T cell metabolism in graft-versus-host disease

Yujing Zou, Benny J. Chen*

Division of Hematologic Malignancies and Cellular Therapy/BMT, Duke University Medical Center, Durham, NC, USA

Abstract

Graft-versus-host disease (GVHD) is a major source of morbidity and mortality following allogeneic hematopoietic stem cell transplant (allo-HSCT), one of the most effective approaches to treat hematopoietic malignancies.[1] However, current prophylaxis regimens and treatments that reduce the detrimental effect of acute GVHD can be offset by increased incidence in opportunistic infections and relapse of the primary malignancy.[2] In addition, the majority of the approaches that inhibit T cell responses are non-specific, resulting in the inhibition of both alloreactive T cells and protective T cells from the donor. Therefore, there is an increase in the demand to develop novel approaches that selectively target alloreactive T cells. One potential means to address this issue is to take advantage of the unique metabolic profile of activated T cells.

Keywords: Graft-versus-host-disease, Metabolism, T cells

1. INTRODUCTION

Graft-versus-host disease (GVHD) occurs when immune responses are directed against foreign antigens. Patients whose immune system or failing organs that need replacement from donors are at risk for GVHD, given that the donor and the recipient are genetically non-identical. In those with aggressive hematologic malignancies or immunodeficiencies, hematopoietic stem cell transplant (HSCT) from an allogeneic donor can be performed as a curative option. However, GVHD accounts for a major source of morbidity and mortality aside from relapse of the primary disease.[1]

T cells, as part of the adaptive immunity, are one of the primary causes for the development of GVHD.[3] In Major histocompatibility (MHC)-mismatched donor-recipient pairs, allogeneic T cells are activated upon recognition of the alloantigen presented by the mismatched-MHC molecule, causing damage in target tissues.[1] Such reactions can also be mediated by minor histocompatibility antigens (MiHAs), which arise from differences in single nucleotide polymorphisms (SNPs) among individuals.[4] In HSCTs, recipient antigen presenting cells (APCs) activate donor T cells to elicit damage in target organs.[3]

Therapies including T cell depletion from the donor bone marrow have been used in the clinic to reduce the risk of GVHD.[5,6] However, this procedure also offsets the graft-versus-tumor (GVT) effect that is required to prevent relapse of the primary disease, particularly in the case of allogeneic HSCT.[7] Since activated alloreactive T cells exhibit a unique fine-tuning profile of metabolic pathways, such signatures can be utilized to improve the treatment of GVHD by using a T cell-specific targeting approach.

2. T CELL METABOLISM

In the resting phase, T cells remain naïve and primarily depend on oxidative phosphorylation (OXPHOS) to sustain survival and trafficking.[8] Compared to aerobic glycolysis, OXPHOS prioritizes energy conservation, which produces 36 Adenosine triphosphate (ATP) molecules in contrast to 2 ATP molecules.[9,10] This mode of metabolism best matches the functional demands of a resting T cell. Although migration through circulation, including secondary lymphoid organs, can be an ATP-exhausting process, immune surveillance is required for resting naïve T cells to screen for foreign antigens prior to activation, hence the requirement for efficient energy production.[8]

T cell priming occurs with T cell receptor (TCR) ligation and stimulation of the costimulatory molecules by APCs. Upon priming, T cell metabolism is fundamentally reprogrammed to adapt to the energetic demands of an activated T cell. Rather than predominately relying on OXPHOS, T cells rapidly increase the rate of aerobic glycolysis.[11] Although OXPHOS is much more efficient in ATP generation, glycolysis provides various intermediate metabolites for nucleotide and amino acid production to support cell growth and division.[8,11]

Early T cell activation occurs from minutes to hours, and is largely independent of transcription and translation.[12] Therefore, although increased aerobic glycolysis is initiated during this phase, glucose uptake and glycolytic enzymes are not yet affected. Rather, during this process, pyruvate dehydrogenase kinase (PDHK1) is activated via TCR ligation to redirect pyruvate to lactate production rather than entering the tricarboxylic acid

* *Address correspondence: Benny J. Chen, Duke University, 905 S. LaSalle St. Durham, NC 27710, USA. E-mail address: benny.chen@duke.edu (B. J. Chen).*

Conflicts of interest: The authors declare no conflicts of interest.

Author contributions and disclosure: Yujing Zou and Benny J. Chen contributed to manuscript writing and final approval of manuscript.

Funding: This study was partially supported by P01CA047741 (B. J. C.) from the National Institute of Health.

Blood Science, (2020) 2, 16-21

Received July 31, 2019; Accepted October 15, 2019.

http://dx.doi.org/10.1097/BS9.0000000000000035

(TCA) cycle.[12] Essentially, PDHK1 acts as a metabolic switch that determines the fate of pyruvate by deactivating pyruvate dehydrogenase through phosphorylation, blocking the conversion of pyruvate into acetyl-CoA. Therefore, PDHK1 activation directly ensures that pyruvate, a metabolic intermediate of glucose, is directed toward aerobic glycolysis.

During late T cell activation, which can take hours to days, various glycolytic enzymes are upregulated to maximize aerobic glycolysis. This second spike of aerobic glycolysis is associated with transcriptional reprogramming in a Myc-dependent manner process.[13] In addition, Akt (also known as protein kinase B) and hypoxia-inducible factor 1 (HIF-1α) activation also contribute to this process.[14] Glucose transporters, primarily Glut1, import extracellular glucose to keep up with the increased demand for glycolysis.[15] During activation, surface expression of glucose transporters is rapidly increased following Akt activation.[15] Late during the T cell activation phase is also accompanied by the clonal expansion phase, during which T cells undergo rapid division. This phase requires the efficient engagement of both glycolysis and OXPHOS. Several metabolites derived from the TCA cycle can be used for de novo synthesis components required for cell growth. Among these metabolites, citrate and oxaloacetate can be used to fuel lipid and nucleotide synthesis, respectively.[16,17]

During the effector phase, aerobic glycolysis continues to play a critical role. The increase in effector functions can be achieved through the overall upregulation of translation as directed by mammalian target of rapamycin complex 1 (mTORC1) activation.[18–20] Not only does aerobic glycolysis meet the demand for growth and rapid cell division, increased biosynthesis also supports the production of effector molecules, including proinflammatory cytokines, IFN-γ, and cytotoxic molecules such as TNF-α and perforin.[12] Overall, the increased output of the proinflammatory molecules can initiate and sustain the damage of alloreactive T cells on recipient tissues. In addition, many glycolytic enzymes act as a switch to control effector cytokine production. When glycolysis is rapidly upregulated, the translation of many cytokines become activated due to alleviated suppression by corresponding glycolytic enzymes.[21] Prior studies demonstrated that T cell effector function, primarily cytokine production, is regulated by aerobic glycolysis through posttranscriptional changes. Specifically, these studies showed that many glycolytic enzymes are linked to cytokine production by binding to AU-rich elements (AREs).[12,21] When not actively engaged in glycolysis (resting T cells), glycolytic enzymes bind to AREs located in the 3′UTR of cytokine mRNAs, blocking their translation. For example, Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) represses *Ifng* translation when the glycolytic flux is low.[22] Besides regulating *Ifng*, the lactic acid dehydrogenase (LDH) can also control the translation of *Tnfa*, *Il2*, limiting the synthesis of proinflammatory cytokines.[12] However, cytotoxic granules including perforin and granzyme B lack the ARE component, and are not under the control of glycolytic enzymes like cytokines.[12] Nevertheless, the translational process is tightly coordinated with aerobic glycolysis through mTORC1 activation, indicating that cytotoxic granules are controlled through translation.

In terms of differences among distinct T cell lineages, $CD4^+$ T cell subsets also display unique signatures of metabolic requirements. Though differences exist, Th1, Th2, and Th17 cells preferentially utilize aerobic glycolysis.[8,23,24] In contrast, regulatory T cells (Tregs) favor fatty acid oxidation (FAO).[25,26] Manipulation of metabolic pathways can also influence T helper cell differentiation.[8] The addition of lipids favors the generation of Tregs, rather than other effector lineages.[27] Similarly, memory T cells predominately rely on FAO,[28,29] accompanied by increased expression of the lipid transporter, *carnitine palmitoyltransferase* 1A (CPT1A), located on the mitochondrial membrane.[30,31] Other relevant metabolic reprogramming of memory T cells include increased mitochondrial biomass and spare respiratory capacity, conferring greater resistance to metabolic stress.[11,28] These metabolic changes are critical to ensure memory T cell survival following an immune response.

Similar to $CD4^+$ T cells, the activation of $CD8^+$ T cells into cytotoxic effector T cells also requires the upregulation of aerobic glycolysis to keep up with the biosynthetic demands.[15] Both rapid division and production of proinflammatory cytokines, as well as cytolytic granules are highly dependent on this process.

3. T CELL METABOLISM DURING GVHD: THE ROLE OF DIFFERENT METABOLIC PATHWAYS IN ALLOREACTIVE T CELLS

3.1. Aerobic glycolysis

Aerobic glycolysis is classically defined in the Warburg effect as the conversion of pyruvate into lactate as opposed to being utilized for TCA cycle even in the presence of sufficient oxygen.[9,32,33] Though a concept familiar to cancer studies as this pathway is highly upregulated in many types of tumor cells, it is now becoming increasingly clear that activated lymphocytes, including T cells, also rely on aerobic glycolysis for rapid biosynthesis and proliferation.[15,34]

Previous studies showed that aerobic glycolysis supports T cell growth and proliferation.[15,35] Although this process is less efficient at generating ATP compared to OXPHOS, the various intermediate metabolites generated through this pathway support cell growth and division. Immediately following TCR ligation, aerobic glycolysis is rapidly initiated through PDHK1, which allows for the conversion of the end product of glycolysis, pyruvate, into lactate rather than feeding the TCA cycle. Subsequently, costimulatory molecule ligation by the APC (CD28) activates the PI3K-Akt-mTOR signaling pathway.[36] Akt can upregulate glycolysis by phosphorylating glycolytic enzymes such as hexokinase (HK) to increase the glycolytic flux.[13,37] In alloreactive T cells, not only is HK1 upregulated, the expression of a second isoform, HK2, is also enhanced to drastically increase the rate of glycolysis.[37] Akt is also critical for the surface trafficking of Glut1 to achieve increased glucose uptake.[38] Downstream of Akt, the mTORC1 is activated and promotes translational efficiency through the phosphorylation of eukaryotic translation initiation factor 4E binding protein 1 (*4EBP*-1) and p70S6 kinase (p70S6K).[39]

We and other have shown that alloreactive T cells preferentially upregulate glycolysis when activated by alloantigens (Fig. 1).[15,37] This was demonstrated by increased glycolytic activity in T cells isolated from transplant recipients, as well as the requirement for Glut1 to mediate GVHD development in a murine bone marrow transplant model. Compared to syngeneic transplants, in which T cells also upregulate glycolysis during homeostatic expansion in response to cytokines or self-ligands, allogeneic transplants differ in that alloreactive T cells upregulate glycolysis much higher than the syngeneic counterparts. In addition, aside from the upregulation of Glut1 by alloreactive T cells, Glut3 expression is also increased to further enhance glycolysis compared to T cells derived from syngeneic recipients.[37]

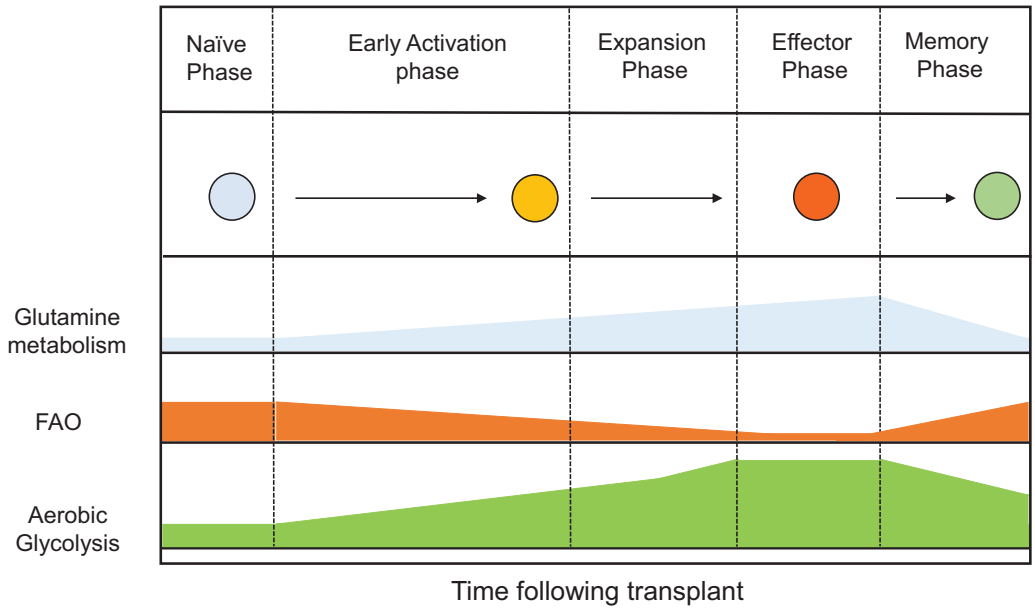

Figure 1. Metabolism of alloreactive T cells. Following the transfer of donor T cells into the allogeneic recipient, resting alloreactive T cells, which rely primarily on fatty acid oxidation (FAO), become activated upon recognition of alloantigens. Early during activation, aerobic glycolysis is immediately upregulated to support the biosynthetic depends required for growth and expansion. Glutamine uptake is also elevated to support biosynthesis. During the effector phase, aerobic glycolysis is required for effector functions such as proinflammatory cytokines. During the transition into the memory phase, aerobic glycolysis is downregulated, along with glutamine metabolism. In contrast, FAO is upregulated to support memory T cell function.

In terms of the role of aerobic glycolysis in $CD4^+$ T cell subset differentiation and function, Th1, Th2, Th17 are pathogenic in the context of GVHD and prefer the use of glycolysis.[40] In contrast, Tregs are suppressive and prevent the progression of GVHD, favoring the use of FAO.[8,41–43] The corresponding key metabolic regulators for each subset differ: while both Th1 and Th17 are regulated by mTORC1,[8] Th17 also requires hypoxia inducible factor 1 subunit alpha (HIF-1α).[44,45] However, Th2 is predominantly dependent on mTORC2.[8,46] Prior murine studies demonstrated that mTOR and mTORC1 are involved in Th1-mediated GVHD progression.[37] In recipients of donor T cells deficient in either components, the number of IFN-γ-producing T cells in the target organ was markedly reduced. It was further determined in this study that the number of induced Tregs (iTregs) was markedly increased in the absence of mTORC1 (*Raptor* KO T cell recipients).[37] This is in line with the finding that glycolysis is required for Th1 differentiation mediated by mTORC1. The absence of mTORC1 hence promotes iTreg differentiation. Moreover, the interference of pathogenic Th17 has been demonstrated to effectively reduce GVHD development, and has been linked to the modulation of Th17 metabolism through the blockade of IL-1 signaling.[47] It was demonstrated that the treatment reduced Th17 induction and was accompanied by a significant decrease in the expression of glycolytic enzymes. Upon transfer of treated donor cells, the severity of GVHD development was markedly reduced, with decreased percentages of Th17 donor T cells and increased induction of iTregs in the target organ.[47]

Although both alloreactive $CD4^+$ and $CD8^+$ T cells rely on glycolysis, there are subtle differences between the metabolic requirements of the two subsets, which may have important clinical implications. In the context of allo-HSCT, $CD4^+$ T cells are more dependent on glycolysis than their $CD8^+$ counterparts.[15] This provides implications for the impact of glycolytic inhibition on GVHD versus GVT. Since the GVT effect is primarily mediated by cytotoxic donor T cells recognizing the tumor antigen, it would be beneficial for $CD8^+$ tumor-specific T cells to survive from glycolytic inhibition.

3.2. OXPHOS

As a critical the energy-conserving component of the catabolic pathway, OXPHOS is a tightly regulated process that allows lymphocytes to adapt to metabolic stress and changes in cellular needs. OXPHOS is tied to the regulation of aerobic glycolysis due to the competition for pyruvate availability. Since aerobic glycolysis is under the control of the energy sensor adenosine monophosphate-activated protein kinase (AMPK), the activity of AMPK can regulate mitochondrial oxidative capacity via OXPHOS.[48–50] In resting T cells and memory T cells, AMPK-mediated oxidative metabolic state promote cell survival and help them adapt to the corresponding energetic needs.[8,51]

In activated T cells, AMPK activation is triggered immediately following T cell activation due to increased LKB1 signaling and escalated intracellular calcium level, which is a transient process.[52,53] This is followed by the activation of mTORC1, which is preceded by the inhibition of AMPK.[48] Therefore, activated T cells have lower AMPK activity and higher glycolytic rate to support growth and effector functions. These findings have implications in the setting of an inflammatory response. Indeed, $CD8^+$ T cells deficient in AMPKα become more potent proinflammatory cytokine producers.[53] Previous studies using murine allo-HSCT models suggest that OXPHOS is actively utilized at comparable levels in both syngeneic and allogeneic BMT in mouse studies.[37] In addition, lower levels of TCA cycle metabolites such as citrate, fumarate, and malate were found in alloreactive T cells compared to T cells derived from syngeneic HSCT transplants.[37] This result points to the possibility that pyruvate molecules were predominately converted to lactate

rather than TCA intermediates, highlighting a dominant role for aerobic glycolysis instead of OXPHOS during GVHD development. Interestingly, ROS production as a result of increased OXPHOS has been shown to be required for T cell activation.[54] It is possible that this mechanism is also utilized by activated alloreactive T cells. Hence, it may be optimal to simultaneously inhibit aerobic glycolysis and OXPHOS, despite a more dominant role for glycolysis.

3.3. Lipid metabolism

During T cell activation, Myc not only mediates transcriptional changes in glucose metabolism, but also regulates genes for fatty acid synthesis (FAS).[13] Moreover, lipid metabolism has been shown to regulate T cell fate.[55] It was demonstrated that enhanced lipid synthesis promotes the proinflammatory effector T cell phenotype while lipid oxidation favors iTreg differentiation.[27]

Previous studies using murine models demonstrated that alloreactive T cells not only displayed a tendency for the accumulation of long-chain fatty acids, but also upregulated enzymes associated with FAS, indicating that FAS may be able to promote GVHD development.[56] In line with this hypothesis, a separate murine study showed that inhibition of FAS by interfering with acetyl-CoA carboxylase 1 (TACC1) prevented clonal expansion of alloreactive T cells in vitro.[57] Furthermore, transfer of treated donor T cells was able to arrest the development of GVHD. Collectively, these findings indicate that the regulation of FAS, a component of anabolic metabolism similar to glycolysis, may be useful to inhibit the pathogenicity of alloreactive T cells.

As the catabolic branch of lipid metabolism, there are also studies testing the role of FAO. However, results from different groups appeared to report conflicting findings about the role of FAO in GVHD.[37,56] Alloreactive T cells have been shown to display increased FAO by Ferrara's group.[56] However, other studies appeared to suggest that FAO plays a less important role, as indicated by the decreased amount of key metabolites required for FAO and TCA cycle in T cells derived from allogeneic HSCT recipients compared to syngeneic recipients in murine models.[37] In addition, fatty acid uptake was also found to be lower in alloreactive T cells, contrary to the former reports. Factors contributing to discrepancies between these reports include the varying use of controls. In the first study, resting cells were used as control while donor T cells derived from syngeneic HSCT were used for the second study. Syngeneic donor T cells may be a more appropriate control in GVHD models as it accounts for background signal contributed by homeostatic proliferation.[58]

3.4. Glutamine metabolism

Glutamine can be used by activated T cells as an alternative carbon source for TCA cycle.[36] The process begins through the conversion of glutamine to glutamate. Eventually, a-ketoglutarate (a-KG), a citrate precursor, is generated via glutaminolysis.[59,60] In addition to replenishing metabolites in TCA cycle, glutamine can also be used as a source for anabolic pathways to support cell growth.[61] The production of a-KG can be used to generate citrate, which forms the backbone during lipid synthesis once converted to acetyl-CoA in the cytosol.[62] In addition, glutamine can also be used for nucleotide synthesis. Specifically, during activation of alloreactive T cells, both $CD4^+$ and $CD8^+$ T cell subsets utilize as substrates for ribose synthesis, promoting DNA replication during proliferation. Another facet of glutamine metabolism in GVHD is the upregulation of glutamine transporters expressions in alloreactive T cells. In particular, the expression of glutamine transporters, including SLC3a2 and SLC5A1, is controlled by Myc.[63] Interestingly, Myc-regulated GLS1 expression further promotes the conversion of glutamine to glutamate.[64,65]

3.5. Pentose phosphate pathway

The pentose phosphate pathway (PPP) is another component of anabolic metabolism, and is preferentially utilized by alloreactive T cells to promote cell growth and proliferation.[37] PPP generates carbon donors (ribose-5 phosphate) for nucleotide synthesis.[66] In alloreactive T cells, the glycolysis intermediate, glucose-6-phosphoate (G-6P), is used as the main substrate for PPP to produce the end product and fuel nucleotide generation.[67,68] Therefore, both PPP and glycolysis activities are enhanced in alloreactive T cells. In addition, PPP also produces NADPH to support the synthesis of antioxidants,[69] potentially alleviating the oxidative stress during T cell activation.

4. TARGETING ALLOREACTIVE T CELL METABOLISM WHILE PRESERVING GVT EFFECT

Activated alloreactive T cells display distinct metabolic signatures to promote their survival, clonal expansion, and proinflammatory effector functions during GVHD development. However, the use of broad immunosuppressant drugs, such as glucocorticoids and calcineurin inhibitors, not only can lead to many complications, but also suppresses both alloreactive and antitumor T cells, thus unable to separate GVHD targeting and the GVT effect. Therefore, studies characterizing the metabolic signatures of alloreactive T cells provide key insights for the development of drugs that will improve the specific inhibition of alloreactive T cells.

For GVHD-targeting, blockade of aerobic glycolysis has shown efficacy for alleviating disease development in murine studies. We have also demonstrated using a murine model for allo-HSCT that the deletion of Glut1 significantly alleviates GVHD development by impairing glycolysis.[15] However, targeting glycolysis with small molecule inhibitors such as 2DG (interfering the HK step of glycolysis),[37] 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3-PO) (inhibiting PFKFB3, a regulatory and a rate-limiting factor in the glycolytic pathway),[37,70,71] and rapamycin (inhibiting mTORC1 activation) may be more practical as a treatment regimen in the clinic.[72–74] This approach may be promising as murine alloreactive T cells have been shown to be susceptible to glycolysis inhibitors in vitro. However, such procedures must be developed with caution to decrease the off-target effects of non-specific targeting if given systemically. Other modulators, such as programmed death 1 (PD-1), have been shown to modulate glucose metabolism in T cells. Ligation of PD-1 reduces the capacity to engage in glycolysis, causing pre-activated T cells to switch the glycolytic program in favor of FAO.[75] Thus, PD-1 ligating elements such as the soluble PD-L1 protein, are able to inhibit alloreactive T cells. The effect of PD-1/PD-L1 interaction has been demonstrated in murine studies, which showed rapid increase in mortality in PD-L1-deficient hosts, compared to wild type recipients with upregulation of PD-L1 during GVHD.[76] On the other hand, AMPK activators, including metformin and AICAR,[77] can be used to suppress aerobic glycolysis in activated T cells by modulating mTORC1. Furthermore, pharmacological activation of AMPK or mTORC1 inhibition may have the potential to promote iTreg polarization while preventing the generation of

pathogenic Th17 cells,[78] as it has been shown in vitro. Another advantage of glycolytic inhibitors is that it has the potential to separate GVHD from the GVT effect, given that tumor-specific T cells, which are $CD8^+$ T cells that mediate direct killing, may be more resistant to metabolic inhibition than their $CD4^+$ counterpart. Although both $CD4^+$ and $CD8^+$T cells upregulate glucose metabolism upon activation, the metabolism of $CD8^+$T cells is more glycolytic while capable of better utilizing glutamine as an alternate energy source, and was demonstrated to be more tolerant to glycolytic inhibition in in vitro assays using murine T cells.[79] In addition, memory $CD8^+$ T cells that mediate GVT preferentially depend on FAO, as opposed glycolysis. Past studies have indicated that the use of glycolytic inhibitors can further enhance the antitumor activity of $CD8^+$ memory T cells, possibly due to increased FAO to compensate for the lack of energy derivation from glycolysis.[80]

Although alloreactive T cells primarily rely on aerobic glycolysis, OXPHOS is also increased, with ROS as a byproduct to support T cell activation. Consequently, alloreactive T cells exhibit an oxidative phenotype and are more susceptible to superoxide-induced apoptosis. In murine allo-HSCT studies, the use of Bz-423 (induces the generation of superoxide by inhibiting mitochondrial F1F0 ATP synthase) alleviated GVHD.[81–83] Therefore, it is tempting to target multiple metabolic pathways simultaneously, such as OXPHOS and aerobic glycolysis, in the clinic.

Inhibitors of lipid metabolism, including etomoxir (suppression of FAO by inhibition of CPT1), can also be used to treat GVHD in the allo-HSCT setting.[56] Etomoxir, which inhibits CPT1, is a potential option to prevent chronic rejection. However, a concern with this approach is that iTregs differentiation may be blocked, as they are also dependent on FAO.[84]

In order to minimize off-target effects and preserve the GVT effect, ex vivo treatment of donor T cells prior to the transplant may deliver a much more precise inhibition. Donor T cells, a heterogeneous pool that contains T cells specific for pathogens, tumor antigens, and alloantigens, can be subjected to ex vivo activation with recipient alloantigens in the presence of metabolic inhibitors such as 2DG. Such treatment would potentially lead to cell death or anergy of activated alloreactive T cells. By contrast, the viability and function of beneficial T cells would be preserved since they cannot react to alloantigens and are less susceptible to glycolytic inhibition during the ex vivo suppression treatment.

5. CONCLUDING REMARKS

The targeting of metabolic pathways utilized by alloreactive T cells have demonstrated promising results in murine models, with increased survival of recipients and reduction in GVHD pathology, as well as decreased incidences of complications due to opportunistic infections. Compared to broadly immunosuppressive regimens currently available in the clinic, the use of metabolic signatures appears as a unique and promising strategy to prevent the development and progression of GVHD. Future studies should also consider the delivery of pharmacological inhibitors in a T cell-specific manner, which will reduce complications caused by systemic administration.

REFERENCES

[1] Zeiser R, Blazar BR. Acute graft-versus-host disease—biologic process, prevention, and therapy. *N Engl J Med* 2017;377(22):2167–2179.

[2] Bleakley M, Riddell SR. Molecules and mechanisms of the graft-versus-leukaemia effect. *Nat Rev Cancer* 2004;4(5):371–380.

[3] Blazar BR, Murphy WJ, Abedi M. Advances in graft-versus-host disease biology and therapy. *Nat Rev Immunol* 2012;12(6):443–458.

[4] Chao NJ. Minors come of age: minor histocompatibility antigens and graft-versus-host disease. *Biol Blood Marrow Transplant* 2004;10(4):215–223.

[5] Hazenberg MD, Otto SA, de Pauw ES, et al. T-cell receptor excision circle and T-cell dynamics after allogeneic stem cell transplantation are related to clinical events. *Blood* 2002;99(9):3449–3453.

[6] Saad A, Lamb LS. Ex vivo T-cell depletion in allogeneic hematopoietic stem cell transplant: past, present and future. *Bone Marrow Transplant* 2017;52(9):1241–1248.

[7] Falkenburg JHF, Jedema I. Graft versus tumor effects and why people relapse. *Hematol Am Soc Hematol Educ Program* 2017;2017(1):693–698.

[8] MacIver NJ, Michalek RD, Rathmell JC. Metabolic regulation of T lymphocytes. *Annu Rev Immunol* 2013;31:259–283.

[9] Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science (New York, NY)* 2009;324(5930):1029–1033.

[10] Zheng J. Energy metabolism of cancer: glycolysis versus oxidative phosphorylation (Review). *Oncol Lett* 2012;4(6):1151–1157.

[11] Pearce EL, Poffenberger MC, Chang CH, Jones RG. Fueling immunity: insights into metabolism and lymphocyte function. *Science (New York, NY)* 2013;342(6155):1242454.

[12] Menk AV, Scharping NE, Moreci RS, et al. Early TCR signaling induces rapid aerobic glycolysis enabling distinct acute T cell effector functions. *Cell Rep* 2018;22(6):1509–1521.

[13] Wang R, Dillon CP, Shi LZ, et al. The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. *Immunity* 2011;35(6):871–882.

[14] Jacobs SR, Herman CE, Maciver NJ, et al. Glucose uptake is limiting in T cell activation and requires CD28-mediated Akt-dependent and independent pathways. *J Immunol* 2008;180(7):4476–4486.

[15] Macintyre AN, Gerriets VA, Nichols AG, et al. The glucose transporter Glut1 is selectively essential for CD4 T cell activation and effector function. *Cell Metab* 2014;20(1):61–72.

[16] Lane AN, Fan TW. Regulation of mammalian nucleotide metabolism and biosynthesis. *Nucleic Acids Res* 2015;43(4):2466–2485.

[17] Baenke F, Peck B, Miess H, Schulze A. Hooked on fat: the role of lipid synthesis in cancer metabolism and tumour development. *Dis Models Mech* 2013;6(6):1353–1363.

[18] Showkat M, Beigh MA, Andrabi KI. mTOR signaling in protein translation regulation: implications in cancer genesis and therapeutic interventions. *Mol Biol Int* 2014;2014:686984.

[19] Gingras AC, Raught B, Sonenberg N. Regulation of translation initiation by FRAP/mTOR. *Genes Dev* 2001;15(7):807–826.

[20] Saxton RA, Sabatini DM. mTOR signaling in growth, metabolism, and disease. *Cell* 2017;168(6):960–976.

[21] Chang CH, Curtis JD, Maggi LB Jr, et al. Posttranscriptional control of T cell effector function by aerobic glycolysis. *Cell* 2013;153(6):1239–1251.

[22] Cham CM, Gajewski TF. Glucose availability regulates IFN-gamma production and p70S6 kinase activation in CD8+ effector T cells. *J Immunol* 2005;174(8):4670–4677.

[23] Yang K, Shrestha S, Zeng H, et al. T cell exit from quiescence and differentiation into Th2 cells depend on Raptor-mTORC1-mediated metabolic reprogramming. *Immunity* 2013;39(6):1043–1056.

[24] Gerriets VA, Kishton RJ, Nichols AG, et al. Metabolic programming and PDHK1 control CD4+ T cell subsets and inflammation. *J Clin Invest* 2015;125(1):194–207.

[25] Buck MD, Sowell RT, Kaech SM, Pearce EL. Metabolic Instruction of Immunity. *Cell* 2017;169(4):570–586.

[26] Michalek RD, Gerriets VA, Jacobs SR, et al. Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4+ T cell subsets. *J Immunol* 2011;186(6):3299–3303.

[27] Cluxton D, Petrasca A, Moran B, Fletcher JM. Differential regulation of human Treg and Th17 cells by fatty acid synthesis and glycolysis. *Front Immunol* 2019;10:115.

[28] Buck MD, O'Sullivan D, Klein Geltink RI, et al. Mitochondrial dynamics controls T cell fate through metabolic programming. *Cell* 2016;166(1):63–76.

[29] Pearce EL, Walsh MC, Cejas PJ, et al. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. *Nature* 2009;460(7251):103–107.

[30] Raud B, Roy DG, Divakaruni AS, et al. Etomoxir actions on regulatory and memory T cells are independent of Cpt1a-mediated fatty acid oxidation. *Cell Metab* 2018;28(3):504–515.e7.

[31] Raud B, McGuire PJ, Jones RG, Sparwasser T, Berod L. Fatty acid metabolism in CD8(+) T cell memory: challenging current concepts. *Immunol Rev* 2018;283(1):213–231.

[32] Lunt SY, Vander Heiden MG. Aerobic glycolysis: meeting the metabolic requirements of cell proliferation. *Annu Rev Cell Dev Biol* 2011;27: 441–464.

[33] Warburg O. On the origin of cancer cells. *Science (New York, NY)* 1956;123:309–314.

[34] Warburg O, Gawehn K, Geissler AW. Metabolism of leukocytes. *Zeitschrift fur Naturforschung Teil B, Chemie, Biochemie, Biophysik, Biologie und verwandte Gebiete* 1958;13b:515–516.

[35] Araujo L, Khim P, Mkhikian H, Mortales CL, Demetriou M. Glycolysis and glutaminolysis cooperatively control T cell function by limiting metabolite supply to N-glycosylation. *eLife* 2017;6: doi: 10.7554/eLife.21330.

[36] Frauwirth KA, Riley JL, Harris MH, et al. The CD28 signaling pathway regulates glucose metabolism. *Immunity* 2002;16(6):769–777.

[37] Nguyen HD, Chatterjee S, Haarberg KM, et al. Metabolic reprogramming of alloantigen-activated T cells after hematopoietic cell transplantation. *J Clin Invest* 2016;126(4):1337–1352.

[38] Wofford JA, Wieman HL, Jacobs SR, Zhao Y, Rathmell JC. IL-7 promotes Glut1 trafficking and glucose uptake via STAT5-mediated activation of Akt to support T-cell survival. *Blood* 2008;111(4):2101–2111.

[39] Josse L, Xie J, Proud CG, Smales CM. mTORC1 signalling and eIF4E/4E-BP1 translation initiation factor stoichiometry influence recombinant protein productivity from GS-CHOK1 cells. *Biochem J* 2016;473 (24):4651–4664.

[40] Coghill JM, Sarantopoulos S, Moran TP, et al. Effector CD4+ T cells, the cytokines they generate, and GVHD: something old and something new. *Blood* 2011;117(12):3268–3276.

[41] Zeng H, Zhang R, Jin B, Chen L. Type 1 regulatory T cells: a new mechanism of peripheral immune tolerance. *Cell Mol Immunol* 2015;12 (5):566–571.

[42] Zhang P, Lee JS, Gartlan KH, et al. Eomesodermin promotes the development of type 1 regulatory T (T_R1) cells. *Sci Immunol* 2017;2(10): doi:10.1126/sciimmunol.aah7152.

[43] Komanduri KV, Champlin RE. Can Treg therapy prevent GVHD? *Blood* 2011;117:751–752.

[44] Shi LZ, Wang R, Huang G, et al. HIF1alpha-dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. *J Exp Med* 2011;208(7):1367–1376.

[45] Dang EV, Barbi J, Yang HY, et al. Control of T(H)17/T(reg) balance by hypoxia-inducible factor 1. *Cell* 2011;146(5):772–784.

[46] Lee K, Gudapati P, Dragovic S, et al. Mammalian target of rapamycin protein complex 2 regulates differentiation of Th1 and Th2 cell subsets via distinct signaling pathways. *Immunity* 2010;32(6):743–753.

[47] Park MJ, Lee SH, Lee SH, et al. IL-1 receptor blockade alleviates graft-versus-host disease through downregulation of an interleukin-1beta-dependent glycolytic pathway in Th17 cells. *Mediators Inflamm* 2015;2015:631384.

[48] Hardie DG, Ross FA, Hawley SA. AMPK: a nutrient and energy sensor that maintains energy homeostasis. *Nat Rev Mol Cell Biol* 2012;13 (4):251–262.

[49] Hardie DG, Ross FA, Hawley SA. AMP-activated protein kinase: a target for drugs both ancient and modern. *Chem Biol* 2012;19(10):1222–1236.

[50] Hawley SA, Fullerton MD, Ross FA, et al. The ancient drug salicylate directly activates AMP-activated protein kinase. *Science (New York, NY)* 2012;336(6083):918–922.

[51] Gerriets VA, Rathmell JC. Metabolic pathways in T cell fate and function. *Trends Immunol* 2012;33(4):168–173.

[52] Tamas P, Hawley SA, Clarke RG, et al. Regulation of the energy sensor AMP-activated protein kinase by antigen receptor and Ca2+ in T lymphocytes. *J Exp Med* 2006;203(7):1665–1670.

[53] MacIver NJ, Blagih J, Saucillo DC, et al. The liver kinase B1 is a central regulator of T cell development, activation, and metabolism. *J Immunol* 2011;187(8):4187–4198.

[54] Franchina DG, Dostert C, Brenner D. Reactive oxygen species: involvement in T cell signaling and metabolism. *Trends Immunol* 2018;39(6):489–502.

[55] Lochner M, Berod L, Sparwasser T. Fatty acid metabolism in the regulation of T cell function. *Trends Immunol* 2015;36(2):81–91.

[56] Byersdorfer CA, Tkachev V, Opipari AW, et al. Effector T cells require fatty acid metabolism during murine graft-versus-host disease. *Blood* 2013;122(18):3230–3237.

[57] Raha S, Raud B, Oberdörfer L, et al. Disruption of de novo fatty acid synthesis via acetyl-CoA carboxylase 1 inhibition prevents acute graft-versus-host disease. *Eur J Immunol* 2016;46(9):2233–2238.

[58] Nguyen HD, Kuril S, Bastian D, Yu XZ. T-cell metabolism in hematopoietic cell transplantation. *Front Immunol* 2018;9:176.

[59] Liu PS, Wang H, Li X, et al. alpha-ketoglutarate orchestrates macrophage activation through metabolic and epigenetic reprogramming. *Nat Immunol* 2017;18(9):985–994.

[60] Arts RJ, Novakovic B, Ter Horst R, et al. Glutaminolysis and fumarate accumulation integrate immunometabolic and epigenetic programs in trained immunity. *Cell Metab* 2016;24(6):807–819.

[61] Choi YK, Park KG. Targeting glutamine metabolism for cancer treatment. *Biomol Ther* 2018;26(1):19–28.

[62] Pearce EL, Pearce EJ. Metabolic pathways in immune cell activation and quiescence. *Immunity* 2013;38:633–643.

[63] Gnanaprakasam JN, Wang R. MYC in regulating immunity: metabolism and beyond. *Genes* 2017;8(3): doi:10.3390/genes8030088.

[64] Goetzman ES, Prochownik EV. The role for Myc in coordinating glycolysis, oxidative phosphorylation, glutaminolysis, and fatty acid metabolism in normal and neoplastic tissues. *Front Endocrinol* 2018;9:129.

[65] Xiao D, Ren P, Su H, et al. Myc promotes glutaminolysis in human neuroblastoma through direct activation of glutaminase 2. *Oncotarget* 2015;6(38):40655–40666.

[66] Weyand CM, Goronzy JJ. Immunometabolism in early and late stages of rheumatoid arthritis. *Nat Rev Rheumatol* 2017;13(5):291–301.

[67] Wang R, Green DR. Metabolic checkpoints in activated T cells. *Nat Immunol* 2012;13(10):907–915.

[68] Filosa S, Fico A, Paglialunga F, et al. Failure to increase glucose consumption through the pentose-phosphate pathway results in the death of glucose-6-phosphate dehydrogenase gene-deleted mouse embryonic stem cells subjected to oxidative stress. *Biochem J* 2003;370((Pt 3)):935–943.

[69] van der Windt GJ, Pearce EL. Metabolic switching and fuel choice during T-cell differentiation and memory development. *Immunol Rev* 2012;249 (1):27–42.

[70] Clem BF, O'Neal J, Tapolsky G, et al. Targeting 6-phosphofructo-2-kinase (PFKFB3) as a therapeutic strategy against cancer. *Mol Cancer Ther* 2013;12(8):1461–1470.

[71] Schoors S, De Bock K, Cantelmo AR, et al. Partial and transient reduction of glycolysis by PFKFB3 blockade reduces pathological angiogenesis. *Cell Metab* 2014;19(1):37–48.

[72] Waickman AT, Powell JD. mTOR, metabolism, and the regulation of T-cell differentiation and function. *Immunol Rev* 2012;249(1): 43–58.

[73] Chi H. Regulation and function of mTOR signalling in T cell fate decisions. *Nat Rev Immunol* 2012;12(5):325–338.

[74] Powell JD, Pollizzi KN, Heikamp EB, Horton MR. Regulation of immune responses by mTOR. *Annu Rev Immunol* 2012;30:39–68.

[75] Patsoukis N, Bardhan K, Chatterjee P, et al. PD-1 alters T-cell metabolic reprogramming by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation. *Nat Commun* 2015;6:6692.

[76] Saha A, Aoyama K, Taylor PA, et al. Host programmed death ligand 1 is dominant over programmed death ligand 2 expression in regulating graft-versus-host disease lethality. *Blood* 2013;122(17):3062–3073.

[77] O'Sullivan D, Pearce EL. Targeting T cell metabolism for therapy. *Trends Immunol* 2015;36:71–80.

[78] Delgoffe GM, Kole TP, Zheng Y, et al. The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment. *Immunity* 2009;30(6):832–844.

[79] Cao Y, Rathmell JC, Macintyre AN. Metabolic reprogramming towards aerobic glycolysis correlates with greater proliferative ability and resistance to metabolic inhibition in CD8 versus CD4 T cells. *PLoS One* 2014;9:e104104.

[80] Sukumar M, Liu J, Ji Y, et al. Inhibiting glycolytic metabolism enhances CD8+ T cell memory and antitumor function. *J Clin Invest* 2013;123 (10):4479–4488.

[81] Gatza E, Wahl DR, Opipari AW, et al. Manipulating the bioenergetics of alloreactive T cells causes their selective apoptosis and arrests graft-versus-host disease. *Sci Transl Med* 2011;3(67):67ra68.

[82] Glick GD, Rossignol R, Lyssiotis CA, et al. Anaplerotic metabolism of alloreactive T cells provides a metabolic approach to treat graft-versus-host disease. *J Pharmacol Exp Ther* 2014;351(2):298–307.

[83] Johnson KM, Chen X, Boitano A, et al. Identification and validation of the mitochondrial F1F0-ATPase as the molecular target of the immunomodulatory benzodiazepine Bz-423. *Chem Biol* 2005;12 (4):485–496.

[84] Siu JHY, Surendrakumar V, Richards JA, Pettigrew GJ. T cell allorecognition pathways in solid organ transplantation. *Front Immunol* 2018;9:2548.