization in FAO in activated T cells. Thus, although like T_{mem} cells, pT_{reg} cells can depend on FAO, the means by which they obtain fatty acids might be different. Whether tT_{reg} cells depend on FAO is yet to be determined.

respectively (**Table 1**). Notably, this not only inhibits T_{eff} cell differentiation but also promotes iT_{reg} cell induction[14,16,17].

T_{reg} cells that develop *in vivo* (tT_{reg} cells) resemble T_{eff} cells in that they depend on glycolysis-driven lipogenesis for their proliferation and functional fitness, with the mevalonate pathway demonstrated to be particularly important in this subset[18]. Interestingly, studies of mouse B16 melanoma tumor models have shown that intratumoral and splenic T_{reg} cells exhibit more glucose uptake than do non-T_{reg} cells[19]. Moreover, *in vivo* blockade of glycolysis and glutaminolysis and enhancement of fatty-acid oxidation (FAO) diminishes the proliferation of T_{reg} cells (although to a lesser degree than the effect on T_{eff} cells) in a model of infection with vaccinia virus and adoptive transfer of T cells[20]. Although such studies have suggested a clear metabolic distinction between tT_{reg} cells and iT_{reg} cells (the latter being probably indicative of pT_{reg} cells as well), these differences are probably context dependent (**Fig. 1a**). Subsequent to those studies, it was reported that human T_{reg} cells isolated *ex vivo* are highly glycolytic and engage in both glycolysis and FAO when cultured *in vitro*[21]. Furthermore, in a model in which human T_{reg} cells were induced *in vitro* by suboptimal TCR stimulation without use of the cytokines TGF-β or IL-10, glycolysis was shown to be required for optimal induction of Foxp3 expression and T_{reg} cell function (discussed in more detail below).

Amino acids, particularly glutamine and leucine, have an essential role in T_{eff} cell differentiation. However, iT_{reg} cells seem to be less dependent on amino acids for their energy needs. For example, depriving CD4$^+$ T cells of glutamine leads to their differentiation toward a T_{reg} cell phenotype[22]. Conversely, the glutamine-derived TCA-cycle intermediate α-ketoglutarate enhances T_H1 differentiation by promoting expression of the transcription factor T-bet[22]. In addition, genetic deficiency in neutral-amino-acid transporters such as Slc7a5 and Slc1a5 diminishes glutamine uptake and glucose metabolism and decreases T_{eff} cell differentiation without effects on the generation of iT_{reg} cells[23,24] (**Fig. 1**).

Interestingly, the metabolic byproducts of tryptophan catabolism, such as kynurenine, promote the generation of iT_{reg} cells by binding to the aryl hydrocarbon receptor[25–27]. Depleting cells of tryptophan can also lead to activation of the amino-acid-starvation response via the

kinase GCN2, with resultant inhibition of T_H17 differentiation[27,28]. Furthermore, T_{reg} cells have been shown to increase their expression of amino-acid-catabolizing enzymes such as ARG1 ('arginase 1'), HDC ('histidine decarboxylase'), TDH ('threonine dehydrogenase') and IL4I1 ('interleukin-4 induced 1'; similar to L-amino-acid oxidase) in skin grafts and bone-marrow-derived dendritic cells, which suggests that T_{reg} cells can modulate the concentration of certain amino acids and their catabolic products in the local milieu, an effect that can itself 'preferentially' enhance suppression mediated by T_{reg} cells[27].

To meet their energetic demands, T_{mem} cells depend on FAO, a process that is dependent upon Cpt1a ('carnitine palmitoyltransferase 1A'), the rate-limiting enzyme for mitochondrial lipid uptake[11]. To support this pathway, T_{mem} cells depend upon both exogenous glycerol and endogenous lipids (**Fig. 1b**). They import exogenous glycerol via IL-7-induced expression of the pore-forming membrane protein AQP9 ('glycerol channel aquaporin 9') and also generate lipids *de novo* from glucose[29]. Furthermore, T_{mem} cells express the enzyme LAL ('lysosomal acid lipase'), which hydrolyzes glucose-derived endogenous cholesterol esters and triacylglyerols to fuel FAO[30]. Both tT_{reg} cells and pT_{reg} cells seem to exhibit some metabolic features that resemble those of T_{mem} cells, in that pT_{reg} cells can depend upon FAO (mirroring iT_{reg} cells), while tT_{reg} cells utilize a glycolytic-lipogenic cholesterol-biosynthetic pathway for both survival and optimal suppressive function, as described in the previous section[13,14,18,31] (**Fig. 1b**). Whether tT_{reg} cells can import exogenous fatty acids and/or use their endogenous stores of triacylglycerides to feed FAO is not yet known (**Fig. 1b**).

Some features of T_{reg} cell metabolism are also closely tied to anatomical location. For example, the dependence of T_{reg} cells on lipid metabolism *in vivo* is particularly evident in tissue-resident T_{reg} cells that localize in non-lymphoid tissues such as visceral adipose tissue (VAT) and intestinal mucosa[32,33]. VAT T_{reg} cells express PPARγ ('peroxisome proliferator-activated receptor-γ'), a master regulator of adipocyte differentiation and function, and have high expression of CD36, a scavenger receptor that facilitates the import of exogenous fatty acids[31,33]. Like other T_{reg} cells, VAT T_{reg} cells also express leptin receptors[32,34,35]. Moreover, adipocytes in the VAT of obese mice have high expression of leptin, which results in overactivation of mTOR in T_{reg} cells and a decrease in the number of T_{reg} cells[35]. Conversely, the VAT of lean aged male mice or mice deficient in leptin or its receptor shows enrichment for T_{reg} cells[31,36]. As an example from another anatomical site, the colon provides an environment rich in short-chain fatty acids, such as butyrate, acetate and propionate, that are generated from the bacterial fermentation of dietary fiber, and this has been closely linked to promoting the generation of T_{reg} cells[32,33,37–39]. One mechanism by which butyrate induces T_{reg} cells in the gut is through inhibition of histone deacetylases, which enhances the acetylation of histone H3 at Lys27 at the *Foxp3* locus and thereby increases *Foxp3* expression[37–39]. Tissue-resident T_{reg} cells are therefore attuned to local metabolic cues that can be exploited for their phenotypic and functional specialization, as well as for 'preferential' survival in the tissue microenvironment.

Metabolic control of T_{reg} cells by mTOR

The mTOR-dependent nutrient-sensing pathway is composed of two distinct complexes: mTORC1 and mTORC2. In T cells, mTORC1 activity is elicited by signaling through the TCR and the co-receptor CD28 (ref. 40), as well as through the expression and activity of nutrient transporters[23,24,41]. For example, the glutamine transporter ASCT2 and the system-L transporter Slc7a5 (which 'preferentially' mediates the uptake of leucine) are both critical for sustained mTORC1-dependent expression of the transcription factor c-Myc and optimal T_H1 and T_H17 differentiation of naive T cells. Notably, either transporter is dispensable for the induction of iT_{reg} cells. Consistent with that, leucine transport is required for the expression of other nutrient transporters, such as Glut1 and CD71, and Glut1 expression itself has emerged as a critical factor in driving glycolysis in T_{eff} cells, whereas T_{reg} cells generated either *in vitro* or *in vivo* develop and function independently of Glut1 (ref. 41). These examples illustrate how amino acids control metabolism in an mTOR-dependent manner, affecting mainly T_{eff} cells and sparing T_{reg} cells. Moreover, one mechanism by which T_{reg} cells achieve suppression relies on the induction, in antigen-presenting cells, of the expression of amino-acid-consuming enzymes such as IDO (upregulated through T_{reg} cell–specific ligation of the costimulatory molecules CD80 or CD86 via the inhibitory receptor CTLA-4) and ARG1 (upregulated by inflammation), which block the proliferation of T_{eff} cells and promote the induction of T_{reg} cells via inhibition of mTOR signaling[42].

The main negative regulator of PI(3)K activity in T cells is the lipid phosphatase PTEN[43,44]. T_{reg} cell–specific deletion of PTEN enhances a glycolytic program in association with compromised function and lineage instability[45]. Moreover, PTEN has been shown to be critical in limiting Akt activity and, consequently, maintaining the transcription-factor activity of Foxo3a in intratumoral T_{reg} cells through the semaphorin Sema4a and neuropilin Nrp1, which suggests that this pathway might be a potential therapeutic target for the potentiation of anti-tumor responses and limiting of T_{reg} cell–mediated tolerance to tumors[46]. It has also been reported that IDO-induced activation and function of T_{reg} cells requires activation of PTEN via the checkpoint receptor PD-1 and that PTEN-deficient T_{reg} cells are compromised in their ability to create a suppressive tumor microenvironment[47]. In activated T cells, PD-1 inhibits the transport and utilization of both glucose and glutamine and alters the metabolic program from glycolysis to FAO, which suggests that PD-1 might affect metabolism through PTEN-mediated control of PI(3)K signaling[48].

Although strong PI(3)K signals clearly have a negative effect on the differentiation, function and stability of T_{reg} cells, this does not indicate that T_{reg} cells are completely PI(3)K independent[45,47,49]. Indeed, an oscillatory nature of mTOR signaling in T_{reg} cells in response to leptin and nutrients has been described[34]. Furthermore, PI(3)K signaling via the TCR and IL-2 maintains functional fitness and suppressive activity via mTORC1-mediated induction of cholesterol and lipid metabolism and upregulation of CTLA-4 expression[18] (**Figs. 1b** and **2**). Interestingly, mTORC1 has a role in inhibiting the mTORC2 pathway in T_{reg} cells, and mTORC2 activity is required for Akt-mediated inhibition of Foxo transcription factors. Given the importance of these transcription factors in the development, maintenance and function of T_{reg} cells, it is possible that the ability of mTORC1 to inhibit mTORC2 contributes to the control of metabolism via maintenance of the activity of Foxo transcription factors in the nucleus[50–52]. This is consistent with the finding that Foxo1 inhibits glycolytic and oxidative metabolism through antagonizing c-Myc function. Of note, Foxo1 also has a role in the memory formation and function of $CD8^+$ T cells[53,54]. $CD8^+$ T_{mem} cells are perhaps the population most similar to T_{reg} cells in their metabolic profile, and we are tempted to speculate that their metabolic properties and reliance on Foxo1 signaling are interrelated.

An alternative to mTOR-driven metabolism is the AMPK-dependent pathway, which promotes mitochondrial oxidative metabolism and suppresses mTOR signaling and glycolysis[55]. A crucial role for AMPK in T_{eff} cells under conditions of limited nutrient availability has been demonstrated[56]. Activated T cells with less access to glucose undergo a metabolic checkpoint marked by decreased mTORC1 activity that limits cell growth, cytokine production and proliferation but maintains cellular ATP levels, cell viability and the ability to resume cytokine production

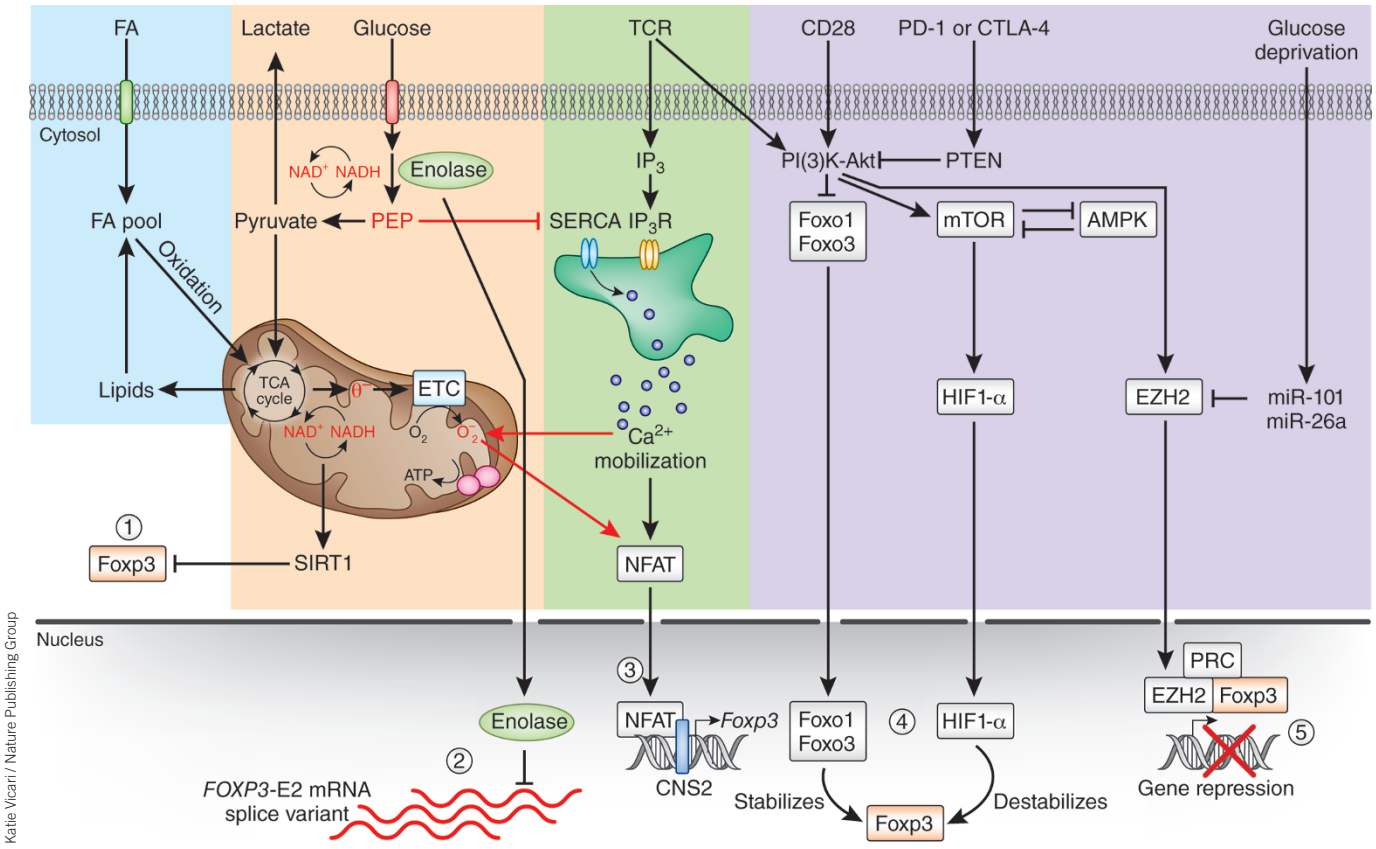

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Figure 2 Effects of metabolism on Foxp3 expression and the generation of T_{reg} cells. There are various scenarios in which T_{reg} cell signaling and metabolic pathways might integrate and potentially affect Foxp3 expression. One of the downstream effects of an enhancement in glycolytic metabolism is the production of metabolic intermediates that can also function as signaling molecules (1). For example, NAD^+ and NADH might control Foxp3 stability via the activation of histone deacetylases such as SIRT proteins, which directly deacetylate Foxp3 in the nucleus and lead to its proteosomal degradation in the cytoplasm. Furthermore, the glycolytic enzyme enolase-1 can repress the *FOXP3* splice variant containing exon 2 (E2) in human T_{reg} cells, and its engagement in glycolysis serves as a mechanism by which glycolysis can control Foxp3 expression (2). Signaling molecules from glycolysis and mitochondrial metabolism (PEP and ROS) activate NFAT via Ca^{2+} mobilization during T cell activation, a process that could potentially affect Foxp3 expression in T_{reg} cells as well (3). Activation of Foxo transcription factors and HIF-1α downstream of the PI(3)K-Akt-mTOR signaling pathway can reciprocally affect Foxp3 expression (4). Finally, the chromatin-modifying enzyme EZH2 that is crucial for the establishment of a repressive T_{reg} cell gene program can be inhibited by specific microRNAs (such as miR-101 and miR-26a) under circumstances of glucose deprivation, which leads to T_{reg} cell instability (5). ETC, electron-transport chain; PRC, polycomb repressive complex; IP_3 and IP_3R, inositol-1,3,4-trisphosphate and its receptor.

following the re-introduction of glucose. In the absence of AMPK during periods of glucose starvation, T cells are deficient in their ability to suppress mTORC1 activation, undergo metabolic reprogramming, and utilize glutamine to generate TCA-cycle intermediates for the support of oxidative phosphorylation.

T_{reg} cells maintain high levels of AMPK activation, which mimics the metabolism of nutrient-deprived T_{eff} cells, although important differences remain, in particular the reliance of T_{reg} cells on lipid oxidation[13]. This aspect of the metabolic profile of T_{reg} cells is particularly evident in the context of deficiency in the receptor ERRα, which leads to a block in glucose oxidation and glucose-dependent lipid synthesis. In this case, lipids restore the differentiation of T_{reg} cells, but pyruvate does not, and neither is able to restore T_H1, T_H2 or T_H17 cells[57]. Interestingly, in the absence of glutamine, the activation of naive T cells (even under T_H1-skewing conditions) results in loss of mTORC1 activity and differentiation into T_{reg} cells[22]. This is dependent on a decrease in intracellular levels of the glutamine-derived metabolite α-ketoglutarate, which identifies a role for intracellular metabolite production in the direct control of cell fate in the context of nutrient availability.

Metabolic control of Foxp3 and T_{reg} cell lineage stability

Instability of the T_{reg} cell lineage is associated with inflammatory conditions and is closely linked to alterations in metabolism[45,46,58,59]. T_{reg} cell–specific deletion of *Atg7* or *Atg5*, which encode two factors essential to autophagy, also leads to compromised stability of the T_{reg} cell lineage due to unchecked control of mTORC1-dependent c-Myc expression and glycolysis[58]. While unrestrained glycolytic activity in T_{reg} cells can contribute to their dysfunction, it is likely that glycolysis also has an important role in supporting this population. In human iT_{reg} cells generated by sub-optimal TCR stimulation, the glycolytic activity of enolase-1 can promote the induction of *FOXP3* splice variants containing exon 2 to confer suppressive activity[60] (**Fig. 2**). After pharmacological blockade of glycolysis with 2-DG during the generation of iT_{reg} cells, non-glycolytic activity of enolase-1 represses *FOXP3* expression, while silencing of enolase-1 under these same conditions restores *FOXP3* transcripts and expression of the exon-2-containing *FOXP3* splice variants. This bifunctionality of a metabolic enzyme, whereby enolase-1 has distinct functions depending on whether or not T_{reg} cells are engaged in glycolysis, has a parallel in T_{eff} cells, in which GAPDH inhibits the production of interferon-γ when disengaged from glycolysis as a result of cellular

Table 1 Potential therapeutic strategies for regulating T_{reg} cell metabolism for immunomodulation

Molecule		Metabolic pathway affected	Mode of drug action and proposed effect	Implications and disease models	Refs.
Surface receptors	CTLA-4 and PD-1	CTLA-4 blocks glycolysis without augmenting FAO; PD-1 blocks glycolysis and enhances FAO in T_{eff} cells by activating ATGL	Checkpoint blockade to enhance T_{eff} cells while potentially inhibiting T_{reg} cells that are reliant on FAO	Cancer therapy	48
	P2X7	Activation of P2X7 via ART by extracellular NAD^+ released during inflammation and cellular damage	Systemic administration of NAD^+ depletes 75–80% of T_{reg} cells in mice; an NAD^+ inhibitor (the single-domain antibody ART2.2) protects T_{reg} cells from NICD	Cancer therapy; autoimmunity	71
	CD39-CD73 and A2AR	Catabolism of ATP to adenosine by T_{reg} cells that in turn inhibits T_{eff} cell responses via A2AR	CD39-CD73 blockade; CD39-CD73 activator; A2AR antagonists in next-generation checkpoint blockade	Cancer therapy; autoimmunity; transplantation	75–77
Intracellular kinases and phosphatases	PTEN	Inhibits PI(3)K-Akt signaling and glycolysis in T_{reg} cells	A PTEN inhibitor causes T_{reg} cell destabilization and tumor regression	Cancer therapy; autoimmunity	45,47,78
	Leptin-mTOR signaling axis	mTOR promotes glycolysis via HIF-1α and inhibits iT_{reg} cells	Rapamycin blocks mTOR and enhances T_{reg} cell proliferation	EAE	17,34
	AMPK	Activates FAO and enhances iT_{reg} cell generation	Metformin activates AMPK signaling and increases lipid oxidation	Allergic asthma	13
Metabolic enzymes	HK	Catalyzes the first rate-limiting reaction in glycolysis	2-DG blocks HK activity and glycolytic pathway	Prolongs allograft survival and diminishes EAE	17,20
	GLS	Converts glutamine to glutamate, the first step in gluatmine oxidation	DON blocks glutamine transport and glutaminase enzymes	DON, along with other metabolic inhibitors (2-DG and metformin), prolongs allograft survival	20
	PDHK1	Inhibits PDH (which catalyzes the conversion of pyruvate to acetyl-CoA) and indirectly enhances glycolysis	DCA blocks PDHK and increases the entry of pyruvate into the TCA cycle	Diminishes colitis, EAE and collagen-II-induced arthritis	16,79
	ACC	Carboxylates acteyl-coA to malonyl-CoA, which is essential for lipid synthesis	SorA blocks ACC and inhibits T_H17 development and favors T_{reg} cells	ACC blockade attenuates EAE	14
	HMGCR	Rate-limiting enzyme involved in the synthesis of cholesterol and isoprenoid lipids that are required for coordinating T_{reg} cell proliferation and optimal induction of CTLA-4 and ICOS in an mTORC1-dependent manner	25-hydroxycholesterol (general lipid-synthesis inhibitor) and drugs such as simvastatin, atorvastatin and lovastatin inhibit HMGCR and impair the suppressive activity of T_{reg} cells	Cancer therapy	18
	IDO	Tryptophan-catabolizing enzyme expressed by dendritic cells that suppresses T cell responses by upregulating PD-1 on T_{reg} cells via amino-acid-sensitive GCN2, which blocks mTOR	IDO-inhibitor drugs can increase Akt phosphorylation in T_{reg} cells; interferon-γ and CTLA-4–Ig can enhance IDO expression in dendritic cells; the small molecule halofuginone activates GCN2 (AAR) and inhibits T_H17 differentiation	Cancer therapy; allogenic bone-marrow transplantation; EAE	47,80,81
	SIRT1	Activated by an increase in the NAD^+/NADH ratio and can directly deacetylate Foxp3, which leads to its proteosomal degradation	Inhibitors EX527 and splitomycin block SIRT1 activity; resveratrol supports mitochondrial biogenesis in a SIRT1-dependent manner	Prolongation of allograft survival	82–84
	Mitochondrial ATPase	Crucial for mitochondrial oxidative phosphorylation	Small-molecule inhibitor (Bz-423) that inhibits mitochondrial F1F0 ATPase in alloreactive T cells that depends on FAO in a GVHD model	Bone-marrow transplantation	85,86
Metabolic intermediates	PEP	One of the products of glycolysis (enhanced by PCK1) that sustains Ca^{2+} mobilization and NFAT signaling by inhibiting SERCA activity	Enhances T_{eff} responses, effect on T_{reg} cell TBD	Anti-tumor immunity	19
	α-KG	Glutamine-derived TCA cycle metabolite	The α-KG analog DMK enhances T-bet expression and T_H1 responses, while a decrease in α-KG can enhance T_{reg} cells	Glutamine deprivation enhances the suppressive activity of T_{reg} cells in an autoimmune colitis model	22
	Mevalonate	A metabolite in the lipid-synthesis pathway downstream of HMGCR	Mevalonate completely reverses the effects of statins (described above) and is involved in maintaining T_{reg} cell functional fitness in an mTORC1-dependent manner	Autoimmunity	18
Chromatin modifiers	EZH2	Associates with Foxp3 to create repressive chromatin but is also crucial for establishing polyfunctionality in T_{eff} cells	Glucose deprivation inhibits EZH2 by microRNAs and T_{eff} cell function; net effect of EZH2 inhibitors on T_{reg} cells is TBD	Cancer; autoimmunity	63,64

HK, hexokinase; GLS, glutaminase; EAE, experimental autoimmune encephalitis; ACC, acetyl-CoA carboxylase; HMGCR, 3-hydroxy-3-methylglutryl-CoA reductase; α-KG, α-ketoglutarate; DMK, dimethyl α-ketoglutarate; ICOS, effector molecule; NICD, NAD^+-induced T cell death; DCA, dichloroacetate; SorA, soraphen; Ig, immunoglobulin; AAR, amino-acid–starvation response; F1F0 ATPase, F-type ATPase (ATP synthase); GVHD, graft-versus-host disease; PCK1, kinase; TBD, to be determined.

reliance on oxidative metabolism[15]. Given the highly proliferative nature of T_{reg} cells, it might not be surprising that they engage glycolysis under certain contexts. This has been directly characterized through analysis of mouse iT_{reg} cell metabolism and in human T_{reg} cells assessed *ex vivo* immediately after isolation or cultured *in vitro*[16,21]. Whether there is a greater reliance on glycolysis in human T_{reg} cells than in mouse T_{reg} cells, under physiological and pathological conditions, and whether this contributes to or diminishes suppressive capacity, remain to be determined.

Under certain contexts, the glycolytic ability of T_{reg} cells might be indispensable. In activated T cells, this shift toward glycolysis is driven by c-Myc[9]. T_H17 cells, however, rely on a HIF-1α-driven glycolytic program[17]. Interestingly, T_H17 cells and iT_{reg} cells demonstrate a reciprocal relationship in which the expression of HIF-1α represents a metabolic 'tipping point' between these two populations. This is true both under hypoxic conditions and normoxic conditions and relies in part on targeting of Foxp3 for degradation through direct binding of HIF-1α[61] (**Fig. 2**). Surprisingly, the HIF-1α pathway has been revealed to induce Foxp3 expression under hypoxic conditions, which constitutes a negative feedback loop for controlling T_{eff} cell responses elicited under inflammatory hypoxic conditions[62]. This again illustrates (as does the role of enolase-1 in T_{reg} cells) that in certain contexts, a glycolytic program might be required for T_{reg} cells to actively engage in suppressive activity.

The regulation of chromatin by the CD28-dependent histone methyltransferase EZH2 is critical in maintaining a Foxp3-dependent gene program in T_{reg} cells, particularly in non-lymphoid tissues and in settings of induced inflammation[63]. However EZH2 also regulates non-T_{reg} cells. Glycolysis-driven EZH2 expression in $CD4^+$ or $CD8^+$ T cells is required for cytokine production in the context of anti-tumor responses[64]. Through glucose restriction imposed by the tumor microenvironment, T cells maintain expression of a set of microRNAs that target EZH2 expression, which results in loss of T cell polyfunctionality and cytokine production. Such studies suggest that glycolytic reprogramming occurs in coordination with chromatin modifications. As T_{reg} cell stability has now been shown to be dependent on EZH2 expression, it remains to be determined if this occurs in a glycolysis-dependent manner (**Fig. 2**).

Interestingly, metabolism might also influence T_{reg} cell stability through direct modulation of Foxp3 expression. Translocation of the transcription factor NFAT into the nucleus downstream of TCR stimulation is crucial for its association with the conserved noncoding sequence CNS2 region and *Foxp3* promoter region[65]. In activated T cells, optimal NFAT activity has been shown to be dependent on reactive oxygen species (ROS) induced by Ca^{2+} mobilization and mitochondrial metabolism[66] (**Fig. 2**). Additionally, glycolytic metabolites such as phosphoenol pyruvate (PEP) can also augment Ca^{2+} mobilization by blocking the ATPase SERCA ('sarcoplasmic or endoplasmic reticulum calcium ATPase')[19] (**Fig. 2**). Given the varying dependence of T_{reg} cells on glycolysis and mitochondrial oxidation and increased levels of mitochondrial ROS in T_{reg} cells[16], it is plausible that glycolytic metabolites such as PEP and mitochondrial ROS might affect Foxp3 expression via the modulation of proximal TCR signaling and NFAT activity in T_{reg} cells.

Foxp3 is also subject to multiple post-translational modifications, as well as post-translational regulation by microRNA. Acetylation of Foxp3, which is dependent on nuclear pools of acetyl-CoA, promotes T_{reg} cell stability, whereby the deacetylase SIRT1 directly targets Foxp3 and subsequently increases the poly-ubiquitination of Foxp3 and its proteasomal degradation. This potentially links metabolic regulation of acetyl-CoA levels with Foxp3 expression and might be particularly relevant in balancing the induction of T_H17 cells versus that of T_{reg} cells, in which reliance on fatty-acid synthesis (T_H17 cells) versus FAO (iT_{reg} cells) can directly affect acetyl-CoA levels. Notably, inhibition of SIRT1 can increase the transcriptional activity of Foxp3 in human T_{reg} cells[67–69]. The activity of SIRT1 is NAD^+ dependent, and it is possible that SIRT1-dependent degradation of Foxp3 is linked to glycolytic flux, in which NAD^+ is regenerated from the fermentation of pyruvate to lactate by metabolic enzymes, such as LDHA, that regulate the NAD^+-NADH balance. The regulation of other intracellular metabolites, such as adenylates that influence AMPK activity, might influence the stability of Foxp3 as well. Whether Foxp3 expression itself can reinforce certain metabolic features that strengthen lineage stability remains to be demonstrated. It is interesting that the gene encoding phosphodiesterase PDE3B, which functions to hydrolyze cAMP and cGMP, has been shown to be one of the Foxp3 targets most downregulated in T_{reg} cells[70]. Although the functional consequences of this are not entirely known, it has been suggested that repression of PDE3B is important for the proliferative fitness of T_{reg} cells and their expression of a set of mitochondrial and biosynthetic proteins.

In addition to having a crucial intracellular role, bioenergetic intermediates such as NAD^+ and ATP can also be released during cell damage and inflammation, in which they can activate the P2X7 (P2 purinergic) receptors that have high expression on T_{reg} cells and thus compromise their function and survival[71]. Conversely, T_{reg} cells, via the pathway of the ATP ectonucleotidase CD39 and the extracellular AMP nucleotidase CD73, are able to metabolize extracellular ATP to adenosine that can bind to adenosine receptors (such as A2AR) on responding conventional T cells and inhibit their function[28]. Together these findings suggest that both intracellular metabolic mediators and extracellular metabolic mediators can modulate the homeostasis and function of T_{reg} cells (**Table 1**).

Modulating T_{reg} cell function by manipulating metabolism

The field of immunometabolism is growing at an exponential rate, and delineation of the different utilization of metabolic pathways by distinct subsets of T cells, including T_{reg} cells, suggests the exciting possibility that this might provide a means for subset-specific targeting (**Table 1**). In the context of transplantation, blocking glycolysis and glutamine metabolism with 2-DG and the glutamine analog DON, respectively, and promoting FAO with metformin prevents the rejection of skin and heart allografts, probably through ablation of all lymphocyte proliferation[20]. With the benefit of greater preservation of immunological function, drugs such as metformin and acetyl-CoA carboxylase inhibitors have been shown to 'preferentially' enhance pT_{reg} cell differentiation while blocking T_H1 or T_H17 differentiation in autoimmune mouse models of allergic asthma and experimental autoimmune encephalitis, respectively[14]. Along similar lines, blocking glycolysis via 2-DG or by inhibition of PDHK can selectively impair T_{eff} cells and improve T_{reg} cell function in experimental autoimmune encephalitis[16,17] (**Table 1**).

In situations in which blocking T_{reg} cell function is desirable, such as in the tumor microenvironment, much attention has been focused on the role of PI(3)K-Akt in T_{reg} cells. A published study has suggested that T_{reg} cells are induced in response to apoptotic tumor cells in an IDO-dependent manner and block T_{eff} cell–mediated tumor elimination[47]. In this setting, pharmacological inhibition of either IDO or PTEN is associated with loss of Foxo3a (a target of Akt), T_{reg} cell destabilization and rapid tumor regression[47]. Unexpectedly, expression of an Akt-insensitive mutant of Foxo1 that is constitutively retained in the nucleus has been shown to 'preferentially' ablate tumor-infiltrating and non–lymphoid-tissue activated T_{reg} cells and thereby lead to enhanced anti-tumor responses by $CD8^+$ T cells. This suggests that an alternative outcome might follow PI(3)K blockade under certain contexts in which retention of Foxo1 in the nucleus of T_{reg} cells could potentially lead to their functional disruption[72].

Concluding remarks

It is apparent from the literature reviewed above that while the understanding of T_{reg} cell metabolism has progressed substantially in only a few years, much remains unknown. While genetic targeting of mice has yielded a great deal of new information, an obvious limitation is the extent to which mouse T_{reg} cells and human T_{reg} cells might differ from each other in their use of, and dependence on, distinct metabolic pathways. Additionally, the majority of studies so far have assessed T_{reg} cell metabolism during differentiation or responses *in vitro*; it is certainly possible that metabolism *in vivo* differs from that *in vitro* due to various contextual features, such as cytokines, antigenic competition, tissue hypoxia and so on. Moreover, T_{reg} cell metabolism *in vivo* varies by the site and type of immune response. Such plasticity of metabolism might be an important consideration in the design of approaches to target T_{reg} cells or their subpopulations for immunomodulation. Finally, a limitation of many *in vivo* models is their frequent inability to distinguish between effects on tT_{reg} cells and those on pT_{reg} cells, which represents a key point, as data increasingly suggest that these two populations might act very differently from a metabolic standpoint. The use of pharmacological agents to manipulate metabolism in animals is an important step forward in the development of clinically relevant strategies, with the understanding, of course, that such drugs probably have effects on all lymphocyte populations, not just T_{reg} cells.

Thus, the setting of *ex vivo* T_{reg} cell population expansion and adoptive immunotherapy might be an ideal one in which to begin to apply immunomodulatory strategies. Such an approach has been used in an adoptive-transfer model of tumor-specific T cells in which limiting glycolysis during *in vitro* proliferation was shown to enhance *in vivo* persistence and anti-tumor efficacy[73].The advent of genetic manipulation by CRISPR-based technologies presents an additional opportunity for the cell-specific manipulation of genes encoding products that control metabolic pathways as well, as might prove useful in enhancing the effectiveness of chimeric-antigen-receptor-based T cell therapies[74]. At present, this would need to be done in the adoptive-cell-therapy setting, but if *in vivo* cell-specific CRISPR targeting can be developed, then direct *in situ* approaches might prove possible one day.

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