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T Cell Energy Metabolism as a Controller of Cell Fate in Transplantation

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Abstract

Purpose of review—This review highlights some of the recent developments in the novel field of immuno-metabolism and the therapeutic potential of the many regulatory components of this immuno-metabolic network for transplantation.

Recent Findings—In response to cytokines, changes in nutrients and other alterations in the local milieu, immune cells are capable of changing internal metabolic pathways to meet their energy demands. Recent studies demonstrate that activated T effectors (Th1 and Th17) are supported by aerobic glycolysis, whereas Tregs and CD8 memory T cells favor fatty acid oxidation and lipid biosynthesis through mitochondrial oxidative phosphorylation. These bioenergetic processes are dependent upon the activation of metabolic sensors such as mTOR and AMPK respectively indicating that the crosstalk between immunity and metabolism can shape the fate and function of immune cells. Finally, exciting new studies suggest that differences in the bioenergetic mechanisms within the various immune subsets may selectively be exploited for regulating immune responses.

Summary—In this review, we will discuss the metabolic signatures adopted by various immune cells during tolerance versus immunity and the promising avenues that can be modulated by targeting metabolic pathways with either nutrition and/or pharmacological intervention for establishing long-term transplantation tolerance.

Keywords

Immuno-metabolism; Transplantation; mammalian target of rapamycin (mTOR); AMP activated protein kinase (AMPK); Regulatory T cells (Tregs)

1. Introduction

Programming the host's immune system to induce allograft tolerance while retaining normal immune responses towards pathogens and tumors has long been the "ultimate" goal of transplantation immunologists [1]. Recent knowledge of the immunoregulatory mechanisms involved in maternal immunity, obesity, type-2 diabetes, over-nutrition associated metabolic

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dysfunction and chronic inflammation is reshaping our understanding of the inter-connectivity between what once appeared to be disparate physiological systems of immunity and metabolism [2], [3], [4]*. The bidirectional coordination between these processes essential for the maintenance of homeostasis is comprised of two aspects. One deals with the effect of immune cells on organs such as adipose tissue and liver that regulate whole body metabolism, while the other deals with the instructive role of metabolism on immune cells in regulating their fate and function [4],[5]**.

In this review, we focus on recent findings in this still-evolving field of immuno-metabolism and discuss how this knowledge can help us reevaluate our understanding of the mechanisms of immune activation and suppression, and potentially design better immunotherapeutic strategies to achieve long-term transplantation tolerance in allograft recipients.

2. Fuel feeds fate and function

Immune cells respond to fluctuations in nutrients, growth factors and oxygen levels in tissue microenvironments (such as lymphoid organs, bone marrow and graft sites), by undergoing metabolic programming, a highly coordinated activity of catabolic and anabolic pathways that produces ATP (adenosine 5'-triphosphate) to provide energy for cellular functions [5], [6]**. Immune cells like most other cells utilize substrates such as glucose, lipids and amino acids to meet their energy demands. Under quiescent conditions, cells metabolize glucose to pyruvate that is further oxidized into acetyl CoA in the mitochondria via the tricarboxylic acid cycle (TCA) cycle (Fig. 1) [7]. Similarly, fatty acids are oxidized to acetyl CoA via fatty acid / β -oxidation (FAO) in the mitochondria [5]. These processes donate electrons to the electron transport chain (ETC) to fuel mitochondrial oxidative phosphorylation (OXPHOS) to generate ATP (Fig. 1).

Upon activation however, cells undergo a metabolic shift to generate ATP primarily by converting glucose to pyruvate and then to lactate, a process known as aerobic glycolysis (Warburg effect) (Fig. 1) [8],[9]. It is accompanied by the oxidation of amino acids such as glutamine that replenishes the TCA cycle (Fig. 1) [9], [10]. This metabolic shift offers a rapid source of ATP and provides necessary biosynthetic precursors required for replication, protein and lipid synthesis vital for growth, proliferation and differentiation, [11], [12],[13]. Here, we will review some of the findings that focus on how metabolism influences the outcome of an immune response.

2A. Metabolism in innate immune cells

In addition to promoting T cell responses, innate immune subsets such as dendritic cells (DCs) and macrophages, induce antigen-specific tolerance by promoting T-cell anergy, deletion or the generation of regulatory T cells (Tregs) [2], [14]. Moreover, M2 (alternate) type macrophages attenuate renal allograft injury by facilitating wound healing and tissue remodeling [15]. Upon activation, DCs undergo a metabolic shift to glycolysis while, M2 macrophages depend upon FAO for their development and function (Fig. 2A) [8], [5],[16].

Another major mechanism essential for the immune-regulatory function of innate cells is amino acid catabolism (Fig. 2A)[17]. Activated DCs and graft endothelial cells express indoleamine 2,3-dioxygenase (IDO), an enzyme that catabolizes tryptophan via the kynurenine (kyn) pathway [18]. Along the same line, activated myeloid-derived suppressor cells (MDSCs) express two enzymes, namely arginase (Arg-1) and nitric oxide synthase (NOS) that catabolize arginine [18]. The induction of these enzymes causes a local depletion of tryptophan and arginine, increases downstream tryptophan metabolites and reactive nitrogen (RNS) and oxygen species (ROS). This results in the repression of protein translation via the blockade of phosphoinositide 3-kinase (PI3K)/ the mammalian target of rapamycin (mTOR) signaling and the activation of general control non-repressed-2 kinase (GCN2), an enzyme involved in the amino acid starvation stress response, in responding T cells leading to their proliferation arrest and apoptosis [2], [17] (Fig. 2A).

IDO is induced upon the engagement of costimulatory molecules CD80/CD86 on DCs and cytotoxic T lymphocyte associated protein-4 (CTLA-4) on Tregs and by interferon- γ (IFN- γ) while Arg-1 is induced by cytokines including transforming growth factor (TGF- β) and interleukin-10 (IL-10) [18]. One of the mechanisms by which CTLA4-immunoglobulin (CTLA4-Ig) suppresses the rejection of pancreatic islet allografts in vivo is in part attributed to its ability to induce IDO in DCs [2],[19]. Also of interest, a small molecule, halofuginone, was found to activate the amino acid starvation response via GCN2 and inhibit Th17 differentiation while enhancing Treg development [20]. Together, these findings open an opportunity for targeting specific metabolic pathways in innate immune cells to optimize their tolerogenic / reparative potential in a transplantation setting [2],[15].

2B. Metabolism in T-effectors (Teff)

Recently activated T cells depend upon the orphan nuclear receptor estrogen-related receptor alpha (ERR α) for upregulation of glycolysis required for T cell growth and proliferation (Fig. 2B) [21]. Moreover, glycolysis is also important for Teff function wherein, it relieves the inhibition on translation of cytokine transcripts such as IL-2 and IFN- γ by engaging the bifunctional glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) that usually binds to the 3' untranslated region (UTR) of the cytokine transcripts and prevents their translation (Fig. 2B) [22]*. Further, glycolysis promotes CD4+ T cell differentiation into various Teff populations (Th1, Th2 and Th17) and its inhibition blocks this process while promoting Tregs [23], [24], [25].

Although glycolysis is critical in controlling T cell responses, the model of glycolysis replacing mitochondrial OXPHOS during T cell activation is not precisely correct, as T cells depend upon mitochondrial OXPHOS as a source of ROS required for optimal T cell activation and expansion [10]. For instance, during graft versus host disease (GVHD) in bone marrow transplantation, where the antigen is ubiquitously present, chronically activated alloreactive T cells increase both glycolysis and mitochondrial OXPHOS through FAO in contrast to proliferating bone marrow cells that are predominantly glycolytic [26]. This preferential metabolic difference was used to selectively block FAO via the use of a small molecule BZ423 that inhibits the mitochondrial ATPase and induces apoptosis of alloreactive T cells. This treatment was shown to reduce GVHD severity without affecting

the bone marrow reconstitution [26]. This suggests that Teff cells rely upon both glycolysis and mitochondrial OXPHOS to achieve optimal activation and functionality, although the extent of reliance upon one process over the other appears to be context dependent [26], [27]*.

2C. Metabolism in Treg cells

Tregs are broadly classified into two types of populations; natural Tregs (nTregs) that are derived from the thymus and induced Tregs (iTregs) that differentiate from naïve T cells in the periphery. Emerging evidence suggests that lipid metabolism is the key metabolic pathway in Treg development and differentiation [28]*. On the one hand, nTregs depend upon lipogenesis for optimal expression of CTLA-4 and ICOS (Inducible T cell-Costimulator) that are required for their suppressive function (Fig. 2C) [28]. On the other hand, iTregs primarily derive their energy from FAO for their development and suppressive function (Fig. 2C) [24]. As such, etomoxir, a drug that blocks FAO (by inhibiting carnitine palmitoyl-transferase-1 (CPT1 α), the first rate limiting enzyme of this process) prevents iTreg generation [24]. Conversely, addition of exogenous fatty acids promotes Treg generation during CD4⁺ T cell differentiation while strongly inhibiting Th1, Th2 and Th17 cytokine producing cells.

In addition, Tregs can metabolize extracellular ATP to perform immunosuppression [29]. The presence of extracellular ATP serves as an indicator of tissue damage and Tregs rapidly degrade ATP via CD39 and CD73 (two members of the ectonucleoside triphosphate diphosphohydrolase (E-NTPDase)) to adenosine that can subsequently bind to A2AR (a type of adenosine receptor) on activated T cells inhibit their activation [29], [30] (Fig. 2C). Moreover, mice deficient of CD39 develop autoimmunity and wasting syndrome associated with poor suppressive capability of Tregs, a condition that is ameliorated by the adoptive transfer of CD39⁺ Tregs [29]. These studies indicate that Tregs depend predominantly upon lipid metabolism for their development and rely upon ATP metabolism in part to mediate their immunosuppressive function [24].

2D. Metabolism in T memory cells

Metabolism of long-lived memory T cells displays a critical overlapping feature with Treg cells and M2 macrophages as they depend upon FAO to meet their metabolic demands. This is facilitated by IL-15 that promotes mitochondrial biogenesis and expression of CPT1 α that is required for FAO [31]. However, unlike iTreg cells that utilize exogenous fatty acids (FA) to fuel FAO, CD8⁺ memory T cells use extracellular glucose to synthesize lipids that are further hydrolyzed to generate free FA by lysosomal acid lipase (LAL) to fuel FAO (Fig. 2D) [32]. Of note, these metabolic features are not invariant, as upon reactivation, human memory T cells have been shown to undergo an immediate-early glycolytic switch for their rapid effector function [33]. This suggests that alloreactive CD8⁺ memory T cells could share some overlapping metabolic features with their Teff counterparts and can be targeted in a similar fashion to inhibit their reactivation during tolerance induction.

3. Key sensors of Metabolism

Metabolic and immune signals converge upon the PI3K / Phosphoinositide dependent kinase-1 (PDK1)/ Protein kinase B (Akt or PKB) signaling pathway initiating a downstream signaling cascade essential for metabolic reprogramming (Fig. 1) [34]. One of major downstream targets is mTOR that couples nutrient/energy sensing to protein translation and cell growth that is crucial for maintaining energy homeostasis [35]*. It is a component of two complexes, mTORC1 and mTORC2 both of which activate a common set of transcription factors. Among the most important of these are hypoxia inducible factor (HIF1 α) and c-myc, that together promote the expression of enzymes required for metabolism in immune cells that affect their fate and function [36], [25], [37]. In innate immune cells, mTOR signaling modulates their function between pro and anti-inflammatory cytokine production such as IL-12 and IL-10 [38], [39]. In CD4⁺ T cells, mTORC1 signaling is specifically crucial for Th1 and Th17 differentiation whereas mTORC2 is vital for Th2 differentiation [40].

The mTOR signaling is negatively regulated by LKB1 (liver kinase B1) and its downstream target AMP activated protein kinase (AMPK), two master nutrient sensors, that sense cellular stress (e.g., limiting ATP levels) and promote FAO in CD8 T cell memory and Treg cells (Fig. 1) [24],[41]. In addition, the phosphatase and tensin homolog (PTEN), an upstream negative regulator of the PI3K/Akt pathway can also inhibit mTOR signaling [42] [43]. AMPK inhibits mTOR signaling via activation of tuberous sclerosis complexes (TSC1/2). Enhancing mTOR activity via TSC1 deletion in T cells augments Th1 and Th17 differentiation while impairing iTreg suppressive function [44]. These findings parallel with studies involving chronic hyperactivation of mTOR occurring in circumstances of obesity and over-nutrition that can block Treg proliferation and potentially impair their function [3], [45]. Further, absence of TSC1/2 can also impair DC differentiation and function, due to hyperactivation of metabolic pathways such as glycolysis, mitochondrial respiration and lipid biosynthesis [46].

Although inhibition of mTOR activity appears to be required for optimal Treg generation and proliferation, recent studies indicate that mTORC1 signaling is actually necessary for optimal Treg suppressive function as complete absence of mTORC1 signaling impairs the lipid biosynthetic pathway required for their function and migration [47] [28]. These recent discoveries indicate that mTOR signaling is dynamic and oscillatory in nature especially in Tregs and hence a targeted approach is required to fully exploit the potential of metabolic immunosuppressive treatments for transplantation.

4. Modulation of Immune Cell Metabolism in Transplantation

Although, the idea of mTOR inhibition to inhibit Teff function and enhance Tregs looks promising and has yielded encouraging results in rodent transplant models, attempts to translate this into humans has proven difficult. Some of the challenges include recent findings indicating that rapamycin selectively targets mTORC1 without affecting mTORC2 signaling [47]. On the one hand, this can induce Akt activation (by phosphorylation at serine 473) by mTORC2 signaling, an event that can destabilize Tregs by inhibiting Forkhead box

protein O (FOXO) transcription factors that are required for Treg development and function and also skew the immune response towards a Th2 phenotype (Fig. 1) [40], [48]. On the other hand, prolonged mTORC1 inhibition could block Treg generation and function by blockade of lipogenesis [3],[47]. Furthermore, inhibition of mTOR signaling with rapamycin or metformin (that activates AMPK) after the peak of Teff immune response (Fig. 1)) can promote CD8 memory T cell responses by enhancing FAO and thus can be detrimental for establishing tolerance [49] [41]. Thus, given the complex nature of mTOR signaling, identifying the proper timing and dosage are critical parameters towards effectively using mTOR inhibitors for metabolic alteration in immune cells in a transplant setting.

Despite these challenges, novel strategies to incorporate mTOR and metabolic inhibitors to already existing regimens are being proposed [50]**. In line with this, here we suggest conceptual approaches for metabolic interventions in this proposed model (Fig 3). Firstly, the addition of IDO/Arg-1 activators such as CTLA4Ig that are already known to cause apoptosis of alloreactive T cells via costimulatory blocking mechanisms may have an augmented tolerogenic effect in the early and late phases by inducing tolerogenic DCs and enhancing Tregs. Secondly, addition of mitochondrial ATPase inhibitors during the induction phase could further augment apoptosis of activated alloreactive T cells along with costimulation blockade regimens. This could be accompanied by the acute inhibition of mTOR (while it is high in alloreactive T cells and low in Treg) via PI3K/mTOR inhibitors that could presumably promote Treg signature genes including (Forkhead box P3) Foxp3 and lipid metabolism in Tregs while inhibiting the activation of alloreactive T cells, thereby tipping the balance towards more Tregs and tolerogenic DCs to achieve tolerance. Addition of glycolytic inhibitors such as ERRA inhibitors or 2 deoxy-glucose (2DG) could also recapitulate the downstream effects of rapamycin [21],[50]. However, like rapamycin / metformin, this step must be proceeded with caution as blockade of glycolysis by 2DG can also promote CD8 memory responses that could be detrimental for allograft survival [51].

Finally, recent studies have shown that altering the nutritional status of a host can also affect the fate and function of Tregs via the adipocytokine leptin-mTOR signaling pathway [52]. Calorie restriction can mimic the effects of rapamycin, by reducing the levels of leptin, and subsequently mTOR activity thereby promoting Tregs while dampening Th1 and Th17 responses [3]. However, calorie restriction is known to activates sirtuins (Sirts), a family of metabolic sensors which are NAD⁺ (nicotinamide adenine dinucleotide [oxidized]) dependent deacetylases that can deacetylate Foxp3 and target it to proteosomal degradation [37], [53]. In particular, Sirt1 deletion/inhibition in Tregs promotes Foxp3 expression and increases Treg function and prolongs allograft survival [54].

5. Conclusions

Although current long-term immunosuppressive regimens have improved the survival of allografts significantly, patients still face the danger of graft rejection and complications associated with toxicities of immunosuppressive drugs such as cardiovascular disease, opportunistic infections and malignancy [29]. Furthermore, treatment comprising of antibody therapy, steroids and calcineurin inhibitors globally inhibit both Teff and Treg populations [50]. The bioenergetic differences exhibited by immune cells at different stages

of tolerance induction therefore serves as promising avenue that can be specifically exploited via the use of mTOR or metabolic inhibitors in combination with dietary therapy to achieve better immune-regulation and functional tolerance to allografts.

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References

* Special Interest

** Outstanding Interest

1. Brandacher G, Margreiter R, Fuchs D. Clinical relevance of indoleamine 2,3-dioxygenase for alloimmunity and transplantation. *Curr Opin Organ Transplant*. 2008; 13:10–15. [PubMed: 18660700]
2. Mellor AL, Munn DH. IDO expression by dendritic cells: tolerance and tryptophan catabolism. *Nat Rev Immunol*. 2004; 4:762–774. [PubMed: 15459668]
3. Procaccini C, Galgani M, De Rosa V, Matarese G. Intracellular metabolic pathways control immune tolerance. *Trends Immunol*. 2012; 33:1–7. [PubMed: 22075206]
- 4**. Odegaard JI, Chawla A. The immune system as a sensor of the metabolic state. *Immunity*. 2013; 38:644–654. This review provides a comprehensive take on the intricate circuitry between immunity and metabolism required for maintenance of homeostasis within the host. [PubMed: 23601683]
- 5**. Pearce EL, Pearce EJ. Metabolic pathways in immune cell activation and quiescence. *Immunity*. 2013; 38:633–643. This review focuses on the instructive and responsive effect of metabolic changes on various immune cells including DCs, Macrophages and T cells on their function. [PubMed: 23601682]
- 6*. Pearce E, Poffenberger M, Chang C-H, Jones R. Fueling immunity: insights into metabolism and lymphocyte function. *Science*. 2013; 342:1242454. This review gives an indepth perspective of the various mechanisms of metabolic regulation in immune cells and highlights the current challenges in this emerging field. [PubMed: 24115444]
7. Vander Heiden M, Cantley L, Thompson C. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science*. 2009; 324:1029–1033. [PubMed: 19460998]
8. Krawczyk C, Holowka T, Sun J, Blagih J, Amiel E, Deberardinis R, Cross J, Jung E, Thompson C, Jones R, et al. Toll-like receptor-induced changes in glycolytic metabolism regulate dendritic cell activation. *Blood*. 2010; 115:4742–4749. [PubMed: 20351312]
9. Wang R, Dillon C, Shi L, Milasta S, Carter R, Finkelstein D, McCormick L, Fitzgerald P, Chi H, Munger J, et al. The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. *Immunity*. 2011; 35:871–882. [PubMed: 22195744]
10. Sena L, Li S, Jairaman A, Prakriya M, Ezponda T, Hildeman D, Wang C-R, Schumacker P, Licht J, Perlman H, et al. Mitochondria are required for antigen-specific T cell activation through reactive oxygen species signaling. *Immunity*. 2013; 38:225–236. [PubMed: 23415911]
11. Pearce EL. Metabolism in T cell activation and differentiation. *Curr Opin Immunol*. 2010; 22:314–320. [PubMed: 20189791]
12. Jones R, Thompson C. Revving the engine: signal transduction fuels T cell activation. *Immunity*. 2007; 27:173–178. [PubMed: 17723208]
13. Matarese G, Colamatto A, De Rosa V. Metabolic fuelling of proper T cell functions. *Immunology Letters*. 2014; 161:174–178. [PubMed: 24365064]
14. Leishman AJ, Silk KM, Fairchild PJ. Pharmacological manipulation of dendritic cells in the pursuit of transplantation tolerance. *Curr Opin Organ Transplant*. 2011; 16:372–378. [PubMed: 21666481]

15. Ricardo SD, Van Goor H, Eddy AA. Macrophage diversity in renal injury and repair. *J Clin Invest*. 2008; 118:3522–3530. [PubMed: 18982158]
16. Huang SC-C, Everts B, Ivanova Y, O'sullivan D, Nascimento M, Smith AM, Beatty W, Love-Gregory L, Lam WY, O'Neill CM, et al. Cell-intrinsic lysosomal lipolysis is essential for alternative activation of macrophages. *Nat Immunol*. 2014; 15:846–855. [PubMed: 25086775]
17. Grohmann U, Bronte V. Control of immune response by amino acid metabolism. *Immunol Rev*. 2010; 236:243–264. [PubMed: 20636821]
18. Thebault P, Condamine T, Heslan M, Hill M, Bernard I, Saoudi A, Josien R, Anegón I, Cuturi MC, Chiffolleau E. Role of IFN γ in allograft tolerance mediated by CD4⁺CD25⁺ regulatory T cells by induction of IDO in endothelial cells. *Am J Transplant*. 2007; 7:2472–2482. [PubMed: 17868070]
19. Grohmann U, Orabona C, Fallarino F, Vacca C, Calcinaro F, Falorni A, Candeloro P, Belladonna ML, Bianchi R, Fioretti MC, et al. CTLA-4-Ig regulates tryptophan catabolism in vivo. *Nat Immunol*. 2002; 3:1097–1101. [PubMed: 12368911]
20. Sundrud MS, Korolov SB, Feuerer M, Calado DP, Kozhaya AE, Rhule-Smith A, Lefebvre RE, Unutmaz D, Mazitschek R, Waldner H, et al. Halofuginone inhibits TH17 cell differentiation by activating the amino acid starvation response. *Science*. 2009; 324:1334–1338. [PubMed: 19498172]
21. Michalek R, Gerriets V, Nichols A, Inoue M, Kazmin D, Chang C-Y, Dwyer M, Nelson E, Pollizzi K, Ilkayeva O, et al. Estrogen-related receptor- α is a metabolic regulator of effector T-cell activation and differentiation. *Proc Natl Acad Sci USA*. 2011; 108:18348–18353. [PubMed: 22042850]
- 22*. Chang C-H, Curtis J, Maggi L, Faubert B, Villarino A, O'Sullivan D, Huang S, van der Windt G, Blagih J, Qiu J, et al. Posttranscriptional control of T cell effector function by aerobic glycolysis. *Cell*. 2013; 153:1239–1251. This study elegantly demonstrates the role of glycolysis in controlling the translation of cytokine transcripts such as IL-2 and IFN- γ . [PubMed: 23746840]
23. Gerriets V, Rathmell J. Metabolic pathways in T cell fate and function. *Trends Immunol*. 2012; 33:168–173. [PubMed: 22342741]
24. Michalek R, Gerriets V, Jacobs S, Macintyre A, MacIver N, Mason E, Sullivan S, Nichols A, Rathmell J. Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4⁺ T cell subsets. *J Immunol*. 2011; 186:3299–3303. [PubMed: 21317389]
25. Shi L, Wang R, Huang G, Vogel P, Neale G, Green D, Chi H. HIF1 α -dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. *J Exp Med*. 2011; 208:1367–1376. [PubMed: 21708926]
26. Gatz E, Wahl D, Opipari A, Sundberg T, Reddy P, Liu C, Glick G, Ferrara J. Manipulating the bioenergetics of alloreactive T cells causes their selective apoptosis and arrests graft-versus-host disease. *Science Translational Medicine*. 2011; 3:67ra68.
- 27*. Byersdorfer C, Tkachev V, Opipari A, Goodell S, Swanson J, Sandquist S, Glick G, Ferrara J. Effector T cells require fatty acid metabolism during murine graft-versus-host disease. *Blood*. 2013; 122:3230–3237. This paper shows that factors of antigen load, duration of stimulation and systemic inflammation can allow T cells to metabolically adapt to increase both glycolysis and mitochondrial respiration to meet their energy demands. [PubMed: 24046012]
- 28*. Zeng H, Yang K, Cloer C, Neale G, Vogel P, Chi H. mTORC1 couples immune signals and metabolic programming to establish T(reg)-cell function. *Nature*. 2013; 499:485–490. This study thoroughly demonstrates the importance of mTORC1 signaling in lipid synthesis required for Treg suppressive function. [PubMed: 23812589]
29. Salcido-Ochoa F, Tsang J, Tam P, Falk K, Rotzschke O. Regulatory T cells in transplantation: does extracellular adenosine triphosphate metabolism through CD39 play a crucial role? *Transplant Rev (Orlando)*. 2010; 24:52–66. [PubMed: 20153159]
30. Romio M, Reinbeck B, Bongardt S, Hüls S, Burghoff S, Schrader J. Extracellular purine metabolism and signaling of CD73-derived adenosine in murine Treg and Teff cells. *AJP: Cell Physiology*. 2011; 301:C530–539. [PubMed: 21593451]

31. van der Windt G, Everts B, Chang C-H, Curtis J, Freitas T, Amiel E, Pearce E, Pearce E. Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development. *Immunity*. 2012; 36:68–78. [PubMed: 22206904]
32. O'Sullivan D, van der Windt GJW, Huang SC-C, Curtis JD, Chang C-H, Buck MD, Qiu J, Smith AM, Lam WY, DiPlato LM, et al. Memory CD8(+) T cells use cell-intrinsic lipolysis to support the metabolic programming necessary for development. *Immunity*. 2014; 41:75–88. [PubMed: 25001241]
33. Gubser PM, Bantug GR, Razik L, Fischer M, Dimeloe S, Hoenger G, Durovic B, Jauch A, Hess C. Rapid effector function of memory CD8+ T cells requires an immediate-early glycolytic switch. *Nat Immunol*. 2013; 14:1064–1072. [PubMed: 23955661]
34. Finlay D, Rosenzweig E, Sinclair L, Feijoo-Carnero C, Hukelmann J, Rolf J, Panteleyev A, Okkenhaug K, Cantrell D. PDK1 regulation of mTOR and hypoxia-inducible factor 1 integrate metabolism and migration of CD8+ T cells. *J Exp Med*. 2012; 209:2441–2453. [PubMed: 23183047]
- 35*. Shimobayashi M, Hall MN. Making new contacts: the mTOR network in metabolism and signalling crosstalk. *Nat Rev Mol Cell Biol*. 2014; 15:155–162. This review gives a thorough update of the downstream signaling pathways affected by mTOR. [PubMed: 24556838]
36. Wang R, Dillon CP, Shi LZ, Milasta S, Carter R, Finkelstein D, McCormick LL, Fitzgerald P, Chi H, Munger J, et al. The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. *Immunity*. 2011; 35:871–882. [PubMed: 22195744]
37. Wang R, Green DR. Metabolic checkpoints in activated T cells. *Nat Immunol*. 2012; 13:907–915. [PubMed: 22990888]
38. Ohtani M, Nagai S, Kondo S, Mizuno S, Nakamura K, Tanabe M, Takeuchi T, Matsuda S, Koyasu S. Mammalian target of rapamycin and glycogen synthase kinase 3 differentially regulate lipopolysaccharide-induced interleukin-12 production in dendritic cells. *Blood*. 2008; 112:635–643. [PubMed: 18492954]
39. Ohtani M, Hoshii T, Fujii H, Koyasu S, Hirao A, Matsuda S. Cutting edge: mTORC1 in intestinal CD11c+ CD11b+ dendritic cells regulates intestinal homeostasis by promoting IL-10 production. *J Immunol*. 2012; 188:4736–4740. [PubMed: 22504639]
40. Delgoffe GM, Pollizzi KN, Waickman AT, Heikamp E, Meyers DJ, Horton MR, Xiao B, Worley PF, Powell JD. The kinase mTOR regulates the differentiation of helper T cells through the selective activation of signaling by mTORC1 and mTORC2. *Nat Immunol*. 2011; 12:295–303. [PubMed: 21358638]
41. Pearce E, Walsh M, Cejas P, Harms G, Shen H, Wang L-S, Jones R, Choi Y. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. *Nature*. 2009; 460:103–107. [PubMed: 19494812]
42. Walsh P, Buckler J, Zhang J, Gelman A, Dalton N, Taylor D, Bensinger S, Hancock W, Turka L. PTEN inhibits IL-2 receptor-mediated expansion of CD4+ CD25+ Tregs. *J Clin Invest*. 2006; 116:2521–2531. [PubMed: 16917540]
43. Garcia-Cao I, Song M, Hobbs R, Laurent G, Giorgi C, de Boer V, Anastasiou D, Ito K, Sasaki A, Rameh L, et al. Systemic elevation of PTEN induces a tumor-suppressive metabolic state. *Cell*. 2012; 149:49–62. [PubMed: 22401813]
44. Park Y, Jin H-S, Lopez J, Elly C, Kim G, Murai M, Kronenberg M, Liu Y-C. TSC1 regulates the balance between effector and regulatory T cells. *J Clin Invest*. 2013; 1–14. [PubMed: 23281402]
45. Mathis D, Shoelson SE. Immunometabolism: an emerging frontier. *Nat Rev Immunol*. 2011; 11:81. [PubMed: 21469396]
46. Wang Y, Huang G, Zeng H, Yang K, Lamb RF, Chi H. Tuberous sclerosis 1 (Tsc1)-dependent metabolic checkpoint controls development of dendritic cells. *Proc Natl Acad Sci USA*. 2013; 110:E4894–4903. [PubMed: 24282297]
47. Zuber J, Hermine O, Chatenoud L, Legendre C. Harnessing regulatory T cells for transplant tolerance in the clinic through mTOR inhibition: myth or reality? *Curr Opin Organ Transplant*. 2011; 16:606–613. [PubMed: 21971509]

48. Kerdiles YM, Stone EL, Beisner DR, Beisner DL, McGargill MA, Ch'en IL, Stockmann C, Katayama CD, Hedrick SM. Foxo transcription factors control regulatory T cell development and function. *Immunity*. 2010; 33:890–904. [PubMed: 21167754]
49. Araki K, Turner A, Shaffer V, Gangappa S, Keller S, Bachmann M, Larsen C, Ahmed R. mTOR regulates memory CD8 T-cell differentiation. *Nature*. 2009; 460:108–112. [PubMed: 19543266]
- 50**. Lo Y-C, Lee C-F, Powell JD. Insight into the role of mTOR and metabolism in T cells reveals new potential approaches to preventing graft rejection. *Curr Opin Organ Transplant*. 2014; 19:363–371. This review gives insight and perspective on using mTOR and metabolic inhibition in transplant tolerance. [PubMed: 24991977]
51. Sukumar M, Liu J, Ji Y, Subramanian M, Crompton J, Yu Z, Roychoudhuri R, Palmer D, Muranski P, Karoly E, et al. Inhibiting glycolytic metabolism enhances CD8+ T cell memory and antitumor function. *J Clin Invest*. 2013; 123:4479–4488. [PubMed: 24091329]
52. Procaccini C, De Rosa V, Galgani M, Abanni L, Cali G, Porcellini A, Carbone F, Fontana S, Horvath TL, La Cava A, et al. An oscillatory switch in mTOR kinase activity sets regulatory T cell responsiveness. *Immunity*. 2010; 33:929–941. [PubMed: 21145759]
53. van Loosdregt J, Vercoulen Y, Guichelaar T, Gent YYJ, Beekman JM, van Beekum O, Brenkman AB, Hijnen D-J, Mutis T, Kalkhoven E, et al. Regulation of Treg functionality by acetylation-mediated Foxp3 protein stabilization. *Blood*. 2010; 115:965–974. [PubMed: 19996091]
54. Beier U, Wang L, Bhatti T, Liu Y, Han R, Ge G, Hancock W. Sirtuin-1 targeting promotes Foxp3+ T-regulatory cell function and prolongs allograft survival. *Molecular and Cellular Biology*. 2011; 31:1022–1029. [PubMed: 21199917]

Key points

- MTOR and AMPK are two central nutrient/ energy sensors that link immunity and metabolism.
- The extent to which T effector cells rely upon metabolic pathways such as glycolysis and mitochondrial oxidative phosphorylation is context – dependent.
- Tregs and CD8 memory T cells pre-dominantly depend upon lipid metabolism constituting FAO and lipogenesis for their differentiation and function.
- Incorporation of glycolytic inhibitors and FAO promoting agents to diminish alloreactive T cell responses and enhance Tregs may serve as a beneficial strategy during tolerance induction.
- Calorie restriction can also serve as a promising alternative to mimic the effects of mTOR inhibition and can increase Treg responsiveness in tolerance induction regimens.

Figure 1. Cross-talk between immune and metabolic signaling pathways

External signals including antigen, costimulation, nutrients, cytokines and metabolic cues converge upon PI3K signaling pathway that results in the phosphorylation and activation of Akt (threonine 308) leading to further downstream activation of two distinct mTOR containing signaling complexes namely mTORC1 and mTORC2. In activated T effector cells, mTORC1 activation leads to increase in protein translation and activation of transcription factors (TFs) such as c-myc and HIF1 α that in turn initiate the glycolytic and glutamine metabolic pathways. Concomitantly, mTORC2 phosphorylates Akt (serine 473), an event that phosphorylates FOXO family of TFs excluding them from nucleus and preventing the induction of Treg genetic program. In Tregs, however, reduced PI3K/AKT/mTOR signaling results in nuclear localization of FOXOs and initiation of Treg genetic program as well as promotion of FAO through LKB1/AMPK signaling axis that inhibits mTOR via TSC1/2 complexes. Furthermore sirtuins (Sirt), a family of NAD⁺ (nicotinamide adenine dinucleotide [oxidized]) dependent deacetylases that sense changes in NAD⁺ [oxidized] /NADH [reduced] redox ratio in cells, deacetylate Foxp3 and target it to proteosomal degradation. The mechanistic actions of various inhibitors are shown in black boxes. (Abbreviations: S6K: Ribosomal protein S6 kinase; 4EBP1: Eukaryotic translation initiation factor 4E binding protein 1; Klf-2: Kruppel like factor-2; Cit: Citrate; α KG: α ketoglutarate; OAA: Oxaloacetate; TCR: T cell receptor; TLR: Toll-like receptor)

Figure 2. Effect of Metabolic regulation on immune cell function

(A) Innate immune cells such as DCs dependent upon glycolytic metabolism during activation while M2 macrophages rely upon FAO via the scavenger receptor CD36. In Addition, amino acid catabolism plays an important role in mediating immunosuppression in these cells. Engagement of CD80/CD86 and pro and anti- inflammatory cytokine signaling induces IDO and Arg-1 that reduces the bioavailability of tryptophan and arginine in the local microenvironment and subsequently results in the release of downstream metabolites that blocks metabolic activity in responding T cells. (B). $ERR\alpha$ signaling stimulates glycolytic metabolism that includes the upregulation of Glut1, glucose uptake and increase in metabolic gene expression in activated T effector cells. During this process GAPDH, a bifunctional glycolytic enzyme that usually binds to the 3'UTR of cytokine transcripts and prevents their translation is engaged in the glycolytic pathway, thereby relieving the inhibition on cytokine translation and promoting T cell effector function. (C). Tregs are capable of degrading extracellular ATP (indicator of tissue injury) into adenosine (via CD39 and CD73) that activates A2AR (adenosine receptors) on Teff cells (B) to generate immunomodulatory signaling cascades (such as cyclic AMP [cAMP] production and $NF\kappa B$ (nuclear factor kappa light chain enhancer of B cells) inhibition [not shown]) in Teff cells. In contrast to Teff, Tregs utilize exogenous fatty acids (FA) to fuel their mitochondrial metabolism during differentiation. They also utilize the lipid biosynthetic pathway for the expression of CTLA4 and ICOS required for their optimal suppressive function. (D). CD8 memory T cells also utilize FAO similar to Tregs. However, in contrast to Tregs they depend upon glucose to synthesize lipids (such as cholesterol esters and triacylglycerols) that are subsequently hydrolyzed in the lysosome via lysosomal acid lipase (LAL) and used for FAO.

Figure 3. Hypothetical model linking mTOR activity and metabolic signatures in alloreactive and regulatory T cells during tolerance induction and maintenance

(A). Shows a hypothetical profile of alloreactive Teff and Treg cell numbers in association with their mTOR activity under no treatment conditions during acute and chronic phases of rejection. The black solid lines represent Teff and Black dotted lines represents Treg populations. Both Teff and Treg display oscillatory mTOR activity that corresponds to their respective metabolic programs during different stages of activation. (B). Shows the altered profile of Teff and Tregs that could be ideally achieved by manipulating cellular metabolism by either drug or diet therapy to reduce the number and activity of alloreactive Teff cells while promoting tolerogenic DCs and Tregs to achieve functional tolerance.