HHS Public Access

Author manuscript

*Curr Opin Hematol*. Author manuscript; available in PMC 2021 December 20.

Published in final edited form as:

*Curr Opin Hematol*. 2020 November ; 27(6): 353–359. doi:10.1097/MOH.0000000000000615.

# Immunometabolism in hematopoietic stem cell transplantation and adoptive cellular therapies

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## Abstract

**Purpose of Review:** Controlling T cell activity through metabolic manipulation has become a prominent feature in immunology and practitioners of both adoptive cellular therapy (ACT) and hematopoietic stem cell transplantation (HSCT) have utilized metabolic interventions to control T cell function. This review will survey recent metabolic research efforts in HSCT and ACT to paint a broad picture of immunometabolism and highlight advances in each area.

**Recent Findings:** In HSCT, recent publications have focused on modifying reactive oxygen species, sirtuin signaling, or the NAD salvage pathway within alloreactive T cells, and to a lesser extent on modulating regulatory T cells. In ACT, metabolic interventions that bolster memory T cell development, increase mitochondrial density and function, or block regulatory signals in the tumor microenvironment (TME) have recently been published.

**Summary:** Metabolic interventions control immune responses. In ACT, efforts seek to improve the *in vivo* metabolic fitness of T cells, while in HSCT energies have focused on blocking alloreactive T cell expansion or promoting regulatory T cells. Methods to identify new, metabolically targetable pathways, as well as the ability of metabolic biomarkers to predict disease onset and therapeutic response, will continue to advance the field towards clinically applicable interventions.

### Keywords

Immunometabolism; hematopoietic stem cell transplantation; adoptive cellular therapy (ACT); CAR-T cells; tumor-infiltrating lymphocytes (TIL)

## Introduction

Controlling T cell activity has become the Holy Grail of clinical immunology and reliance on oxidative versus glycolytic metabolism exerts significant influence over T cell fate. However, to make metabolic control a clinical reality, it is crucial to understand metabolism within specific environmental contexts. We will begin by reviewing the

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Conflict of Interest

None

influence of metabolism on T cell activation and differentiation, then cover recent advances in understanding T cell metabolism during graft-versus-host disease (GVHD), metabolism-based methods to improve adoptive cellular therapies (ACT), and a role for metabolic biomarkers following hematopoietic stem cell transplantation (HSCT).

## The role of T cell metabolism in differentiation and activation

In broad strokes, T cell metabolism can be divided into oxidative phosphorylation (OXPHOS), which uses the proton gradient established by the electron transport chain (ETC) to generate ATP, and glycolysis, where glucose is converted into lactate in the absence of oxidation. OXPHOS generates ATP more efficiently, but glycolysis supports one carbon metabolism, a necessity during rapid T cell expansion[1]. Naïve and memory cells are metabolically quiescent, while effector T cells (Teff) aggressively increase their metabolism to support proliferation and effector functions. In the classic paradigm, naïve and memory T cells adopt an oxidative phenotype, while Teff rely more heavily on glycolysis[2]. Regulatory T cells (Treg) adopt their own unique metabolic signature[3]. However, these categories were largely established *in vitro* and it is increasingly clear that *in vitro* metabolic pathways do not necessarily reflect those utilized by T cells *in vivo*[4,5], with ample evidence that Teff can adopt an oxidative phenotype[6–8]. In allogeneic HSCT, the intent is to inhibit Teff while promoting Treg, while the goal for ACT is to do the opposite. Despite these opposing objectives, common ways to understand and manipulate T cell metabolism link these fields together and much can be learned from understanding the techniques and targets employed by the other side.

## Cellular Metabolism in GVHD

The challenge facing T cell manipulation during allogeneic HSCT is how to mitigate GVHD-causing T cells while still preserving graft-versus-tumor (GVT) responses. One way to achieve this goal is by improving the Treg/Teff ratio, either by decreasing allogeneic Teff numbers or increasing the number or stability of the Treg population[9–11].

## Metabolic control of alloreactive T cells

Reactive oxygen species (ROS) propagate several aspects of T cell-mediated immunity including intracellular signaling downstream of the T cell receptor (TCR)[12,13]. High levels of ROS were found in the liver, spleen, and bone marrow during acute GVHD [14,15] and promotion of ROS scavenging, through T cell overexpression of thioredoxin-1 (Trx1), reduced GVHD development [14]. Systemically limiting ROS with apocynin, a pharmacologic NADPH oxidase inhibitor, similarly reduced GVHD clinical scores and improved recipient survival [15]. Interestingly, Trx1 overexpression decreased glutamine uptake in liver T cells, but not those recovered from the spleen [14], suggesting metabolic reliance based in part on cellular location. Intracellular ROS levels are controlled by redox pathways, and the transcription factor Nuclear factor erythroid 2–related factor 2 (Nrf2) controls expression of an array of antioxidant genes. Transplantation of Nrf2-deficient T cells reduced GVHD clinical scores and improved post-transplant survival [16]. Interestingly, systemic treatment with arsenic trioxide, which upregulates NRF2 globally,

minimized GVHD pathogenesis [17], highlighting the need for further study of Nrf2's role in the post-transplant environment.

Sirtuins are class III histone deacetylases (HDACs) and Sirtuin 1 (SIRT1) controls cellular metabolism by deacetylating a broad range of transcription factors and transcriptional co-regulators. SIRT3 is a mitochondrially-associated HDAC which inhibits intracellular ROS generation. Transplantation of SIRT1-deficient T cells decreased GVHD by limiting CD4+ cell division and minimizing interferon gamma (IFN-γ) production. Transplantation of SIRT3-deficient T cells also decreased GVHD severity and improved post-transplant mortality [18], but ROS levels actually decreased in these cells, a likely consequence of reduced T cell activation. Sirtuins and T cells both require nicotinamide (NAD) as a co-factor for their function, making them dependent upon the NAD salvage pathway, a process catalyzed by nicotinamide phosphoribosyl-transferase (NAMPT) and intricately linked to glucose metabolism [19]. Serum NAMPT levels increased in acute GVHD models and pharmacologic blockade of NAMPT limited liver- and spleen-associated donor CD4+ and CD8+ T cell numbers, with improved clinical scores and reduced post-transplant weight loss [20]. Interestingly, NAMPT inhibition exerted anti-leukemia effects independent of its impact on GVHD T cells, while SIRT1, SIRT3, and NRF2-deficient donor T cells mitigated GVHD while still preserving GVT responses [18,21].

## Metabolic augmentation of Treg

Both genetic and pharmacologic downregulation of ROS favored FoxP3 expression, the transcription factor synonymous with Treg generation. T cell overexpression of Trx1 increased Foxp3 levels in recipient spleens [14] and recipients of Nrf2 knock-out (KO) cells had greater Treg percentages with increased expression of Helios, a marker of thymus-derived Tregs [16]. Inhibition of the NAD salvage pathway also improved Treg/Teff ratios [20] and transplantation of SIRT1 KO T cells increased Foxp3 percentages, with fewer Treg converting into INF-γ producing Th1 cells [21]. A caveat to interpreting studies where the gene is knocked out in all T cells is whether the metabolic intervention directly impacts Tregs themselves, or simply lessens systemic inflammation, which improves Treg stability.

Treg administration can improve the post-transplant Treg/Teff balance and adoptive transfer of $T_{reg}$ lacking vimentin mitigated GVHD to a greater degree than WT $T_{reg}$, a consequence of enhanced oxidative metabolism and concomitant increase in suppressive capacity [22]. Infusion of CD4CD8 double negative (DN) Treg cells also prevented xenogeneic GVHD [23] and levels of DN Treg correlated with chronic GVHD (cGVHD) severity [24]. Teff co-cultured with DN Treg decreased mammalian target of rapamycin (mTOR) signaling [25], but whether this decrease was simply a consequence of reduced Teff proliferation is unclear.

## Metabolic control in non-T cells

Retinoic acid (RA), a metabolite of Vitamin A, is critical for development of committed CD4 and CD8 T cells and during GVHD, RA is pro-inflammatory and high levels correlate with more severe disease. A lack of RA synthesis in host DCs impaired Teff function,

increased donor Treg frequency, and improved survival [26]. These changes were not seen if intestinal epithelial cells (IECs) lacked RA synthesis, suggesting that RA metabolism in host DCs (> donor DCs) is critical to regulate Treg/Teff ratios. Previous studies have also described salutary effects of butyrate on intestinal epithelial cells (IECs) [27]. Expression of the G-protein coupled receptor GPR43 was necessary for butyrate's protective effects and epithelial repair was secondary to secretion of local cytokines and subsequent activation of the inflammasome [28].

## Metabolic challenges in adoptive cellular therapy

Repurposing a patient's own immune system to combat cancer is a unique form of ACT and includes expansion of tumor infiltrating lymphocytes (TIL) and creation of chimeric antigen receptor (CAR) T cells. While these methods seek to reinvigorate underperforming immune responses, they face many metabolic challenges. T cells obtained from tumor sites or the peripheral blood are often terminally differentiated or "exhausted" and require significant activation *in vitro*. Unfortunately, this stimulation drives terminal differentiation, leading to poor activity and limited persistence upon return to the host [29]. After transfer, T cells need to survive the harsh metabolic landscape of the *in vivo* environment, made worse in fighting leukemia because leukemic cells block T cell acquisition of glucose [30]. Furthermore, there is a clear correlation between loss of cancer-targeting T cells and relapse [31]. Thus, techniques to support or encourage the metabolic health of tumor-specific T cells are critical.

Memory T cells (Tmem) adopt a phenotype skewed towards oxidative metabolism and encouraging T cells to become metabolically more "memory-like" gives them a critical adaptation upon *in vivo* transfer. For example, CAR T cells bearing the CD28 co-stimulatory domain adopt a glycolysis-driven phenotype, with aggressive early activation followed by poor *in vivo* persistence. In contrast, CAR T cells bearing the 4–1BB co-receptor relied on oxidative metabolism, with a slower early expansion but improved overall persistence [32]. Promoting a more oxidative phenotype can be addressed in two main ways. The first utilizes metabolic inhibitors, nutrient withdrawal/supplementation, cytokine addition, and checkpoint blockade to alter the environment in which T cells expand or ultimately function. The second approach genetically modifies T cells to delete unwanted mediators, block ineffective metabolic signaling, or promote advantageous metabolic pathways.

## External Manipulation: Targeting the T cell Environment

T cell expansion is classically glycolysis-driven. Thus, growing T cells in 2-deoxyglucose (2DG), an inhibitor of hexokinase, increased the percentage of memory-like cells and improved their tumor clearance in a mouse model of melanoma[33]. In addition, the IL-2 used during *in vitro* expansion induces glycolysis by upregulating expression of lactate dehydrogenase (LDH), the terminal step in glycolysis. Expansion in IL-21 reduced LDH induction and increased shuttling of pyruvate into the mitochondria. Simultaneous pharmacological blockade of LDH enhanced memory formation with reduced expression of exhaustion markers.[34] Glutamine blockade similarly restricted T cell differentiation and improved anti-tumor function post-transfer, with enhanced generation of memory cells[35]. ROS levels also impacted *in vitro* differentiation of ACT T cells. Addition of

the anti-oxidant N-acetylcysteine during expansion produced less-differentiated stem and central memory cells which were more functional [36,37], while ROS induction conversely increased the percentage of more differentiated effector memory cells.

Targeting T cells in the TME is another potential opportunity to metabolically augment T cell activity Targeting programmed death 1 (PD-1) improves T cell function in the TME[38]. Recently, it has become clear that PD-1 signaling is intricately linked to cellular metabolism. The majority of transcripts altered by PD-1 ligation involved metabolic pathways, with blunting of both glycolysis and oxidative metabolism[39]. In other work, T cells expressing an oncolytic vaccinia virus increased influx of T cells into the TME, however these cells showed poor activity and decreased mitochondrial reserve. This metabolic dysfunction was reversed through enhanced leptin signaling as leptin delivered to the TME via vaccinia virus expression improved T cell mitochondrial density and enhanced spare respiratory capacity (SRC), ultimately resulting in more efficient tumor clearance[40]. Other factors, including the balance between CD4+ and CD8+ T cells, influence CD8+ T cell subset formation and function. These factors could also improve the effectiveness of ACT. Recent evidence from a viral model suggests that CD8+ T cells generated with adequate CD4+ T cell help enhance their cytolytic capacity, SRC, and proliferation upon secondary activation exposure compared to cells without adequate CD4+ help [41]. Thus, improving CD4+ T cell yields during *in vitro* expansion may be the best guarantee of functional CD8 T cells *in vivo*.

## Internal Manipulation: Genetically Modifying T cells

Blockade of specific metabolic pathways can be done externally as discussed above, but a more permanent way to alter T metabolic choice is through genetic manipulation. For example, T cells can be modified to ignore messages of metabolic dysfunction transmitted by transforming growth factor-beta (TGF-β), which in the TME blocks both T cell glycolysis and oxidative metabolism [42]. As expected, deletion of the TGF-β receptor in adoptively transferred cells improved overall anti-tumor activity.[43] Alternatively, oxidative proteins can be overexpressed to promote advantageous metabolism. Targets for this endeavor include the mitochondrial fusion protein Opa1 and those controlling mitochondrial biogenesis, like peroxisome proliferator activated receptor co-activator 1-alpha (PGC1α). These metabolic interventions are modeled after characteristic changes in Tmem mitochondrial density and organization and Opa1 overexpression in ACT generated more memory-like T cells with improved *in vivo* efficacy [44]. Similarly, increased expression of PGC1α enhanced central memory T cell formation with characteristic increased SRC and augmented proliferation upon antigen re-challenge[45]. In addition to these well-described targets, additional methods to screen for metabolically influential proteins have been developed. Using a CRISPR/Cas9 library screen to knock down candidate genes, REGNASE-1 was identified as playing a key role in metabolic exhaustion. Previously known as an RNAse with RNA binding activity, REGNASE-1 depleted T cells showed enhanced anti-tumor activity[46]. This finding synergized with simultaneous knockdown of additional proteins from the same screen, indicating that further targets may continue to be identified through this method.

## Metabolism-based biomarkers

While metabolic manipulation has shown promising results in GVHD and ACT, identifying patients who would benefit most from specific interventions remains an area of active investigation. Allogeneic transplantation in humans is followed by major changes in recipient serum metabolic profiles through a combination of microbial dysbiosis [47] and transplantation-related alteration of host metabolism. Patient samples experienced a significant decrease in tryptophan metabolites at the onset of acute GvHD, including a large decrease in microbiota-produced compounds such as 3-indoxyl sulfate (3-IS) [48]. Previous studies correlated low urinary 3-IS levels with GVHD severity, while increased indole intake improved GVHD-associated weight loss and extended post-transplant survival [49]. Dietary intake of choline has an effect opposite of indoles and oral administration of the choline derivative Tri-methylamine N-oxide (TMAO) worsened GVHD, an effect driven by inflammasome activation, enhanced M1 macrophage polarization, and production of IL-1β [50]. Conversely, treatment of allogeneic recipients with arsenic trioxide decreased macrophage M1 polarization and improved GVHD [51]. These data suggest that reducing choline-mediated macrophage inflammation, while providing protective indoles or short-chain fatty acids, could synergistically protect against GVHD.

Amino acids levels also have predictive value post-transplant. Metabolomic analysis on day 0 (prior to HSCT infusion) identified 20 metabolites associated with GVHD [52], among them a decrease in 2-aminobutyric acid. In addition, total plasma amino acids decreased on day +7 in patients who later developed severe acute GVHD [53], while decreased levels of the branched chain amino acids leucine and isoleucine on day 100 predicted cGVHD development [54]. It is hypothesized that amino acids are consumed as tissues are damaged and immune cell activation programs (including cytokine production) are initiated. In contrast to amino acids, complex lipid products including medium- and long-chain fatty acid (FA), polyunsaturated FA, diacylglycerol, and primary/secondary bile acids increased in patients destined to develop or already experiencing acute GvHD [48,52]. FA levels also increased in future cGVHD patients [54]. A separate approach combining lipidomics and transcriptomics revealed a robust increase in glycerophospholipid (GPL) metabolism and allowed construction of an aGvHD risk score with both diagnostic and prognostic value [55]. Together these data suggest that serum metabolic profiles can function as indirect indicators of T cell activation and thus serve to both gauge GVHD as well as evaluate responses to therapeutic interventions.

The use of biomarkers to predict success in ACT has focused mainly on expression of inhibitory ligands in the TME and serum cytokine production. Monitoring tumors for PDL1 expression is a potential marker for the utility of checkpoint inhibition, although this approach does not guarantee successful therapy [56]. Similarly, measurement of inflammatory cytokines such as IL-6 have been used to indicate CAR-T cell activity [57]. Assessments of true metabolic intermediates may help shed light, as it has in GVHD, on the metabolic activity of tumors and suggest therapies to best target them.

## Conclusion

The study of T cell metabolism has opened many doors for understanding and controlling T cell immunity. During GVHD, defining metabolic pathways in Treg and alloreactive Teff will allow for GHVD-specific interventions with preservation of physiologic and graft-versus-tumor responses. In ACT, recognizing the metabolic dysfunction caused by *in vitro* T cell expansion and the TME can direct interventions to specifically augment metabolic health during key times to improve anti-tumor efficacy. Despite promising preclinical work, the clinical utility of many interventions continues to fall short. Thus, research focusing on predictive biomarkers may identify patients at risk for GVHD, those who will benefit most from specific interventions (e.g. checkpoint inhibitor therapy), or better predict treatment efficacy in both HSCT and ACT. Overall, our ongoing gains in understanding T cell metabolism will increase the ability to manipulate metabolic pathways to clinical advantage and deliver exciting new therapies to patients who are most at-risk.

## Acknowledgements

This work was supported by grants to CB from the National Institute of Health—NHBLI (K08 HL123631, R01 HL144556), the Department of Defense (CA180681), the Hyundai Motor Company (Hope on Wheels Scholar grant), the American Society of Hematology (Scholar award), and the Be the Match Foundation (Amy Strelzer Manasevit award) and to EB from the Burroughs Wellcome Fund (Physician Scientist Incubator program), the NCI (CA082084), the NIH (K12 HD052892), and the St. Baldrick's Foundation (Fellowship award).

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**Key Points**

1. In HSCT, modulation of ROS levels, sirtuin signaling and the NAD salvage pathway were used to decrease Teff and increased Treg post-transplant.
2. In ACT, augmentation of oxidative metabolism and a decrease in inhibitory signaling (e.g. TGF-β) overcame metabolic dysfunction caused by *in vitro* expansion and influences of the TME
3. Metabolic biomarkers could be used to predict the onset of GVHD as well as the potential response to therapy in both HSCT and ACT.