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Suppression of Glut1 and Glucose Metabolism by Decreased Akt/mTORC1 Signaling Drives T Cell Impairment in B Cell Leukemia

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Leukemia can promote T cell dysfunction and exhaustion that contributes to increased susceptibility to infection and mortality. The treatment-independent mechanisms that mediate leukemia-associated T cell impairments are poorly understood, but metabolism tightly regulates T cell function and may contribute. In this study, we show that B cell leukemia causes T cells to become activated and hyporesponsive with increased PD-1 and TIM3 expression similar to exhausted T cells and that T cells from leukemic hosts become metabolically impaired. Metabolic defects included reduced Akt/mammalian target of rapamycin complex 1 (mTORC1) signaling, decreased expression of the glucose transporter Glut1 and hexokinase 2, and reduced glucose uptake. These metabolic changes correlated with increased regulatory T cell frequency and expression of PD-L1 and Gal-9 on both leukemic and stromal cells in the leukemic microenvironment. PD-1, however, was not sufficient to drive T cell impairment, as in vivo and in vitro anti–PD-1 blockade on its own only modestly improved T cell function. Importantly, impaired T cell metabolism directly contributed to dysfunction, as a rescue of T cell metabolism by genetically increasing Akt/mTORC1 signaling or expression of Glut1 partially restored T cell function. Enforced Akt/mTORC1 signaling also decreased expression of inhibitory receptors TIM3 and PD-1, as well as partially improved antileukemia immunity. Similar findings were obtained in T cells from patients with acute or chronic B cell leukemia, which were also metabolically exhausted and had defective Akt/mTORC1 signaling, reduced expression of Glut1 and hexokinase 2, and decreased glucose metabolism. Thus, B cell leukemia–induced inhibition of T cell Akt/mTORC1 signaling and glucose metabolism drives T cell dysfunction. The Journal of Immunology, 2016, 197: 2532–2540.

Immunological defects are frequent complications and lead to increased susceptibility to infections in B cell leukemia patients (1, 2). Chemotherapeutic treatments, such as those provided in acute leukemia, can reduce production of hematopoietic cells and disrupt bone marrow niches to contribute to reduced immune function. Furthermore, targeted therapies for chronic B cell malignancies can have major impacts on nonleukemic immune cells (3). Additionally, differentiated immune cells can also be actively suppressed prior to therapy (4–7). Despite the distinct natures of B cell–derived acute lymphoblastic and chronic lymphocytic leukemia, each can lead to T cell dysregulation independent of treatment (7–9) that resembles functional impairment and exhaustion of T cells chronically stimulated with viral Ag (10, 11). T cell exhaustion is thought to be important to prevent excessive inflammation and tissue damage and is marked by phenotypic changes including expression of inhibitory receptors such as PD-1 and TIM3 as well as decreased activation, proliferation, and inflammatory cytokine production upon restimulation (12, 13). In B cell malignancies, broad T cell stimulation (14, 15) and aberrant expression of immunomodulatory ligands or cytokines by leukemic and stromal cells may also promote T cell exhaustion (16, 17). The molecular mechanisms by which B cell leukemia promotes T cell dysfunction, however, are uncertain.

Metabolic pathways must be tightly regulated to allow normal proliferation and T cell effector function. Altered metabolic regulation may thus contribute to impaired T cell function in leukemia. While resting T cells use an oxidative metabolism, T cell activation promotes effector T cells to induce the glucose transporter Glut1 and

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Abbreviations used in this article: CLL, chronic lymphocytic leukemia; HK2, hexokinase 2; mAkt, myristoylated Akt; mTORC1, mammalian target of rapamycin complex 1; 2-NBDG, 2-[(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose; PerCP, peridinin chlorophyll protein complex; Treg, regulatory T cell.

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hexokinase 2 (HK2) and upregulate a program termed aerobic glycolysis (18). The PI3K/Akt/mammalian target of rapamycin complex 1 (mTORC1) pathway is critical in this metabolic reprogramming, and activation of Akt/mTORC1 drives expression and cell surface trafficking of Glut1 (19, 20) and promotes glycolytic metabolism (21). Consistent with a key role for aerobic glycolysis in effector T cells, Glut1-deficient T cells have impaired proliferation and are unable to efficiently elicit inflammation in vivo (22). As T cells differentiate into functionally distinct subsets, however, each population is metabolically unique. In particular, CD4+ regulatory T cells (Tregs) primarily use oxidative metabolism and can be immunosuppressive independent of PI3K/Akt/mTORC1 signaling and upregulate Glut1 and aerobic glycolysis. Importantly, restoring T cell metabolism through Akt activation or expression of Glut1 was sufficient to improve T cell function, and activation of Akt in T cells delayed progression of leukemia. Taken together, these data demonstrate that inhibition of T cell glucose metabolism is a mechanism by which leukemia promotes T cell dysfunction. Restoring T cell metabolism may therefore provide a new avenue to promote immunological function in leukemia.

In this study, we examine the mechanism of B cell leukemia–associated T cell dysfunction and show that inhibition of T cell metabolism contributes to impaired T cell function in both acute and chronic B cell leukemia. We show that functional exhaustion of T cells from leukemic hosts occurs with reduced ability of T cells to activate Akt/mTORC1 signaling and upregulate Glut1 and aerobic glycolysis. Importantly, restoring T cell metabolism through Akt activation or expression of Glut1 was sufficient to improve T cell function, and activation of Akt in T cells delayed progression of leukemia. Taken together, these data demonstrate that inhibition of T cell glucose metabolism is a mechanism by which leukemia promotes T cell dysfunction. Restoring T cell metabolism may therefore provide a new avenue to promote immunological function in leukemia.

Materials and Methods

Mice

C57BL/6J and BALB/c mice were purchased from The Jackson Laboratory (Bar Harbor, ME). T cell–specific Glut1 transgenic and myristoylated Akt (mAkt) mice on the C57BL/6J background were previously described and metabolically characterized (28, 29). Because FLS.12 cells were generated on the BALB/c background (30), mice were crossed with BALB/c, and C57BL/6J × BALB/c F1 mice were used as hosts for FLS.12 cell transfers. Mice were bred and housed under specific pathogen-free conditions at Duke University Medical Center. All experiments were performed under protocols approved by the Institutional Animal Care and Use Committee. Six- to eight-wk-old transgenic or nontransgenic littermates were used for all experiments.

FLS.12 leukemia model

Murine pro-B cell FLS.12 cells retrovirally transduced with MSCV-BCR/Ab1-IRE5-GFP were cultured in RPMI 1640 with 10% FCS (Gemini Bio-Products) as described (31) and tested Mycoplasma negative. In some experiments, 0.03 μg/ml IFN-γ (eBioscience) was added to culture media to induce inhibitory ligands. For in vivo experiments, cells were washed in PBS and 0.05–0.1 × 10⁶ cells were injected i.v. for immunization experiments. 0.05–0.1 × 10⁶ BCR/Ab1 FL5.12 cells were irradiated (30 Gy) and injected s.c. 7 d prior to i.v. injections. At specified time points, splenocytes were isolated and RBCs lysed using ACK buffer (Lonza). For anti-CD1d treatment experiments, mice were immunized with irradiated FL5.12 cells 7 d prior to injection of live cells. After injection of leukemic cells, mice were treated with i.p. administration of PD-1 blocking Ab (250 μg per mouse) or isotype control every 3 d for the course of 12 d.

Patients and blood samples

PBMCs from 37 chronic lymphocytic leukemia (CLL) patients (32 patients in cohort 1 [Duke University, Durham, NC] and 5 patients in cohort 2 [Academic Medical Center, Amsterdam, the Netherlands]) and healthy donors, and bone marrow mononuclear cells from five B cell acute lymphoblastic leukemia patients (Vanderbilt University, Nashville, TN) were isolated by density gradient centrifugation. All subjects were de-identified and gave written informed consent according to protocols approved by Institutional Review Boards of the collecting center in accordance with the Declaration of Helsinki. The diagnosis and staging (using the Rai system) of CLL were determined according to International Workshop on Chronic Lymphocytic Leukemia criteria (32). Samples were analyzed fresh (CLL cohort 1) or after cryopreservation (CLL cohort 2 and acute lymphocytic leukemia). Where fresh samples were analyzed for in vitro activation and metabolic assays, the distribution of patient subgroups in relationship to disease characteristics was not normalized.

T cell purification and stimulation

Human PBMCs and murine splenocytes were cultured in complete RPMI 1640 with 10% FBS and 1% penicillin/streptomycin and stimulated by addition of anti-CD3 with or without anti-CD28 to cell cultures (eBioscience). For selected experiments, murine splenocytes were cultured with 0.03 μg/ml IFN-γ (eBioscience). In some cases, T cells were purified using pan T cell isolation kits (Miltenyi Biotec) and stimulated with plate-bound anti-CD3 and anti-CD28 (eBioscience) or anti-CD3−, anti-CD2−, and anti-CD28−coated beads (Miltenyi Biotec).

Flow cytometry

Expression of T cell surface markers was measured by flow cytometry (MACSQuant; Miltenyi Biotec) and analyzed with FlowJo software (Tree Star, Ashland, OR). The following anti-human Abs were used: CD4 Violet Blue, CD25 PE, CD8 peridinin chlorophyll protein complex (PerCP) (all Miltenyi Biotec), CD69 FITC (BD Biosciences), CD8 allophycocyanin, CD19 Violet Blue, CD71 allophycocyanin, CTLA4 PE, CD200 FITC, CD200R PE, Ga9 PerCP, PD-1 allophycocyanin, PD-1 allophycocyanin, TIM3 FITC, LAG3 PerCp, BTLA FITC, CD160 FITC, and CD244 allophycocyanin (all eBioscience). The following anti-mouse Abs were used: galecetin-9 PerCP (BioLegend), CD4 Violet Blue, CD8 PerCP, CD9 allophycocyanin, CD69 allophycocyanin, CD25 PE, CD71 PE, CTLA4 PE, CD200 FITC, CD200R PE, Ga9 PerCP, PD-L1 allophycocyanin, PD-1 PerCP, TIM3 allophycocyanin, LAG3 PE, BTLA FITC, CD160 FITC, and CD244 allophycocyanin (all eBioscience). Glut1, HK2, and pS6 were measured by intracellular flow cytometry of paraformaldehyde-fixed and methanol-permeabilized cells using monoclonal rabbit anti-Glut1 (ab652; Abcam), anti-HK2 (131196; Abcam), and anti-pS6 (4858; Cell Signaling Technology) in the presence of rat serum and Fc Block, followed by anti-rabbit PE. Intracellular cytokine production was measured after 5 h of stimulation with PMA (50 ng/ml; Sigma-Aldrich) and ionomycin (750 ng/ml; Calbiochem) in the presence of GolgiPlug (eBioscience). Cells were permeabilized with Cytofix/Cytoperm Plus (BD Biosciences) and stained with IL-2 PE and IFN-γ allophycocyanin (BD Biosciences). Transcription factor staining was performed using the mouse regulatory T cell staining kit (eBioscience) and Foxp3 PE Abs (eBioscience). Cell proliferation was measured using CellTrace Violet (Invitrogen).

Metabolic assays

Glycolysis measurements were normalized to cell number and have been described previously (19). Glucose uptake was measured using the fluororescent glucose analog 2-N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)aminoo-2-deoxy-g-glucose (2-NBDG) as described previously (33). Briefly, cells were cultured in glucose-free media and incubated in 50 μM 2-NBDG for 45 min. Fluorescence of selected T cell subpopulations was measured by flow cytometry.

Statistical analysis

Data involving two groups were analyzed using a paired or unpaired two-tailed Student t test. Data of more than two groups were analyzed using two-way ANOVA with Bonferroni posttest. Statistical analysis was performed using Prism (GraphPad Software, La Jolla, CA).

Results

Experimental B cell lymphoblastic leukemia induces T cell metabolic dysfunction in vivo

B cell leukemia can impair T cell function, but the molecular mechanisms and whether changes in T cell metabolism contribute to T cell dysfunction were unknown. To address this gap, a murine model of B cell leukemia was employed in which BCR/Ab1 transduced murine
pro–B cell FL5.12 cells were adoptively transferred into syngeneic immunocompetent hosts. Leukemic B cells accumulated in unimmunized mice and led to decreased survival (Supplemental Fig. 1A). When immunized with irradiated FL5.12 cells prior to transfer of live leukemic cells, however, recipients were capable of rejecting the leukemia, demonstrating the potential of immune regulation. Indeed, nonirradiated leukemic B cells did not alter endogenous T cells, as the percentage of activated, CD25-, CD69-, and CD71-positive splenic T cells increased as leukemia progressed (Supplemental Fig. 1B). Expression of inhibitory receptors was next measured on T cells from leukemia-bearing animals. Increased expression of PD-1 was measured on CD4+ and CD8+ T cells. Additionally, CD4+ T cells showed elevated LAG3 expression and both CD4+ and CD8+ T cells showed a trend toward increased TIM3 expression (Fig. 1A).

T cells from leukemia-bearing mice became functionally impaired independent of any treatment and failed to respond to in vitro activation over time. Consistent with in vivo T cell activation, CD69, CD25, and CD71 each showed progressively impaired in vitro reinduction as leukemia progressed (Fig. 1B). The proliferative capacity of T cells was also reduced, as both CD4+ and CD8+ T cells from leukemia-bearing mice became incapable of robust proliferation upon in vitro stimulation (Supplemental Fig. 1C). Importantly, splenic T cells isolated from mice injected with FL5.12 leukemia were metabolically affected and failed to increase cell size and glucose uptake upon in vitro anti-CD3 stimulation (Fig. 1C). These defects correlated with a failure of stimulated T cells from leukemia-bearing animals to induce Glut1 and HK2 or activate the Akt/mTORC1 pathway and phosphorylate S6 (Fig. 1D). Nevertheless, the leukemic environment was required for the persistence of the T cell metabolic dysfunction, as T cells purified and removed from leukemic cells showed elevated stimulation-induced glucose uptake and cell size comparable to controls (Fig. 1E). Collectively, these data show that leukemic cells alter the T cell microenvironment to induce T cell stimulation and expression of inhibitory receptors and activation markers that correlate with impaired T cell function and metabolism after restimulation.

**Inhibitory ligands and regulatory T cells are present in leukemic microenvironment**

The failure of T cells from leukemia-bearing animals to properly activate upon secondary stimulation may have been due to insufficient activation or altered costimulation provided by leukemic and stromal cells. However, leukemic cells (GFP+) and stromal cells expressed similar levels of B7-1, B7-2, and B7-H2 as splenocytes not exposed to leukemia (Supplemental Fig. 1D). Furthermore, increased TCR activation, CD28 costimulation, or addition of IL-2 each failed to restore T cell function from leukemia-bearing mice when stimulated in the presence of leukemic cells (Supplemental Fig. 1E–H). Inhibitory pathways, such as PD-1/PD-L1, can modulate PI3K/Akt/mTOR signaling (27, 34) and may contribute to T cell metabolic dysfunction in leukemia. The expression of PD-L1 and Gal-9, a TIM3 ligand, was analyzed on leukemic cells and surrounding noncancerous cells. IFN-γ can induce PD-L1 (35), and BCR/Abl FL5.12 cells expressed low levels of PD-L1 that increased...
upon IFN-γ stimulation (Fig. 2A). A population of PD-L1^{high} cells was found in spleens from leukemia-bearing animals that was comprised of both GFP^{+} leukemic and GFP^{−} nonleukemic cells. Gal-9 was similarly expressed on splenocytes from leukemia-bearing mice and was predominantly from GFP^{−} nonleukemic cells (Fig. 2B). The percentage of PD-L1^{+} and Gal-9^{+} splenic cells increased over time in animals with leukemia (Fig. 2C). Inhibitory ligands can also induce suppressive Tregs (36, 37), and spleens of mice injected with FL5.12 leukemic cells were analyzed to determine whether leukemia promoted Tregs. Consistent with induction of inhibitory signals, the percentage of Foxp3^{+} cells among CD4^{+} T cells increased as leukemia progressed (Fig. 2D).

Although multiple mechanisms may contribute to T cell dysfunction in leukemia-bearing mice, PD-1 plays a prominent role in T cell exhaustion and is associated with poor outcome in acute and chronic B cell leukemia (9, 38). To test whether PD-1 contributes to T cell defects in this model of experimental B cell leukemia, we treated leukemic mice with anti–PD-1 blocking Abs in vivo. Mice were immunized with a small number of irradiated FL5.12 cells 7 d prior to leukemia transfer to prime for an antileukemic T cell response. Anti–PD-1 treatment of leukemia-bearing mice failed to significantly decrease the percentage of GFP^{+} leukemic cells in spleens (Fig. 2E). Anti-PD-1–treated animals showed a trend toward decreased splenocyte count (Fig. 2F) and a trend toward prolonged survival (Fig. 2G). Baseline expression of LAG3, PD-1, TIM3, CD25, and CD71 as well as activation-induced T cell glucose uptake, cell size, and expression of CD25 and CD71 were not different between controls and anti-PD-1–treated mice (Supplemental Fig. 2A–C). However, anti–PD-1 was sufficient to increase Glut1 expression on CD8^{+} T cells from leukemia-bearing animals (Fig. 2H). Nevertheless, it appeared that PD-1 was, on its own, insufficient to account for all T cell defects. Consistent with this conclusion, in vitro PD-1 blockade also failed to restore the ability of T cells to properly activate (Supplemental Fig. 3A). To identify additional inhibitory molecules expressed on chronically stimulated T cells that may contribute to T cell dysfunction, we repeatedly stimulated human T cells in vitro to mimic in vivo chronic T cell activation (Supplemental Fig. 3B). Similar to T cells from leukemia-bearing mice, chronically stimulated T cells were unable to increase 2-NBDG uptake and phosphorylation of S6 upon restimulation (Fig. 2I). In addition to PD-1, chronically stimulated T cells also increased expression of the inhibitory receptor CD200R (Fig. 2J, Supplemental Fig. 3C). Thus, chronic stimulation induces expression of T cell inhibitory receptors that correlate with

![Figure 2](http://www.jimmunol.org/)

**FIGURE 2.** Leukemia-associated T cell dysfunction is correlated with immunosuppressive microenvironment but can be recapitulated by chronic in vitro stimulation. **(A and B)** Expression of PD-L1 and Gal-9 on FL5.12 cells in vitro and on splenocytes from control mice (n = 2) or injected mice (n = 5) ex vivo or after overnight culture with or without IFN-γ. **(C)** Expression of PD-L1 and Gal-9 on control splenocytes (n = 6) or splenocytes from mice 14 (n = 3) or 20 d (n = 4) after injection of FL5.12 cells. **(D)** Foxp3 expression in CD4^{+} splenic T cells from control mice (n = 2) or injected mice (n = 3 per group) analyzed by flow cytometry. **(E–H)** BALB/c mice were immunized with low-dose (0.02 × 10^{6}) irradiated BCR/Abl FL5.12 cells 7 d prior to injection of live cells. After injection of leukemic cells, mice were treated with i.p. administration of PD-1 blocking Ab (250 μg per mouse) every 3 d for the course of 12 d. Splenic T cells were analyzed 20 d after injection. Percentage of GFP^{+} cells in spleen (E) and total splenocyte count (F) on day 20. **(G)** Survival of FL5.12–infected mice without treatment (n = 7), immunized and treated with IgG isotype (n = 7), and immunized and treated with anti-PD-1 (n = 7). (H) Glut1 expression on T cells from animals treated as in (E–G) was measured with flow cytometry. **(I and J)** Purified healthy human blood T cells were repeatedly stimulated with low-dose anti-CD3-, anti-CD28-, and anti-CD2-coated beads for indicated times with or without rest until restimulation on day 21 (Supplemental Fig. 3B). Glucose uptake, pS6 on CD4^{+} and CD8^{+} T cells (I), and expression of specified markers on CD4^{+} T cells (J) was measured with flow cytometry. Data in (C) and (D) are means ± SD. Differences in survival were analyzed using a log-rank (Mantel–Cox) test. Data in (I) are representative of four to seven chronically stimulated and four to six control PBMCs samples. Data in (J) are means ± SEM from three donors. *p < 0.05, **p < 0.01, ***p < 0.001.
impaired ability to upregulate mTORC1 signaling and glucose uptake upon restimulation.

Rescue of T cell Akt/mTORC1 signaling and Glut1 expression decreases leukemia-associated T cell dysfunction

The Akt/mTORC1 pathway is critical in T cell metabolic reprogramming (21), yet mTORC1 activity was decreased in T cells in the murine leukemia model. Next, mice with T cell–specific transgenic expression of constitutively active Akt (mAkt) in which T cells show increased glucose uptake and glycolysis (28) were used to test whether restoration of Akt/mTORC1 signaling could improve function of T cells from leukemia-bearing animals. mAkt mice and control littersmates were injected with leukemic FL5.12 cells, and disease progression and T cell phenotypes were assessed (Fig. 3A). mAkt transgenic CD8+ T cells from leukemia-bearing mice had smaller cell size, lower baseline expression of CD25 and CD71 (Supplemental Fig. 3D), and higher levels of phosphorylated S6 upon stimulation (Fig. 3B). Stimulated mAkt transgenic T cells from leukemia-bearing mice showed increased ability to induce CD69, CD25, and CD71 after in vitro stimulation (Supplemental Fig. 3E) and also to produce inflammatory cytokines IL-2 and IFN-γ (Fig. 3C, 3D). This was accompanied by decreased expression of the inhibitory receptors TIM3 and PD-1 on a subset of mAkt transgenic CD8+ T cells (Fig. 3E, 3F). Importantly, progression of leukemia was significantly delayed in mAkt transgenic mice (Fig. 3G–I), and disease burden positively correlated with T cell expression of PD-1 (Supplemental Fig. 3F). Thus, inhibition of Akt/mTORC1 signaling mediates B cell leukemia-associated T cell dysfunction that both functionally impairs T cells and may prevent antileukemic immunity.

The Akt/mTORC1 pathway affects a wide range of cellular functions, including, but not limited to, induction of Glut1 and glucose metabolism (28, 39). Given that T cell Glut1 expression and glucose uptake were affected in mice with B cell leukemia, we tested whether leukemia-induced suppression of Glut1 expression contributed to T cell dysfunction and examined the effects of leukemia on T cells with constitutive Glut1 expression. Animals with T cell–specific transgenic expression of Glut1 and increased T cell glucose uptake (20) were used as hosts for FL5.12 leukemia. Unlike with T cells with constitutively active Akt, progression of leukemia was unaffected by T cell expression of Glut1 (Supplemental Fig. 3G–I). Baseline expression of inhibitory receptors LAG3, PD-1, and TIM3 was also not changed on T cells by constitutive Glut1 expression in leukemia-bearing animals (Supplemental Fig. 3J). However, Glut1 expression was sufficient to partially rescue the ability...
of T cells from leukemia-bearing mice to induce CD25, CD69, and CD71 expression after anti-CD3 stimulation (Fig. 4A, 4B). The production of IL-2 by stimulated CD4+ T cells was also significantly increased, and IFN-γ production showed a trend to increase in Glut1 transgenic CD8+ T cells (Fig. 4C, 4D). These data demonstrate that impaired Glut1 induction contributes to T cell functional defects in B cell leukemia–bearing hosts.

**T cells from human B cell leukemia patients show decreased glucose transport, glycolysis, and mTORC1 activity**

Our data in the mouse model demonstrate that B cell leukemia leads to T cell functional defects in part through metabolic suppression. To determine whether similar impairments occur in patients with B cell leukemia, we examined T cell activation and metabolic phenotypes of peripheral blood T cells from healthy donors and leukemia patients (described in Supplemental Table IA). Similar to our experimental model, T cells from CLL patients had increased expression of activation markers CD25, CD69, and CD71 ex vivo. PD-1 expression was significantly increased on CD4+ T cells and CD160 was elevated on CD8+ T cells from CLL patients (Fig. 5A, Supplemental Fig. 4A, 4B). Whereas the expression of PD-L1 and Gal-9 on PBMCs was not increased, elevated CD200 was found on CD19+ B cells of CLL patients (Supplemental Fig. 4C, 4D). T cells from CLL patients also showed impaired induction of activation markers CD25 and CD71 upon in vitro stimulation (Fig. 5B). T cells from CLL patients were next analyzed to determine whether the observed activation defects correlated to alterations in T cell glucose metabolism. Whereas resting T cells from CLL patients had similar glucose uptake capacity to those from healthy donors, induction of glucose uptake (Fig. 5C) and glycolysis (Fig. 5D) was impaired in activated T cells from CLL patients. Activated T cells in healthy donors showed an increase in the expression of Glut1, HK2, and phospho-S6. T cells from CLL patients, however, showed a mixed response with notable defects in the measured metabolic parameters (Fig. 5E).

Similar metabolic defects were also observed in a second cohort of CLL patients when compared with age-matched healthy control donors (Supplemental Fig. 4E). Likewise, T cells from patients with acute BCR/Ab1+ B cell lymphoblastic leukemia (described in Supplemental Table IB) also showed defects in glucose uptake and Glut1 and HK2 expression upon in vitro stimulation (Fig. 5F–H, Supplemental Fig. 4F). Whereas no correlation of disease status and clinical parameters to the metabolic phenotype was observed, these data show that T cells in human B cell leukemia patients are metabolically impaired. Metabolic suppression of T cells is thus a mechanism of T cell dysfunction that may predispose to infections and mortality in B cell leukemia.

**Discussion**

Although having a distinct biology and prognosis, both acute and chronic B cell–derived leukemia share immune defects that predispose for infections and can contribute to decreased antitumor immunity. These immune defects are mediated in part by treatment-unrelated T cell dysfunction (7–9), but the mechanisms are uncertain. Tight

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**FIGURE 4.** Reduced Glut1 expression contributes to dysfunction of T cells from leukemia-bearing hosts. Splenocytes from not injected mice with T cells with (n = 3) or without (n = 3) Glut1 transgenic and FL5.12-injected mice with T cells with (n = 20) or without (n = 10) Glut1 transgenic cells were stimulated overnight with anti-CD3. (A) Representative data of CD25 expression. Numbers represent percentage of positive cells in no transgenic and Glut1 transgenic animals. (B) CD25, CD71, and CD69 expression measured with flow cytometry. (C and D) Production of IL-2 and IFN-γ was assessed after additional 5 h stimulation with PMA/ionomycin. Numbers in each quadrant represent percentage of events. *p < 0.05, **p < 0.01, ***p < 0.001. tg, transgenic.
regulation of T cell metabolism is critical for T cell fate and function (18), and dysregulation of key metabolic pathways may contribute to T cell dysfunction. In this study, we show that T cells in leukemia patients and leukemia-bearing animals are metabolically suppressed and unable to efficiently increase glucose metabolism upon stimulation. This appears to be due to chronic in vivo stimulation leading to a phenotype similar to that described for T cell exhaustion. Importantly, these metabolic impairments are critical for T cell dysfunction, as genetic rescue of Akt signaling or glucose uptake partially restored T cell activation and function.

T cell exhaustion has been previously associated with altered cell metabolism, but this link remained largely indirect. Myc is critical for increased T cell metabolism upon activation (40), and decreased cMyc expression was reported in T cells exhausted upon chronic viral infection (41). Additionally, stabilization of HIF1α has been shown to reduce T cell exhaustion (42), and overexpression of phosphoenolpyruvate carboxykinase 1 improved antitumor immunity in a mouse melanoma model (43). Recently, intratumoral nutrient competition was implicated as a regulator of T cell function in a mouse sarcoma model (44). The metabolic impairments of exhausted and dysfunctional cells themselves and the role of T cell metabolic dysfunction under normoxic conditions to mediate these states, however, remain unclear. In the present study, we add to these findings and directly demonstrate that B cell leukemia induces a metabolic dysfunction of endogenous T cells that is dependent on reduced Akt/mTORC1 signaling and an inability to upregulate Glut1 and glycolysis. These metabolic defects became apparent after in vitro stimulation and required presence of leukemic cells in a coculture model. Furthermore, these metabolic defects constituted a mechanism of T cell functional dysfunction because rescue of metabolism improved T cell function and in the case of increased Akt signaling delayed disease progression, suggesting restoration of antileukemic immunity. Although it remains unclear to what extent the dysfunction of T cells in CLL reflects exhaustion of T cells in chronic viral infections or solid tumors, each shares features of expression of inhibitory receptors and resistance to activation. Suppression of metabolism may, therefore, be a shared feature of these T cell impairments.

Despite an increase of reported leukemia-specific Ags, the source of T cell activation and impairments remain uncertain (45–47). T cells in our murine and human models appeared to be stimulated at a low level in vivo, presumably in a polyclonal fashion. Additionally, repeated polyclonal in vitro stimulation of naive T cells induced a phenotype similar to in vivo-stimulated T cells. The observed decrease of T cell responsiveness and impaired upregulation of metabolism may therefore result from a combination of chronic Ag stimulation and polyclonal activation. The relative contribution of Ag-specific and unspecific T cell activation in vivo may vary, thus leading to heterogeneity in the phenotype of T cells from leukemic hosts. Consistent with pre-existing activation, T cells from leukemic hosts showed a trend toward increased basal expression of Glut1, and a subpopulation of these T cells presented increased baseline glucose uptake. However, the T cell metabolic defects remained apparent only in the presence of leukemic cells. These results support a model in which leukemia-associated activation of T cells in vivo induces a T cell phenotype with increased...
expression of multiple inhibitory receptors that suppress T cell metabolism and activation.

PD-1 was upregulated on a subset of T cells from leukemia mice, and increased expression of PD-L1 was detected on cells in the leukemic microenvironment. PD-1 ligation inhibits PI3K/Akt/mTOR signaling and reduces Myc expression (27, 34). These pathways are critical to allow activated T cells to undergo metabolic reprogramming necessary for effector function and their inhibition would prevent T cell activation and function (40, 48, 49).

In vivo blockade of PD-1 alone was insufficient to completely restore T cell function and induce antitumor immunity in our murine B cell leukemia model. Nevertheless, anti-PD-1 therapy increased Glut1 expression on CD8+ T cells from leukemic hosts, suggesting that PD-1 signaling contributed to leukemia-induced T cell dysfunction by metabolic suppression. Blockade of PD-1/PD-L1 interaction has proven promising to reactivate exhausted T cells in some solid tumors (50) and more recently in a mouse model of CLL (17, 51). PD-1 expression is associated with poor outcome in CLL (9) and PD-1 blockade alone or in combination with other therapies may therefore provide an approach for CLL patients.

We found that T cells from leukemic hosts expressed inhibitory receptors whose ligands were expressed in leukemic microenvironment. CD200 for example was highly upregulated on CLL cells, and PD-200R signaling might have contributed to metabolic dysfunction of non-Tregs in B cell leukemia by Treg induction. Furthermore, CD200 blockade can increase Ag-specific T cell responses in human CLL (52). Blockade of multiple immune checkpoints may therefore be necessary to induce sufficient antitumor immunity in B cell leukemia. In addition to inhibition of T cell inhibitory pathways, direct metabolic modulation of T cells in vivo is also possible, but it may impact leukemic cells to potentially complicate therapy. It may be more feasible to modulate T cell metabolism ex vivo. In this approach, modulation of T cell metabolic pathways or alteration of in vitro culture conditions prior to cellular therapies such as treatment with chimeric Ag receptor T cells may decrease the observed T cell exhaustion (53) and increase efficiency.

Taken together, our studies show that alteration of key T cell metabolic pathways critical for T cell activation and function such as Akt/mTORC1 and those involved in glucose uptake and glycolysis is an important part of T cell dysregulation in B cell leukemia. It is likely that similar metabolic impairments contribute to an altered T cell phenotype in other settings, including chronic viral infection. As these metabolic pathways are essential for proper T cell responses in both protective and antineclemic immunity, modulation of T cell metabolism may represent a new therapeutic avenue for leukemia patients.

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Disclosures
The authors have no financial conflicts of interest.

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