

UCSF

UC San Francisco Previously Published Works

Title

Increased glucose metabolic activity is associated with CD4+ T-cell activation and depletion during chronic HIV infection

Permalink

<https://escholarship.org/uc/item/6zd6152h>

Journal

AIDS, 28(3)

ISSN

0269-9370

Authors

Palmer, Clovis S
Ostrowski, Matias
Gouillou, Maelenn
[et al.](#)

Publication Date

2014-01-28

DOI

10.1097/qad.0000000000000128

Peer reviewed



Published in final edited form as:

AIDS. 2014 January 28; 28(3): 297–309. doi:10.1097/QAD.000000000000128.

Increased glucose metabolic activity is associated with CD4⁺ T-cell activation and depletion during chronic HIV infection

Clovis S. Palmer^a, Matias Ostrowski^b, Maelenn Guillo^c, Louis Tsai^d, Di Yu^d, Jingling Zhou^a, Darren C. Henstridge^e, Anna Maisa^a, Anna C. Hearps^{a,f}, Sharon R. Lewin^{a,f,g}, Alan Landay^h, Anthony Jaworowski^{a,f}, Joseph M. McCuneⁱ, and Suzanne M. Crowe^{a,f,g}

^aCentre for Biomedical Research, Burnet Institute, Melbourne, Australia

^bInstituto de Investigaciones Biomédicas en Retrovirus y SIDA. Facultad de Medicina, Buenos Aires, Argentina

^cCentre for Population Health, Burnet Institute, Melbourne

^dLaboratory of Molecular Immunomodulation, School of Biomedical Sciences, Monash University, Clayton

^eCellular and Molecular Metabolism Laboratory, Baker IDI Heart and Diabetes Institute

^fDepartment of Infectious Diseases, Monash University

^gInfectious Diseases Department, The Alfred hospital, Melbourne, Australia

^hDepartment of Immunology/Microbiology, Rush University Medical Center, Chicago, Illinois

ⁱDivision of Experimental Medicine, Department of Medicine, University of California, San Francisco, San Francisco, California, USA

Abstract

Objectives—Glucose metabolism plays a fundamental role in supporting the growth, proliferation and effector functions of T cells. We investigated the impact of HIV infection on key processes that regulate glucose uptake and metabolism in primary CD4⁺ and CD8⁺ T cells.

Design and methods—Thirty-eight HIV-infected treatment-naive, 35 HIV+/combination antiretroviral therapy, seven HIV+ long-term nonprogressors and 25 HIV control individuals were studied. Basal markers of glycolysis [e.g. glucose transporter-1 (Glut1) expression, glucose uptake, intracellular glucose-6-phosphate, and L-lactate] were measured in T cells. The cellular markers of immune activation, CD38 and HLA-DR, were measured by flow cytometry.

© 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins

Correspondence to Clovis Palmer, Centre for Biomedical Research, Burnet Institute, 85 Commercial Road, Melbourne, VIC 3004, Australia. Tel: +61 3 8506 2389; cpalmer@burnet.edu.au.

Author Contributions: C.S.P. conceived the project; C.S.P., S.M.C., J.M.M., A.L., and S.L. provided ideas and designed experiments; C.S.P., M.O., L.T., D.Y., and J.Z. performed experiments; C.S.P., S.M.C., J.M.M., A.J., M.O., M.G., and D.H. interpreted the data; C.S.P. and M.G. analyzed data; C.S.P. wrote the manuscript with editing provided by S.M.C., J.M.M., A.J., M.O., D.H., A.C.H., S.L.; and C.S.P., S.M.C., A.C.H., A.M. and J.Z. recruited patients and provided data.

Conflicts of interest

The authors declare no competing financial interest.

Results—The surface expression of the Glut1 is up-regulated in CD4⁺ T cells in HIV-infected patients compared with uninfected controls. The percentage of circulating CD4⁺Glut1⁺ T cells was significantly increased in HIV-infected patients and was not restored to normal levels following combination antiretroviral therapy. Basal markers of glycolysis were significantly higher in CD4⁺Glut1⁺ T cells compared to CD4⁺Glut1⁻ T cells. The proportion of CD4⁺Glut1⁺ T cells correlated positively with the expression of the cellular activation marker, HLA-DR, on total CD4⁺ T cells, but inversely with the absolute CD4⁺ T-cell count irrespective of HIV treatment status.

Conclusion—Our data suggest that Glut1 is a potentially novel and functional marker of CD4⁺ T-cell activation during HIV infection. In addition, Glut1 expression on CD4⁺ T cells may be exploited as a prognostic marker for CD4⁺ T-cell loss during HIV disease progression.

Keywords

CD4⁺ cells; combination antiretroviral therapy; glucose; glucose transporter-1; HIV; immune activation; inflammation; lymphocytes; metabolism

Introduction

Glucose is the major cellular fuel which supports T-cell growth and survival [1]. Several immune functions with relevance to HIV infection depend on adequate glucose supply, including T-cell activation [2], T-cell-mediated antiviral responses, and other T-cell effector functions [1,3].

The pathogenesis of HIV disease *in vivo* is characterized by chronic immune activation, inflammation, and increased oxidative stress [4-6]. Even in the presence of effective combination antiretroviral therapy (cART), evidence of chronic immune activation may be observed and is associated with and predictive of incomplete CD4⁺ T-cell recovery, as well as increased morbidity and mortality [7-12]. Immune activation is characterized by high levels of T-cell activation, measured by CD38 and human leukocyte antigen D-related (HLA-DR) expression on peripheral CD4⁺ and CD8⁺ T cells [13,14].

Upon activation, the energy demands of T cells increase dramatically and they undergo a metabolic switch in glucose metabolism from oxidative phosphorylation to aerobic glycolysis, so that growth, proliferation, and effector functions can be supported [15] (and as reviewed in references [16-19]). In peripheral tissues, glucose is transported into cells by glucose transporters (Gluts) that carry hexose sugars across the cell membrane. Gluts comprise a family of at least 13 members including the proton-myoinositol co-transporter, H⁺-coupled myoinositol co-transporter. Glucose transporter-1 (Glut1) is a class 1 glucose transporter that has high affinity for glucose and is the primary glucose transporter on T cells [20,21].

Few studies have evaluated the role of HIV infection on glucose metabolism in leukocytes and these have been conducted exclusively *in vitro* [22-24]. Given the sustained energy requirements of activated T cells (as reviewed in references [18] and [25]) we hypothesized that T cells would up-regulate Glut1 expression and increase glucose transport in the context

of HIV infection. In the present study, we analyzed key steps of glucose metabolism in T cells from HIV-infected individuals (both treatment-naive and cART-treated), including cell surface expression of Glut1 on lymphocyte subpopulations, glucose uptake, and glycolytic flux analysis. Thus far, our study represents the most comprehensive glucose metabolic analysis in T cells from HIV-infected individuals. Identification of metabolic dysregulation of the immune system during HIV infection could uncover novel mechanisms and potential drug targets to reduce immune activation and to support CD4⁺ T-cell recovery in some patients.

Methods

Study participants

The study population included untreated HIV-infected individuals [progressors and long-term nonprogressors (LTNPs)], HIV-infected patients on cART, and HIV seronegative controls (see Table 1). Patients were recruited from the community, the Infectious Diseases Unit at The Alfred Hospital in Melbourne Australia, and from the Clinical Research Core Repository at the University of Washington, Seattle, USA. Informed consent was obtained from all participants and the study was approved by the ethics committee at the participating institutions. Fresh blood samples from individuals recruited in Melbourne (45, 51, and 100% of the total study population of HIV-infected/treatment-naive, HIV+/cART, and HIV-negative individuals, respectively) were collected in EDTA, citrate, or heparin anticoagulant tubes and processed within 1 h of venipuncture; cryopreserved peripheral blood mononuclear cells (PBMCs) were shipped from University of Washington to Melbourne in liquid-phase nitrogen. The main exclusion criteria included self-reported co-infection with hepatitis C virus (HCV), active malignancy, vaccination, physical trauma, or surgery within 3 weeks prior to participation. In some experiments, a representative subpopulation was analyzed in which there were no statistically significant differences between the subpopulation and the whole group in terms of sex, age, CD4⁺ T-cell count, and viral load.

Peripheral blood mononuclear cell preparation

Peripheral blood mononuclear cells were isolated by density gradient centrifugation (Lymphoprep, Axis Shield, Dundee, Scotland), as previously described [26], and cryopreserved in 10% dimethyl sulfoxide (DMSO, Sigma-Aldrich, St Louis, Missouri, USA) and 90% autologous plasma.

Immunophenotyping

Fresh PBMCs were prepared and stained on ice for 30 min as previously described [27], using the following pretitrated antibodies: CD3-phycoerythrin (PE), CD4-PerCP, CD8-allophycocyanin (APC), CD27-APC, CD45-RA-PE, CD38-PE, and HLA-DR-fluorescein (FITC) (BD Biosciences, San Jose, California, USA). Cells were acquired on a FACSCalibur (BD Biosciences) and analyzed using FlowJo software, version 8.8 (Tree Star Inc., Ashland, Oregon, USA). Cryopreserved PBMCs (>90% viability) were rested for 24 h in supplemented RPMI-1640 medium [(10% human serum, penicillin/ streptomycin (Invitrogen), 2 mmol/l L-glutamine (Invitrogen, Carlsbad, California, USA)] prior to staining.

Glucose transporter-1 detection

Extracellular Glut1 expression was quantified on freshly isolated or cryopreserved PBMCs by flow cytometry using Glut1 antibody [MAB1418 clone (R&D Systems, Minneapolis, Minnesota, USA)] conjugated with FITC or APC to detect cell surface Glut1 [28-30]. A pilot analysis of Glut1 expression on T cells revealed that the cryopreservation and thawing process did not affect its expression on T cells. Intracellular Glut1 (Glut1_{c-term}) was detected using an unconjugated monoclonal anti-body against Glut1_{c-term} (Abcam) and a goat antimouse FITC-conjugated secondary antibody. Cells were surface stained as above and permeabilized using the Intra Stain kit (Dako, Campbellfield, Victoria, Australia). Cells were acquired on a FACSCalibur.

Gene expression analysis

Total CD4⁺ T cells were isolated from cryopreserved PBMCs using magnetic bead-based negative selection (Stemcell Technologies, Vancouver, British Columbia, Canada). mRNA extraction and quantitative real-time PCR were performed as previously described [31], using the following primer sets: β -actin – forward: 5'-3': AGGCATCCTCACCTGAAGT, reverse: 5'-3': GCG TACAGGGATAGCACAGC; Glut1 – forward: 5'-3': TCTGGCATCAACGCTGTCTTC, reverse: 5'-3': CGATACCGGAGCCAATGGT; and modified cycle conditions: 508C for 2 min, 958C for 10 min, and 40 cycles of (958C, 30 s; 608C, 1 min).

Plasma cytokine and biochemical analysis

Cytokines, 25-hydroxy vitamin D, insulin, high-sensitivity C-reactive protein (CRP), D-dimer, and bio-chemical analyses were conducted by Cardinal Bio-research Pty Ltd (Australia).

Glucose uptake assay

The fluorescently-labeled glucose analog, 2-N-(7-nitro-benz-2-oxa-1, 3-diazol-4-yl) amino)-2 deoxyglucose (2-NBDG) (Invitrogen), was used to measure glucose uptake. Cryopreserved PBMCs were thawed and recovered for 24 h at 37°C, 5% CO₂ in supplemented RPMI-1640 medium. Cells were then treated with 2-NBDG, washed twice with 1× phosphate-buffered saline (PBS), stained for cell surface markers, resuspended in 1× PBS, and analyzed within 15 min on a FACSCalibur.

Glucose-6-phosphate assay

Briefly, 5×10^6 cryopreserved cells were rested in the supplemented RPMI-1640 medium with or without glucose (glucose-limiting media) for 4 h. The cells were then pelleted, washed once in ice-cold 1× PBS, and resuspended in 50 μ l of ice-cold 1× PBS. Cells were sonicated for 30 min on 'high setting' (Sonician Pty Ltd, Thebarton, South Australia, Australia). Cell lysates were centrifuged at 10 000 r.p.m. at 4°C for 5 min and glucose-6-phosphate (G-6-P) levels were determined in the supernatant using a G-6-P assay kit (Biovision, San Francisco, California, USA).

L-lactate assay

Secreted L-lactate concentrations in cell-free culture supernatants were determined by using the Glycolysis Cell-Based Assay Kit (Cayman Chemical, Ann Arbor, Michigan, USA). For intracellular L-lactate determination, cryopreserved cells were allowed to recover for 24 h in supplemented RPMI-1640 medium and suspensions were stained using the Glycolysis Cell-Based Assay Kit (Cayman Chemical). The cells were washed once in wash buffer (0.5% FCS/1× PBS), stained with cell surface markers, and resuspended in 1× PBS prior to analysis. The highly colored intracellular formazan was detected in the FL3 channel on a FACSCalibur.

Statistical analysis

The nonparametric Mann–Whitney test was used for comparison of unpaired data and the Wilcoxon matched-pairs signed-rank test was used to analyze paired data. Measures of central tendency are expressed as median and interquartile range (IQR 25th, 75th percentile), unless otherwise stated. Linear regression was applied to assess the relationship between different covariates. Markers with a significant value of less than 0.05 in univariate analyses were entered in a multivariate linear regression model and the final model was derived through a process of backward elimination. Spearman rank test was used for correlation analyses. *P* values less than 0.05 were considered significant. All statistical analyses were performed using GraphPad Prism (version 6.0; GraphPad Software, La Jolla, California, USA) or Stata (version 11; Stata Corp., College Station, Texas, USA).

Results

Participant clinical characteristics

Demographic and clinical characteristics of participants are summarized in Table 1. A total of 105 participants including 38 HIV-infected treatment-naïve (HIV+/naive), seven HIV-infected treatment-naïve LTNP, 35 HIV-infected cART-experienced (HIV+/cART), and 25 HIV seronegative (HIV−) control individuals were recruited. LTNPs were infected with HIV for more than 10 years, and were not on cART. The median CD4⁺ T-cell counts in the HIV+/naive and HIV+/cART groups were 400 and 479 cells/μl, respectively (*P* = 0.08). Plasma concentrations of tumor necrosis factor (TNF) were significantly elevated in the HIV+/naive (*P* = 0.005) and HIV+/cART (*P* = 0.02) groups relative to the HIV-group.

HIV infection is associated with an increased percentage of circulating CD4⁺ T cells expressing glucose transporter-1

Figure 1a–e illustrates the gating strategy used to evaluate Glut1 expression on T cells. The percentage of CD3⁺CD4⁺ T cells that expressed Glut1 (referred to as CD4⁺Glut1⁺ T cells) in HIV+/naive participants was significantly higher (median 23.8%) than that found in HIV-controls (median 5.2%; *P* < 0.0001) and remained so after commencing cART (median 11.7%; *P* = 0.0002). The median percentage of CD4⁺Glut1⁺ T cells in LTNPs was only 11.6% (Fig. 1f, left panel). The mean fluorescent intensity (MFI) of Glut1 on CD4⁺ T cells from HIV+/naive individuals (median 13.3, range 5.8 – 45.6) was also significantly higher than that found on CD4⁺ T cells from HIV-individuals (median 11.1, range 5.0 – 15.3; *P* =

0.02, data not shown). In a subgroup of 17 HIV+/naive individuals recruited at University of Washington (Seattle, USA) commencing cART and analyzed 2.1 ± 1.3 years after initiation of therapy, the proportion of CD4⁺Glut1⁺ T cells decreased significantly from a median of 30.9 to 16.5% ($P = 0.002$) (Fig. 1f, right panel). Over this time, their CD4⁺ T-cell count increased from a median of 233 cells/μl (range 11 – 488) to 433 cells/μl (range 123 – 1090). Figure 1g illustrates that Glut1 was expressed on virtually all CD8⁺ T cells, irrespective of HIV or treatment status; there were no significant differences in the levels of Glut1 expression on CD8⁺ T cells between the groups.

In a subset of representative samples (based on CD4⁺ cell count), we observed increased intracellular Glut1 in CD4⁺ T cells from HIV+ patients irrespective of treatment status (Fig. 1h,i). Further, the level of Glut1 mRNA was also significantly higher in CD4⁺ T cells from HIV+/naive compared to HIV-individuals ($P = 0.03$; Fig. S1a, <http://links.lww.com/QAD/A445>). The Glut1 mRNA correlated significantly with the percentage of CD4⁺Glut1⁺ T cells ($P = 0.0007$; Fig. S1b, <http://links.lww.com/QAD/A445>).

These data suggest that transcription, synthesis, and cell membrane trafficking of Glut1 in CD4⁺ T cells from HIV-infected individuals are higher compared with cells from uninfected controls. Additional data on intracellular Glut1 and mRNA expression in a larger sample size will be required to confirm this. Noteworthy, there was a weak inverse relationship between the percentage of CD4⁺Glut1⁺ T cells and time on cART ($r = -0.40$, $P = 0.02$; Fig. S1c, <http://links.lww.com/QAD/A445>). Therefore, at least in some patients on cART, Glut1 expression on CD4⁺ T cells might be a function of duration of viral suppression and/or CD4⁺ T-cell count.

The frequencies of glucose transporter-1R T cells are higher in effector CD4⁺ T-cell subpopulations

In a subset of 10 HIV-controls, 12 HIV+/naive and 8 HIV+/cART patients, we measured Glut1 expression on CD4⁺ effector, naive, memory, and effector-memory cells, as defined by their expression of CD45RA and CD27. Glut1 was expressed on a higher percentage of effector and effector-memory CD4⁺ T cells than of naive and memory CD4⁺ T cells, irrespective of HIV or treatment status. The CD4⁺ naive and memory subpopulations in HIV-individuals showed only a small fraction of CD4⁺Glut1⁺ T cells (median 5.2 and 10.3%, respectively); in HIV+/naive individuals, by contrast, these populations showed significantly increased Glut1 expression (median 13.3%; $P = 0.02$ and 25.5%; $P = 0.006$, respectively). The expression of Glut1 on naive and memory T cells remained significantly elevated ($P = 0.001$ and $P < 0.0001$, respectively) in HIV+/cART patients (Fig. 1j). The fraction of Glut1⁺ cells was similar in each of the CD4⁺ subpopulations measured from the different patient groups (Fig. 1k). The proportions of effector and effector-memory CD4⁺ T cells were higher in HIV+/naive patients than in HIV-controls and there was a positive correlation between the percentage of circulating CD4⁺Glut1⁺ T cells and the frequency of these subpopulations (Fig. S2a–c, <http://links.lww.com/QAD/A445>). Data were unavailable to determine the absolute number of CD4⁺Glut1⁺ T cells in HIV-individuals, but HIV+/naive patients had higher absolute CD4⁺Glut1⁺ T cells than did HIV+/cART patients (Fig. S2d, <http://links.lww.com/QAD/A445>). In sum, increased percentages of circulating

CD4⁺Glut1⁺ T cells during HIV infection might not only be attributed to increased fractional representation of effector and effector-memory CD4⁺ T cells but may also reflect an absolute increase in the number of these cells in blood.

The specificity of glucose transporter-1 detection

Given published concerns about the specificity of the R&D Glut1 antibody [32], we conducted two independent Glut1 overexpression experiments and confirmed increased cell surface reactivity of the R&D Glut1 antibody on HEK293T cells overexpressing Glut1 (Fig. S3a,b, <http://links.lww.com/QAD/A445>). Using a different commercially available antibody, we confirmed by western blot that the cells were indeed overexpressing Glut1 (Fig. S3c, <http://links.lww.com/QAD/A445>). Interestingly, the R&D Glut1 antibody showed strong reactivity to permeabilized NIH3T3 cells transfected with Glut1-expressing lentivirus (Fig. S3d,e, <http://links.lww.com/QAD/A445>). However, no reactivity occurred using R&D Glut1 antibody on nonpermeabilized NIH3T3 cells that were overexpressing Glut1, presumably due to defects in Glut1 trafficking in these cell lines. In addition, we demonstrated significant cell surface reactivity of R&D Glut1 antibody on the highly metabolically active and paraformaldehyde-fixed Jurkat cell and N2a cells (positive control for cell surface Glut1 [33]) (Fig. S4, <http://links.lww.com/QAD/A445>).

CD4⁺Glut1⁺ T cells have high expression of activation and proliferation markers

HIV infection is associated with immune activation [9,11,12], as is reflected in this study by elevated plasma concentrations of TNF (Table 1) and by an increased frequency of peripheral blood CD4⁺CD38⁺HLA-DR⁺ and CD8⁺CD38⁺HLA-DR⁺ cells in HIV+/naive and HIV+/cART patients compared with HIV-controls (Fig. 2a). In subgroup of 17 HIV+/naive individuals commencing cART and analyzed 2.1 ± 1.3 years after initiation of therapy, the proportion of CD4⁺ and CD8⁺ T cells co-expressing CD38 and HLA-DR appeared to have more rapidly declined than the percentage of CD4⁺Glut1⁺ T cells (Fig. 2b vs. Fig 1f, right panel). Figure 2(c–e) shows that markers of T-cell activation were significantly higher in the Glut1⁺ population than in the Glut1[−] population in all study groups. Time-course experiments showed that Glut1 expression occurred early during the activation of CD4⁺ T cells (Fig. S5a, <http://links.lww.com/QAD/A445>). In contrast to the other activation markers and as expected [34], there was a rapid increase in percentage of CD4⁺Glut1⁺ T cells expressing CD69, followed by a time-dependent decrease in expression of CD69 on these cells (Fig. S5b,c, <http://links.lww.com/QAD/A445>).

HIV infection increases glucose uptake and glycolytic activity in CD4⁺ T cells

To associate Glut1 expression with glucose metabolic activity in CD4⁺ cells, we selected samples that were within 2 SDs of the mean value of Glut1⁺CD4⁺ T-cell percentage from the respective groups. CD4⁺ T cells from HIV+/naive individuals took up more glucose over time than do CD4⁺ T cells from HIV− and HIV+/cART patients (Fig. 3a). After 60 min of incubation, the MFI of intracellular 2-NBDG was significantly higher in the CD4⁺ T cells from HIV+/naive individuals than in cells from HIV− or HIV+/cART patients (Fig. 3b), and this correlated significantly with Glut1 expression on CD4⁺ T cells ($r = 0.70$, $P = 0.005$, $n = 24$, data not shown). Notably, CD4⁺Glut1⁺ T cells from HIV+/naive and HIV+/cART

patients took up more glucose than CD4⁺Glut1⁺ T cells from HIV⁻ individuals (Fig. 3c). Confirmatory activation experiments showed that the presumably blast cells that expressed more Glut1 were also highly positive for 2-NBDG (Fig. 3d,e).

Intracellular retention of glucose occurs by phosphorylation of glucose to G-6-P and is catalyzed by hexokinases. We therefore measured the intracellular concentrations of G-6-P in purified unstimulated CD4⁺ T cells. Jurkat cells were used as positive controls (Fig. 3f). The levels of intracellular G-6-P were significantly higher in CD4⁺ T cells from HIV⁺/naive individuals compared to HIV⁻ ($P = 0.0009$) and when compared to HIV⁺/cART ($P = 0.005$) (Fig. 3g), consistent with an increased transport of glucose in these cells.

We extended the above observations to show that CD4⁺ T cells from HIV⁺/naive individuals secreted significantly more L-lactate into the culture medium than HIV⁻ individuals (Fig. 3h). Using Jurkat cells and anti-CD3/CD28-stimulated PBMCs as positive controls (Fig. 3i,j), we confirmed by flow cytometry that the intracellular concentration of L-lactate was significantly higher in Glut1⁺ compared with Glut1⁻ cells from HIV⁺/naive individuals ($P = 0.01$) (Fig. 3k).

A high frequency of glucose transporter-1-expressing CD4⁺ T cells is associated with markers of HIV disease progression

A significant inverse correlation was found between the percentage of CD4⁺Glut1⁺ T cells and the percentage of CD4⁺ T cells ($P < 0.0001$) and absolute CD4⁺ cell count ($P = 0.0002$) in peripheral blood of HIV⁺/naive individuals (Fig. 4a). This was also true when HIV⁺/cART patients were analyzed separately ($r = -0.53$, $P = 0.001$, $n = 35$ for percentage CD4⁺ T cells and $r = -0.50$, $P = 0.004$, $n = 27$ for absolute CD4⁺ cell count; data not shown). There were no significant correlations between plasma concentrations of glucose and insulin, and the percentage of CD4⁺Glut1⁺ T cells, suggesting that peripheral glucose homeostasis is an unlikely factor influencing Glut1 expression on CD4⁺ T cells in this setting.

Multivariate analysis was conducted to determine which covariates were associated with the percentage of circulating CD4⁺Glut1⁺ T cells. Only the total percentage of CD4⁺ T cells and MFI of HLA-DR on CD4⁺ T cells were independently associated with the percentage of circulating CD4⁺Glut1⁺ T cells in the peripheral blood of patients (Supplement Table 1, <http://links.lww.com/QAD/A444>). In HIV⁺/naive individuals, the percentage of CD4⁺Glut1⁺ T cells and known correlates of CD4⁺ T-cell activation had a comparably inverse relationship with the percentage of CD4⁺ T cells (Fig. 4b), but the percentage of CD4⁺Glut1⁺ T cells in HIV⁺/cART patients showed the strongest correlation with total CD4⁺ T-cell percentage (Fig. 4c). On the contrary, the percentage of CD4⁺CD38⁺HLA-DR⁺ T cells had the strongest correlation with viral load in HIV⁺/naive individuals (Fig. 4d). The relationship between CD8⁺ T-cell activation and total CD4⁺ T-cell percentage was relatively weak in HIV⁺/naive and HIV⁺/cART patients (Fig. S6a,b, <http://links.lww.com/QAD/A445>). Conversely, there was a strong correlation between the levels of CD38 expression on CD8⁺ T cells and viral load in HIV⁺/naive individuals (Fig. S6c, <http://links.lww.com/QAD/A445>).

Multivariate analysis (described in Supplemental methods, <http://links.lww.com/QAD/A446>) was used to compare the predictive strength of CD4⁺Glut1⁺ percentage with established predictors of HIV disease progression. Compared with known variables of T-cell activation, the percentage of CD4⁺Glut1⁺ T cells was the only independent predictor of CD4⁺ cell count and CD4⁺ percentage (Supplemental Table 2a,b, <http://links.lww.com/QAD/A444>). In contrast, the percentage of CD8⁺CD38⁺HLA-DR⁺, the MFI of HLADR on CD4⁺ T cells, and the MFI of Glut1 on CD4⁺ T cells were independently associated with viral load in HIV+/naive individuals.

Discussion

We report here that HIV infection is associated with increased glucose metabolism in T cells. In HIV+/naive individuals compared to uninfected controls, there is a substantial increase in the percentage of circulating CD4⁺ T cells that express the glucose transporter, Glut1, and this percentage remained elevated despite virologic suppression on cART. The percentage of CD4⁺Glut1⁺ T cells correlates inversely with the percentage and absolute CD4⁺ T-cell count, irrespective of treatment status. HIV+/naive and HIV+/cART patients have an increased proportion of Glut1-expressing naive and memory CD4⁺ T cells compared with HIV- controls. The expression of Glut1 on total CD4⁺ T cells reflects their activation status as demonstrated by significantly higher expression levels of both CD38 and HLA-DR in the Glut1⁺ vs. Glut1⁻ population in all patient study groups, supporting a critical role for Glut1 in activated T cells, and confirming and extending in-vitro reports [32,35,36]. Multivariate analysis indicates that the percentage of circulating CD4⁺Glut1⁺ T cells is independently associated with both the percentage and the levels of activation of CD4⁺ T cells. In HIV+/cART patients, the percentage of CD4⁺Glut1⁺ cells has a broader dynamic range and correlates more strongly with CD4⁺ T-cell loss than the percentage of CD4⁺ or CD8⁺ T cells co-expressing CD38 and HLA-DR. Finally, CD4⁺Glut1⁺ T cells take up more glucose and have higher glycolytic activity than do CD4⁺Glut1⁻ T cells, a metabolic phenomenon characteristic of other viral responses in different cell types [37]. In contrast to CD4⁺ T cells, there were no significant changes in the cell surface expression Glut1 and glucose uptake by CD8⁺ T cells in HIV-infected patients.

Conflicting reports have been published concerning the specificity of the R&D antibody that was used in our experiments to detect Glut1 [32,38]. In one case, that antibody failed to detect endogenous Glut1 in cells, including Jurkat cells known to express abundant levels of Glut1 [32]. However, we observed strong immune reactivity of the R&D Glut1 antibody on paraformaldehyde-fixed Jurkat cells and on unfixed N2a cells, suggesting that in some situations the Glut1 epitope may be masked by post-transcriptional modifications [32,39]. We also observed that, under the conditions of our experiments, this antibody detected a dramatic increase in Glut1 levels following T-cell activation that was highly correlated with increased glucose uptake. Although we cannot fully explain the discrepancy between our observations and those reported in ref. [32], we speculate that they may be related to different protocols to achieve T-cell activation (e.g. 24 h vs. 2–4 days) and/or to subtle differences in staining protocols. Interestingly, the R&D Glut1 antibody detected intracellular but not cell surface Glut1 in NIH3T3 cells that were overexpressing Glut1, an observation that is not consistent with the suggestion that the antibody interacts with a

different cell surface protein that is associated with Glut-1 overexpression in transformed cells [32]. More recently, this antibody has been shown by others to be specific for Glut1 [40] and has been used to evaluate Glut1 expression on cell surfaces [28,29] including T cells in a cohort of HIV-infected individuals [30]. We have also clearly shown increased intracellular Glut1 (using a Glut-1_{cterm} antibody), increased Glut1 mRNA, and increased glucose uptake in CD4⁺ T cells in HIV⁺/naive individuals, all of which is consistent with increased glucose metabolic activity.

Recent metabolomics analyses of HIV-infected primary CD4⁺ T cells *in vitro* have shown a profound increase in intracellular levels of key glycolytic metabolites with a concomitant increase in glucose uptake when compared with HIV-uninfected cells in the same culture [23], suggesting that direct HIV infection of CD4⁺Glut1⁺ T cells may contribute at least in part to the increased glycolytic activity in CD4⁺Glut1⁺ T cells in some HIV⁺ patients. Remarkably, in HIV⁺ patients, we observed a paradoxical increase in the frequency of Glut1⁺ T cells in the naive and memory subpopulations. This could potentially allow these 'resting' cells to be more permissive for productive infection, as shown directly by experiments demonstrating that IL-7-induced Glut1 expression enabled HIV infection in naive CD4⁺ T cells in the absence of activating stimuli [24]. The origin of the heightened glucose metabolism in CD4⁺ T cells in HIV⁺ patients is unknown but may be a result of elevated cytokines such as interferon (IFN) γ , interleukin (IL)-2, and IL-7 [19,41], and/or persistent inflammatory signals such as translocated microbial products [42]. However, direct HIV infection of CD4⁺ T cells may be an additional contributor of increased glucose metabolic activity, especially in untreated patients, supporting the observation of increased glucose metabolic activity by HIV *in vitro* [23].

It has been suggested by several groups that Glut1 is a T-cell activation marker based on its increased expression on T cells activated *in vitro* [32,35,36]. However, none of these investigators evaluated the expression of established activation markers on these cells, nor did they examine co-expression of activation markers with Glut1. Our data suggest that Glut1 is a potential marker of CD4⁺ T-cell activation in the context of HIV infection, although it might be expressed in a small proportion of cells independently of the activation markers evaluated here. Interestingly, HIV/cART patients with low CD4⁺ T-cell count have elevated percentages of CD4⁺Glut1⁺ T cells even when their CD38 and HLA-DR levels on CD4⁺ T cells returned to almost normal. It is possible that CD4⁺ T cells may lose CD38 and HLA-DR with the suppression of HIV in cART-treated individuals, but retain metabolic activation markers like Glut1. This may be interpreted as a homeostatic response to drive the increase of CD4⁺ T cell. Compared with the activation markers CD38 and HLA-DR, Glut1 is unique because it is up-regulated on CD4⁺, but not CD8⁺ T cells in HIV⁺ patients. It will also be interesting to determine whether subpopulations of CD4⁺Glut1⁺ T cells preferentially contain HIV viral DNA, especially in those cells that lack the expression of the traditional activation markers.

What are the biological consequences of increased glucose metabolic activity in CD4⁺ T cells in HIV⁺ patients? Glut1-mediated glucose metabolic pathways are proposed as critical regulators of HIV infection in human primary CD4⁺ T cells and T-cell lines in cell culture [22,24]. In recent reviews, hyperactivation of aerobic glycolysis in CD4⁺ T cells during HIV

infection has been hypothesized to foster the apoptosis and destruction of such cells [19,43]. Indeed, a high rate of glycolysis in cells increases the concentrations of metabolites such as L-lactate which induce acidosis and can trigger apoptosis, either through the p53 pathway or by acid-induced collapse of the transmembrane H(+) gradient [44,45]. In addition, Glut1 is recognized as a key transporter for vitamin C [46], and under oxidative stress, it can be oxidized to ascorbate free radical which may also contribute to cell death [47].

In conclusion, our study identifies the glucose metabolic machinery as a component of HIV-associated T-cell activation and provides a rationale for exploratory approaches for therapeutic interventions. We also identified Glut1 as a potentially novel marker of CD4⁺ T-cell activation of HIV disease progression. One limitation of this study is the small sample size and cross-sectional analysis design. Longitudinal analysis using a larger sample size will shed more light on the role of glucose metabolism in HIV disease progression. Another limitation of the study is that we had access to cells from only a limited number of patients within each group for several experiments, raising the possibility that some interpretations may be affected by patient selection bias. In addition, in-vitro studies to assess the effects of targeted pharmacological and genetic inhibition of glycolysis in CD4⁺ T cells may help to clarify a mechanism and direct link between glucose metabolism and CD4⁺ T-cell activation. The maturation of different functional subsets of T cells such as Tregs, Th1, Th2, and Th17 are dependent on distinct metabolic programming [48]. It will be of interest to evaluate how changes in glucose metabolic activity affect the functions of these cells during HIV infection and the course of HIV disease progression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

C.S.P. would like to thank Mr Geoffrey Radford for his secretarial and managerial support, and Professor Geoffrey A. Pietersz and Dr Louise Swainson for proof reading and review of the manuscript. We also acknowledge Dr Naomi Taylor for critical discussions.

The authors would like to acknowledge The Alfred Hospital and Clinical Research Core Repository and Specimen Collection Service of the University of Washington, USA through the support of an NIH grant [P30 AI027757] for clinical samples. The authors gratefully acknowledge the contribution to this work of the Victorian Operational Infrastructure Support Program received by the Burnet Institute.

Sources of support: This research was funded by a 2010 developmental grant (CNIHR) from the University of Washington Center for AIDS Research (CFAR), an NIH funded program under award number AI027757 which is supported by the following NIH Institutes and Centers (NIAID, NCI, NIMH, NIDA, NICHD, NHLBI, NIA), and the Australian Centre for HIV and Hepatitis Virology Research (ACH²). C.S.P is a recipient of the CNIHR and ACH² grant. SMC is a recipient of a National Health and Medical Research Council of Australia (NHMRC) Principal Research Fellowship.

References

1. Jacobs SR, Herman CE, MacIver NJ, Wofford JA, Wieman HL, Hammen JJ, et al. Glucose uptake is limiting in T cell activation and requires CD28-mediated Akt-dependent and independent pathways. *J Immunol.* 2008; 180:4476–4486. [PubMed: 18354169]

2. Marko AJ, Miller RA, Kelman A, Frauwirth KA. Induction of glucose metabolism in stimulated T lymphocytes is regulated by mitogen-activated protein kinase signaling. *PLoS One*. 2010; 5:e15425. [PubMed: 21085672]
3. Cham CM, Driessens G, O'Keefe JP, Gajewski TF. Glucose deprivation inhibits multiple key gene expression events and effector functions in CD8R T cells. *Eur J Immunol*. 2008; 38:2438–2450. [PubMed: 18792400]
4. Gil L, Martinez G, Gonzalez I, Tarinas A, Alvarez A, Giuliani A, et al. Contribution to characterization of oxidative stress in HIV/AIDS patients. *Pharmacol Res*. 2003; 47:217–224. [PubMed: 12591017]
5. Kitchen CM, Yeghiazarian L, Hoh R, McCune JM, Sinclair E, Martin JN, et al. Immune activation, CD4R T cell counts, and viremia exhibit oscillatory patterns over time in patients with highly resistant HIV infection. *PLoS One*. 2011; 6:e21190. [PubMed: 21701594]
6. Deeks SG. HIV infection, inflammation, immunosenescence, and aging. *Annu Rev Med*. 2011; 62:141–155. [PubMed: 21090961]
7. Hunt PW, Martin JN, Sinclair E, Epling L, Teague J, Jacobson MA, et al. Valganciclovir reduces T cell activation in HIV-infected individuals with incomplete CD4R T cell recovery on antiretroviral therapy. *J Infect Dis*. 2011; 203:1474–1483. [PubMed: 21502083]
8. Hunt PW, Landay AL, Sinclair E, Martinson JA, Hatano H, Emu B, et al. A low T regulatory cell response may contribute to both viral control and generalized immune activation in HIV controllers. *PLoS One*. 2011; 6:e15924. [PubMed: 21305005]
9. Deeks SG. Immune dysfunction, inflammation, and accelerated aging in patients on antiretroviral therapy. *Top HIV Med*. 2009; 17:118–123. [PubMed: 19890183]
10. Hunt PW, Martin JN, Sinclair E, Bredt B, Hagos E, Lampiris H, et al. T cell activation is associated with lower CD4R T cell gains in human immunodeficiency virus-infected patients with sustained viral suppression during antiretroviral therapy. *J Infect Dis*. 2003; 187:1534–1543. [PubMed: 12721933]
11. Hunt PW, Cao HL, Muzoora C, Ssewanyana I, Bennett J, Emenyonu N, et al. Impact of CD8R T-cell activation on CD4R T-cell recovery and mortality in HIV-infected Ugandans initiating antiretroviral therapy. *AIDS*. 2011; 25:2123–2131. [PubMed: 21881481]
12. Fernandez S, Tanaskovic S, Helbig K, Rajasuriar R, Kramski M, Murray JM, et al. CD4R T-cell deficiency in HIV patients responding to antiretroviral therapy is associated with increased expression of interferon-stimulated genes in CD4R T cells. *J Infect Dis*. 2011; 204:1927–1935. [PubMed: 22006994]
13. Deeks SG, Kitchen CM, Liu L, Guo H, Gascon R, Narvaez AB, et al. Immune activation set point during early HIV infection predicts subsequent CD4R T-cell changes independent of viral load. *Blood*. 2004; 104:942–947. [PubMed: 15117761]
14. Liu Z, Cumberland WG, Hultin LE, Kaplan AH, Detels R, Giorgi JV. CD8R T-lymphocyte activation in HIV-1 disease reflects an aspect of pathogenesis distinct from viral burden and immunodeficiency. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1998; 18:332–340. [PubMed: 9704938]
15. Michalek RD, Gerriets VA, Jacobs SR, Macintyre AN, MacIver NJ, Mason EF, et al. Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4R T cell subsets. *J Immunol*. 2011; 186:3299–3303. [PubMed: 21317389]
16. Finlay D, Cantrell DA. Metabolism, migration and memory in cytotoxic T cells. *Nat Rev Immunol*. 2011; 11:109–117. [PubMed: 21233853]
17. Fox CJ, Hammerman PS, Thompson CB. Fuel feeds function: energy metabolism and the T-cell response. *Nat Rev Immunol*. 2005; 5:844–852. [PubMed: 16239903]
18. MacIver NJ, Jacobs SR, Wieman HL, Wofford JA, Coloff JL, Rathmell JC. Glucose metabolism in lymphocytes is a regulated process with significant effects on immune cell function and survival. *J Leukoc Biol*. 2008; 84:949–957. [PubMed: 18577716]
19. Palmer CS, Crowe SM. The role of glucose and lipid metabolism in the pathogenesis of HIV-1 infection. *Curr Trends Immunol*. 2012; 13:37–50.

20. Wieman HL, Wofford JA, Rathmell JC. Cytokine stimulation promotes glucose uptake via phosphatidylinositol-3 kinase/Akt regulation of Glut1 activity and trafficking. *Mol Biol Cell*. 2007; 18:1437–1446. [PubMed: 17301289]
21. 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; 111:2101–2111. [PubMed: 18042802]
22. Sorbara LR, Maldarelli F, Chamoun G, Schilling B, Chokekijcahi S, Staudt L, et al. Human immunodeficiency virus type 1 infection of H9 cells induces increased glucose transporter expression. *J Virol*. 1996; 70:7275–7279. [PubMed: 8794382]
23. Hollenbaugh JA, Munger J, Kim B. Metabolite profiles of human immunodeficiency virus infected CD4R T cells and macrophages using LC–MS/MS analysis. *Virology*. 2011; 415:153–159. [PubMed: 21565377]
24. Loisel-Meyer S, Swainson L, Craveiro M, Oburoglu L, Mongellaz C, Costa C, et al. Glut1-mediated glucose transport regulates HIV infection. *Proc Natl Acad Sci U S A*. 2012; 109:2549–2554. [PubMed: 22308487]
25. Michalek RD, Rathmell JC. The metabolic life and times of a T-cell. *Immunol Rev*. 2010; 236:190–202. [PubMed: 20636818]
26. Palmer C, Hampartzoumian T, Lloyd A, Zekry A. A novel role for adiponectin in regulating the immune responses in chronic hepatitis C virus infection. *Hepatology*. 2008; 48:374–384. [PubMed: 18666256]
27. Hearps AC, Maisa A, Cheng WJ, Angelovich TA, Lichtfuss GF, Palmer CS, et al. HIV infection induces age-related changes to monocytes and innate immune activation in young men that persist despite combination antiretroviral therapy. *AIDS*. 2012; 26:843–853. [PubMed: 22313961]
28. Jain P, Manuel SL, Khan ZK, Ahuja J, Quann K, Wigdahl B. DC-SIGN mediates cell-free infection and transmission of human T-cell lymphotropic virus type 1 by dendritic cells. *J Virol*. 2009; 83:10908–10921. [PubMed: 19692463]
29. Tanaka A, Jinno-Oue A, Shimizu N, Hoque A, Mori T, Islam S, et al. Entry of human T-cell leukemia virus type 1 is augmented by heparin sulfate proteoglycans bearing short heparin-like structures. *J Virol*. 2012; 86:2959–2969. [PubMed: 22238310]
30. Chou JP, Ramirez CM, Wu JE, Effros RB. Accelerated aging in HIV/AIDS: novel biomarkers of senescent human CD8R T cells. *PLoS One*. 2013; 8:e64702. [PubMed: 23717651]
31. Ludlow LE, Zhou J, Tippet E, Cheng WJ, Hasang W, Rogerson SJ, et al. HIV-1 inhibits phagocytosis and inflammatory cytokine responses of human monocyte-derived macrophages to *P. falciparum* infected erythrocytes. *PLoS One*. 2012; 7:e32102. [PubMed: 22363802]
32. Kinet S, Swainson L, Lavanya M, Mongellaz C, Montel-Hagen A, Craveiro M, et al. Isolated receptor binding domains of HTLV-1 and HTLV-2 envelopes bind Glut-1 on activated CD4R and CD8R T cells. *Retrovirology*. 2007; 4:31. [PubMed: 17504522]
33. Shin BC, McKnight RA, Devaskar SU. Glucose transporter GLUT8 translocation in neurons is not insulin responsive. *J Neurosci Res*. 2004; 75:835–844. [PubMed: 14994344]
34. Testi R, Phillips JH, Lanier LL. Leu 23 induction as an early marker of functional CD3/T cell antigen receptor triggering. Requirement for receptor cross-linking, prolonged elevation of intracellular [CaRR] and stimulation of protein kinase C. *J Immunol*. 1989; 142:1854–1860.
35. Manel N, Kinet S, Battini J-L, Kim FJ, Taylor N, Sitbon M. The HTLV receptor is an early T-cell activation marker whose expression requires de novo protein synthesis. *Blood*. 2003; 101:1913–1918. [PubMed: 12393496]
36. Maratou E, Dimitriadis G, Kollias A, Boutati E, Lambadiari V, Mitrou P, et al. Glucose transporter expression on the plasma membrane of resting and activated white blood cells. *Eur J Clin Invest*. 2007; 37:282–290. [PubMed: 17373964]
37. Yu Y, Clippinger AJ, Alwine JC. Viral effects on metabolism: changes in glucose and glutamine utilization during human cytomegalovirus infection. *Trends Microbiol*. 2011; 19:360–367. [PubMed: 21570293]
38. Takenouchi N, Jones KS, Lisinski I, Fugo K, Yao K, Cushman SW, et al. GLUT1 is not the primary binding receptor but is associated with cell-to-cell transmission of human T-cell leukemia virus type 1. *J Virol*. 2007; 81:1506–1510. [PubMed: 17108050]

39. Asano T, Katagiri H, Takata K, Lin JL, Ishihara H, Inukai K, et al. The role of N-glycosylation of GLUT1 for glucose transport activity. *J Biol Chem.* 1991; 266:24632–24636. [PubMed: 1761560]
40. Nakayama T, Kamiguchi H, Akagawa K. Syntaxin 1C, a soluble form of syntaxin, attenuates membrane recycling by destabilizing microtubules. *J Cell Sci.* 2012; 125:817–830. [PubMed: 22421360]
41. Chehtane M, Khaled AR. Interleukin-7 mediates glucose utilization in lymphocytes through transcriptional regulation of the hexokinase II gene. *Am J Physiol Cell Physiol.* 2010; 298:C1560–C1571. [PubMed: 20200205]
42. Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med.* 2006; 12:1365–1371. [PubMed: 17115046]
43. Nicoletti F, Fagone P, Meroni P, McCubrey J, Bendtzen K. mTOR as a multifunctional therapeutic target in HIV infection. *Drug Dis Today.* 2011; 16:715–721.
44. Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis? *Nat Rev Cancer.* 2004; 4:891–899. [PubMed: 15516961]
45. Gatenby RA, Gawlinski ET, Gmitro AF, Kaylor B, Gillies RJ. Acid-mediated tumor invasion: a multidisciplinary study. *Cancer Res.* 2006; 66:5216–5223. [PubMed: 16707446]
46. Montel-Hagen A, Kinet S, Manel N, Mongellaz C, Prohaska R, Battini JL, et al. Erythrocyte Glut1 triggers dehydroascorbic acid uptake in mammals unable to synthesize vitamin C. *Cell.* 2008; 132:1039–1048. [PubMed: 18358815]
47. Munoz E, Blazquez MV, Ortiz C, Gomez-Diaz C, Navas P. Role of ascorbate in the activation of NF-kappaB by tumour necrosis factor-alpha in T-cells. *Biochem J.* 1997; 325:23–28. Pt 1. [PubMed: 9224625]
48. Powell JD, Pollizzi KN, Heikamp EB, Horton MR. Regulation of immune responses by mTOR. *Annu Rev Immunol.* 2012; 30:39–68. [PubMed: 22136167]

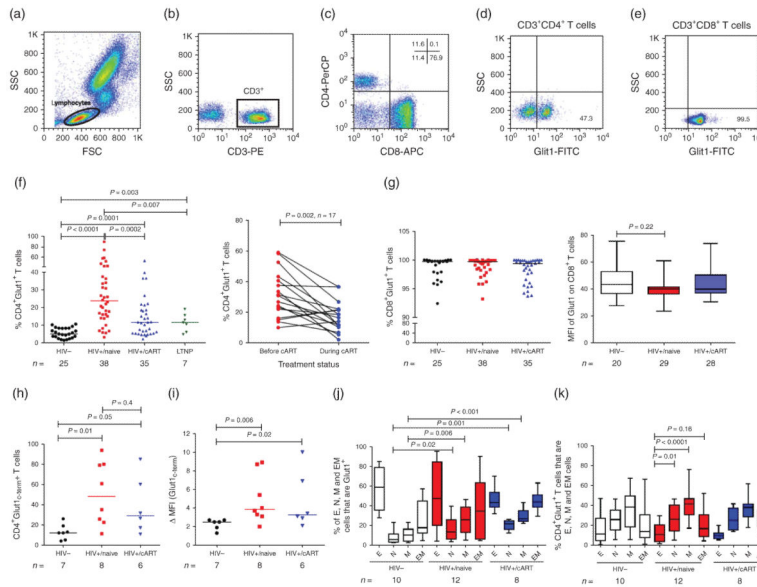


Fig. 1. Glut1 expression is increased on CD4⁺ T cells from HIVR/naive patients and is not restored to baseline by cART
 Within 1 h of collection, samples of whole blood were analyzed by flow cytometry for Glut1 expression on CD4⁺ and CD8⁺ T cells. (a) Lymphocytes (circled) were defined using side scatter (SSC) and forward scatter (FSC) characteristics. (b, c) Gating strategy showing CD3⁺ T cells within the lymphocyte-gated population which were then further defined based on CD4⁺ and CD8⁺ surface expression. (d, e) Representative flow cytometric dot plot of Glut1 expression on CD4⁺ T and CD8⁺ T cells in peripheral blood from HIV+/naive patients. (f) Percentage of CD4⁺Glut1⁺ T cells in peripheral blood from HIV⁻, HIV+/naive, HIV+/cART, and LTNP patients (left panel), and percentage of CD4⁺ T cells in peripheral blood of HIV+/naive patients before and during cART that express Glut1 (right panel). (g) Percentage of CD8⁺ T cells that are Glut1⁺ (left panel) and MFI of Glut1 on CD8⁺ T cells (right panel). (h) Percentage of CD4⁺ T cells that are Glut1_{c-term}⁺ and (i) MFI of Glut1_{c-term} on CD4⁺ T cells. (j) Median percentage of each CD4⁺ T-cell subpopulation that is Glut1⁺. (k) Median percentage of CD4⁺Glut1⁺ T cells expressing markers of functional subpopulations. Subpopulations were defined by their expression of CD45RA and CD27 to identify effector (E, CD45RA⁺CD27⁻), naive (N, CD45RA⁺CD27⁺), memory (M, CD45RA⁻CD27⁺), and effector-memory (EM, CD45RA⁻CD27⁻) cells. The nonparametric Mann–Whitney *t* test was used to evaluate significant differences between the median values of each group. Wilcoxon matched-pairs sign rank *t* test was used to analyze changes between paired data sets. Horizontal lines within histograms represent median value. Whiskers represent minimum and maximum values. cART, combination antiretroviral therapy; Glut1, glucose transporter-1; LTNP, long-term nonprogressor.

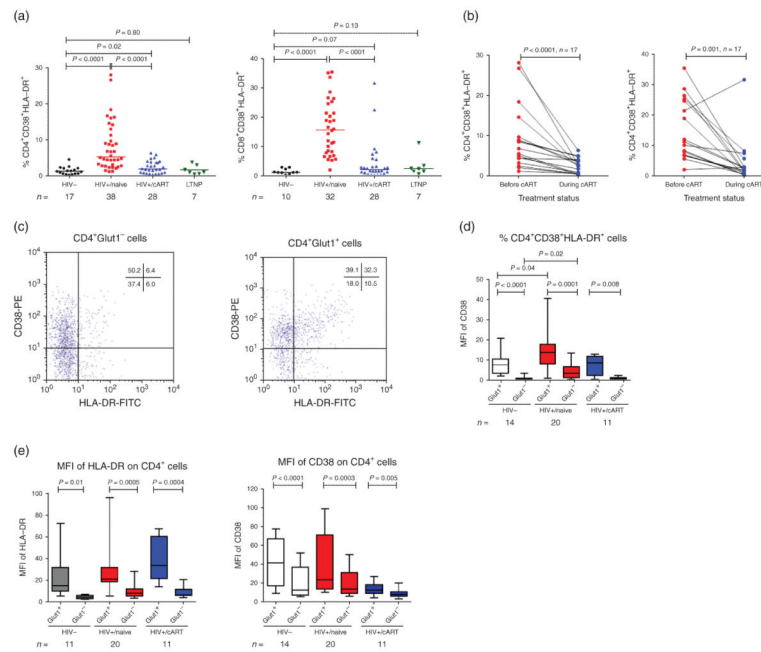


Fig. 2. Evaluation of T-cell activation and expression of activation markers on CD4⁺Glut1⁺ T cells

(a) The percentage of CD4⁺ (left panel) and CD8⁺ (right panel) T cells that co-express CD38 and HLA-DR was measured in whole blood from HIV⁻, HIV⁺/naive, and HIV⁺/cART patients using flow cytometry. (b) The percentage of CD4⁺ (left panel) and CD8⁺ (right panel) T cells that co-express CD38 and HLA-DR patients before and during cART. (c) Flow cytometric dot plot showing the expression of CD38 and HLA-DR on CD4⁺Glut1⁻ and CD4⁺Glut1⁺ T cells from a representative HIV⁺/naive patient. (d) The percentage of CD4⁺Glut1⁺ and CD4⁺Glut1⁻ cells that co-express CD38 and HLA-DR was measured by flow cytometry on whole blood. (e) MFI of HLA-DR (left panel) and CD38 (right panel) expressed on CD4⁺Glut1⁺ and CD4⁺Glut1⁻ T cells from the patients analyzed in (d). White histograms: HIV⁻ ($n = 14$); red histograms: HIV⁺/naive ($n = 20$); and blue histograms: HIV⁺/cART patients ($n = 11$). The nonparametric Mann–Whitney t test was used to evaluate significant differences between the median values of each group, whereas significant differences between Glut1⁺ and Glut1⁻ subsets were evaluated using the Wilcoxon matched-pairs sign rank t test. Horizontal lines within histograms represent median value. Whiskers represent minimum and maximum values. cART, combination antiretroviral therapy; Glut1, glucose transporter-1; MFI, mean fluorescent intensity.

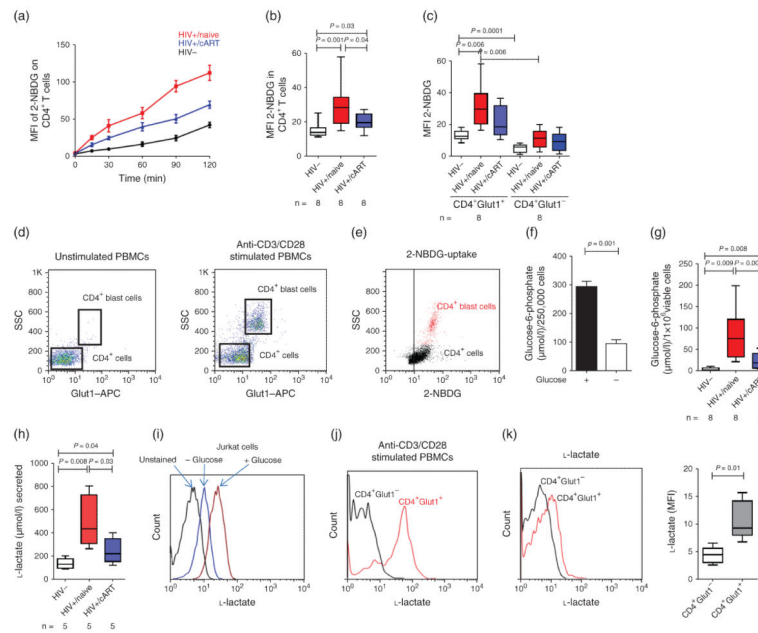


Fig. 3. Effects of HIV status on glucose uptake and glycolysis in CD4⁺ T cells

(a) The kinetics of glucose uptake were compared using three representative HIV⁻, HIV⁺/naive, and HIV⁺/cART patients who had low (3.6 ± 1.6 , black symbols), high (55.8 ± 17.1 , red symbols), and moderate (15.6 ± 2.2 , blue symbols) percentages of CD4⁺Glut1⁺ T cells, respectively. PBMCs were treated with 15 mmol/l 2-NBDG for the indicated times ($n = 3$ per group). Cells were washed and internalized 2-NBDG was detected by flow cytometry, as described in the Methods section. (b) Uptake of 2-NBDG in CD4⁺ T cells present in PBMCs incubated for 60 min with 15 mmol/l 2-NBDG ($n = 8$ per group). (c) Uptake of 2-NBDG by CD4⁺ T cells in the same donors analyzed in (b), stratified for Glut1 expression. (d) Dot plot of cells gated within the CD4⁺ T population to identify Glut1-expressing CD4⁺ T cells present in unstimulated (left panel) or anti-CD3/CD28-stimulated (right panel) PBMCs from a representative HIV⁻ individual. (e) CD4⁺ blast cells (red) and CD4⁺ cells (black) present within PBMCs stimulated with anti-CD3/CD28 beads and incubated for 60 min with 2-NBDG were overlaid onto the SSC vs. 2-NBDG dot plot. Data are from PBMCs from the same representative HIV-uninfected donor sample used in (d). (f) Concentrations of intracellular glucose-6-phosphate (G-6-P) in Jurkat cells cultured in glucose-containing (11 mmol/l) and glucose-deprived (0 mmol/l) RPMI-1640 medium for 4 h. (g) Basal concentrations of intracellular G-6-P in purified CD4⁺ T cells incubated in glucose containing RPMI-1640 for 4 h. (h) Basal secretion of L-lactate into culture medium by 1×10^6 purified viable CD4⁺ T cells incubated for 24 h in glucose (11 mmol/l) containing RPMI-1640. (i) Representative histogram showing intracellular L-lactate levels determined by flow cytometry in Jurkat cells cultured for 24 h in the absence (blue) or presence (brown) of 11 mmol/l glucose. (j) Representative histogram showing intracellular L-lactate levels in CD4⁺Glut1⁺ (red) and CD4⁺Glut1⁻ (black) T cells in PBMCs from a representative HIV⁻ control individual and stimulated for 24 h with anti-CD3/CD28 microbeads. (k) Intracellular L-lactate staining in Glut1⁻ (black) and Glut1⁺ (red) cells present in CD4⁺ T cells purified from a representative HIV⁺/naive individual. Horizontal lines within histograms represent

median value and whiskers represent minimum and maximum values. The nonparametric Mann–Whitney t test was used to evaluate significant differences between the median values of each group, whereas significant differences between Glut1⁺ and Glut1⁻ subsets were evaluated using the Wilcoxon matched-pairs sign rank t test. 2-NDBG, 2-N-(7-nitrobenz-2-oxa-1, 3-diazol-4-yl) amino)-2 deoxyglucose; cART, combination antiretroviral therapy; Glut1, glucose transporter-1; PBMCs, peripheral blood mononuclear cells; SSC, side scatter characteristic

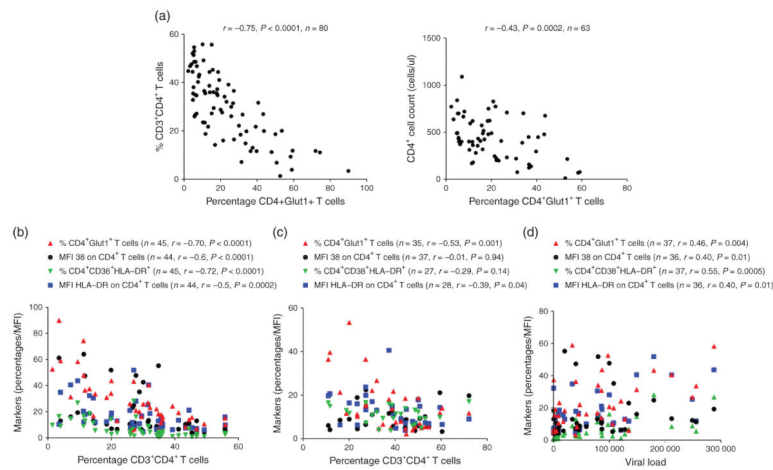


Fig. 4. Relationship between percentage CD4⁺Glut1⁺ T cells and total CD4⁺ T-cell percentage, CD4⁺ T-cell count, and HIV viral load in HIV-infected patients

(a) Spearman's correlations between the percentage (left panel) and absolute number (right panel) of CD3⁺CD4⁺ T cells and the percentage of CD4⁺Glut1⁺ T cells in the peripheral blood of HIV+/naive and HIV+/cART patients. (b, c) Comparative relationship between the percentage of CD4⁺Glut1⁺ T cells and markers of CD4⁺ T-cell activation, and the percentage of CD3⁺CD4⁺ T cells in (b) HIV+/naive and (c) HIV+/cART patients. (d) Comparative relationship between the percentage of CD4⁺Glut1⁺ T cells and markers of CD4⁺ T-cell activation, and HIV viral load in HIV+/naive patients. cART, combination antiretroviral therapy; Glut1, glucose transporter-1.

Table 1

Clinical characteristics of study groups.

Variables	n	Groups				P value		
		HIV- (A)	HIV+/naive (B)	HIV+/CART (C)	LTNP (D) ^a	A vs. B	B vs. C	A vs. C
Sex (M/F)	105	24/1	37/1	35/0	7	–	–	–
BMI (kg/m ²)	82	22.4 (22–25)	24.7 (22–27)	24.5 (21–27)	21.5 (16–29)	0.48	0.42	0.57
Age, years, mean (SD)	93	45.2 ± 20.5	42.3 ± 10.7	44.6 ± 11.5	54.0 ± 7.0	0.83	0.46	0.82
MSM (self-reported)	67	11/25	22/38	28/35	6/7	–	–	–
CD4 ⁺ T-cell count (cells/μl)	63	–	400.0 (223–483)	479.0 (245–710)	493.0 (372–594)	–	0.08	–
Nadir CD4 ⁺ T-cell count (cells/μl)	30	–	–	388 (38–500)	–	–	–	–
Time on cART, years, mean (SD)	30	–	–	6.1 (6.2)	–	–	–	–
% CD3 ⁺ CD4 ⁺ T cells	105	54.5 (35–45)	27.5 (14–36)	39.2 (27–50)	35.0 (35–45)	<0.0001	0.0008	0.0001
CD4 ⁺ /CD8 ⁺ ratio	94	1.7 (1–2)	0.5 (0.2–0.7)	0.8 (0.5–1.2)	0.6 (0.6–1)	<0.0001	0.001	<0.0001
Viral load (copies/ml)	72	–	86000 (39500–128000)	<502385	(203–10963)	–	–	–
Plasma glucose (mmol/l)	80	4.7 (4.5–5.2)	4.7 (4.5–5.1)	4.9 (4.5–5.7)	5.8 (5.1–7.8)	0.64	0.32	0.26
Plasma insulin (μU/ml)	48	5.9 (3.0–14.7)	4.4 (3.1–5.9)	3.7 (2.2–27.5)	–	0.50	>0.10	0.87
Plasma triglyceride (mmol/l)	64	0.9 (0.6–1.3)	1.0 (0.8–1.6)	1.4 (0.9–2.1)	0.7 (0.7–1.5)	0.30	0.22	0.03
Plasma HDLCH (mmol/l)	62	1.1 (0.8–1.4)	0.7 (0.6–1.0)	0.8 (0.7–1.0)	1.0 (0.8–1.1)	0.003	0.54	0.005
Plasma cholesterol (mmol/l)	60	4.2 (3.2–5.6)	4.0 (3.0–4.7)	3.3 (3.2–4.4)	4.2 (3.5–4.6)	0.40	0.34	0.06
Creatinine (μmol/l)	79	74.0 (67.0–84.0)	76.0 (66.0–88.4)	74.5 (69.0–80.2)	88.4 (79.6–97.2)	0.64	0.60	0.90
hsCRP (mg/l)	47	0.8 (0.5–1.4)	1.3 (0.7–4.4)	0.90 (0.5–4.7)	–	0.07	0.10	0.40
D-dimer (μg/ml)	48	0.8 (0.5–1.0)	0.9 (0.7–1.6)	0.77 (0.4–1.1)	–	0.06	0.16	0.38
Plasma vitamin D (nmol/l)	48	46.5 (34.0–99.0)	74.0 (41.3–88.5)	78.9 (36.8–99.7)	–	0.24	0.40	0.30
TNF (pg/ml)	48	6.0 (3.6–7.5)	8.7 (6.9–14.3)	8.2 (5.5–12.9)	–	0.005	0.28	0.02

cART, combination antiretroviral therapy; hsCRP, high-sensitivity C-reactive protein; LTNP, long-term nonprogressor; TNF, tumor necrosis factor. LTNP samples were obtained from the Clinical Research Core Repository at the University of Washington, Seattle, USA(UW). Continuous variables are expressed in median (interquartile range), unless otherwise indicated. Categorical variables are expressed in mean (±SD). The nonparametric Mann–Whitney test was used to evaluate significant difference between each group.

^a Selected analysis of group D; BMI, $P = 0.40$ D vs. C; age, $P = 0.009$ D vs. B, $P = 0.03$ D vs. C; $P = 0.24$ D vs. A; CD4⁺ T-cell count, $P = 0.30$ D vs. B; %CD3⁺CD4⁺ T cells, $P = 0.03$ D vs. B, $P = 0.81$ D vs. C; CD4⁺/CD8⁺ ratio, $P = 0.003$ D vs. A, $P = 0.08$ D vs. B, $P = 0.64$ D vs. C; viral load, $P = 0.0003$ D vs. B; plasma triglyceride, $P = 0.08$ D vs. C.