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Brief Reviews

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Emerging Role and Characterization of Immunometabolism: Relevance to HIV Pathogenesis, Serious Non-AIDS Events, and a Cure

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Immune cells cycle between a resting and an activated state. Their metabolism is tightly linked to their activation status and, consequently, functions. Ag recognition induces T lymphocyte activation and proliferation and acquisition of effector functions that require and depend on cellular metabolic reprogramming. Likewise, recognition of pathogen-associated molecular patterns by monocytes and macrophages induces changes in cellular metabolism. As obligate intracellular parasites, viruses manipulate the metabolism of infected cells to meet their structural and functional requirements. For example, HIV-induced changes in immune cell metabolism and redox state are associated with CD4$^+$ T cell depletion, immune activation, and inflammation. In this review, we highlight how HIV modifies immunometabolism with potential implications for cure research and pathogenesis of comorbidities observed in HIV-infected patients, including those with virologic suppression. In addition, we highlight recently described key methods that can be applied to study the metabolic dysregulation of immune cells in disease states. *The Journal of Immunology*, **2016, 196: 4437–4444.**

All cells need nutrients to meet their bioenergetic requirements; cellular metabolism adapts to match those demands. A fundamental, yet often unrecognized, change that occurs in immune cells is their metabolic reprogramming that facilitates transformation from a resting to an active state, or differentiation. The metabolic shift usually occurs via increased expression of nutrient transporters [e.g., glucose transporters (1, 2)], increased generation of glycolytic enzymes, greater glycolytic flux, and increased rate of oxidative phosphorylation (OxPhos). The increased metabolic demands observed in activated T cells and monocytes are associated with immune activation and inflammatory responses, respectively (3).

Observations that HIV infection is strongly associated with elevated plasma IL-7 (4) and that the virus overwhelmingly infects activated, but not resting, CD4$^+$ T cells established the putative role of glycolysis in HIV pathogenesis (5–7). Loisel-Meyer et al. (8) were the first to provide direct evidence for the role of glucose transporter 1 (Glut1) in regulating HIV entry into CD4$^+$ T cells and thymocytes. We (9) subsequently demonstrated that Glut1 is a persistent metabolic activation marker of HIV$^+$ "effector" CD4$^+$ T cells and monocytes, remaining elevated in treated, chronic HIV infection. Increased aerobic glycolysis, a hallmark of cancer, drives cancerous growth (10); however, its role in the pathogenesis of HIV infection is only beginning to emerge, with technical advances allowing measurement of metabolic activities in immune cells.

This review focuses on how changes in glucose metabolic profile and redox potential of T cells and monocytes contribute to HIV pathogenesis, including immune activation, serious

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Abbreviations used in this article: cART, combination antiretroviral therapy; ECAR, extracellular acidification rate; FAO, fatty acid oxidation; FCCP, carbonyl cyanide p-trifluoromethoxyphenylhydrazone; Glut1, glucose transporter 1; G6P, glucose-6-phosphate; NBDG, (*N*-(7-nitrobenz-2-oxa-1, 3-diazol-4-yl) amino)-2 deoxyglucose; OCR, oxygen consumption rate; OxPhos, oxidative phosphorylation; ROS, reactive oxygen species; SNAE, serious non-AIDS event; Tfh, follicular helper T; Treg, regulatory T cell.

non-AIDS events (SNAEs), and HIV reservoir persistence in the era of combination antiretroviral therapy (cART). We summarize the newly available techniques that facilitate understanding of the immune-metabolic dysfunction in chronic inflammatory diseases.

Metabolic features of T cell subsets

T cell function is intimately linked to cellular metabolism (11, 12). Cells use two major pathways for energy generation: glycolysis and OxPhos. After activation, metabolically quiescent naive T cells switch from OxPhos to glycolysis, providing energy and biosynthetic precursors for cell proliferation and effector functions. The metabolic transition is mediated, in part, by activation-induced increases in Glut1 surface expression. Exiting functional activation, memory T cells revert back to OxPhos, but with increased mitochondrial mass and spare respiratory capability (additional mitochondrial capacity to produce energy under stress) compared with naive cells (13) (Fig. 1). Intriguingly, specific T cell functional subsets possess unique metabolic profiles essential for their differentiation and function. $CD4^+$ T cell effector subsets, Th1, Th2, and Th17, primarily rely on aerobic glycolysis (14). In contrast, regulatory T cells (Tregs) use less glycolysis but more fatty acid oxidation (FAO), a feature also seen in $CD8^+$ memory T cells (15, 16). Higher total cellular and cell surface Glut1, as well as increased glycolysis, are present in Th1, Th2, and Th17 cells compared with Tregs (15). Indeed, blocking glycolysis inhibits proinflammatory Th17 cell development while promoting anti-inflammatory Treg generation (17). Th17 cells also rely on acetyl-CoA carboxylase 1–mediated de novo fatty acid synthesis; thus, induction of the glycolytic-lipogenic axis is central for the development of Th17 cells but not Tregs. Blocking de novo fatty acid synthesis using the acetyl-CoA carboxylase–specific inhibitor soraphen A restrains the development of Th17 cells in mice and attenuates Th17 cell–mediated autoimmune disease (18).

Compared with other effector $CD4^+$ T cell subsets, follicular helper T (Tfh) cells demonstrate reduced metabolic function, as shown by reduced glucose uptake, maximal respiratory capacity, and extracellular acidification rate, a proxy for glycolysis (19). Notably, Bcl6, the transcription factor that directs Tfh cell differentiation, directly binds and suppresses expression of Glut1 (20).

Tfh cells and HIV reservoir persistence. Tfh cell frequency is substantially higher in HIV-infected patients and SIV (the nonhuman primate counterpart to HIV)-infected rhesus macaque monkeys compared with noninfected controls. This reflects an increase in absolute Tfh cell numbers rather than a ratio change caused by depletion of non-Tfh populations (21–23). Perreau et al. (23) showed the expanded Tfh cells carried the highest number of HIV DNA copies, and cART reduced the proportion of HIV^+ Tfh cells. In addition, Tfh cells were more efficient at producing replication-competent virus in vitro than other CD4 subsets (23). Thus, Tfh cells expand and proliferate during HIV infection and represent a major compartment of HIV production and replication.

How do Tfh cells constitute a major reservoir for HIV replication and production? Data suggest that cytotoxic $CD8^+$ T cells are inefficient in controlling Tfh cell infection, possibly because most are excluded from B cell follicles where Tfh cells are located (24). Additionally, the metabolic quiescence of Tfh cells resulting from reduced glycolysis may lower their sensitivity to HIV-induced cell death and contribute to HIV persistence in this subset.

T cell metabolic dysfunction during HIV infection

HIV infection is associated with changes in T cell metabolism critical for viral replication but is also linked to T cell activation and depletion (3). The hyperactive (20, 21) and exhaustive (22, 23) phenotype associated with $CD4^+$ T cells during HIV infection contributes to a gradual decline in CD4 T cell numbers and reduced functionality of surviving cells (25, 26). HIV-associated $CD4^+$ T cell depletion occurs via two major pathways (27). Productive infection of $CD4^+$ T cells induces apoptosis, mediated by caspase-3 activation (28), and abortive infection induces pyroptosis, mediated by caspase-1 activation (29). We observed that high glucose metabolic activities correlated with $CD4^+$ T cell depletion, and enhanced glycolytic activity in $CD4^+$ T cells was associated with T cell activation in HIV-infected adults (9). These findings are supported by in vitro studies. Metabolic profiling revealed increased levels of glycolytic intermediates in HIV-1–infected activated $CD4^+$ T cells (30). Furthermore, HIV infection increases glycolytic flux in $CD4^+$ T cells, and inhibition of glycolysis reduces viral production (7). The consequences of dysfunctional glucose metabolism in T cells and monocytes during HIV infection were reviewed comprehensively (3); however, metabolic dysfunction may impose unique responses in different subpopulations of immune cells. For example, the metabolic demands of effector $CD4^+$ T cells and the shift from the high ATP–producing OxPhos to the less-efficient energy-producing aerobic glycolysis may encourage "CD4 T cell metabolic catastrophe" and cell death (3). Increased glucose uptake and glycolytic metabolism provide precursors for HIV biosynthesis, as well as intermediary enzymes that may disengage glycolysis and regulate HIV reactivation (31). Association of hexokinase with the outer mitochondrial membrane of HIV-infected macrophages may facilitate survival of these cells (32), whereas HIV proteins induce oxidative stress by disrupting mitochondrial biogenesis (33, 34) (Fig. 2).

Although cART is largely successful in preventing peripheral $CD4^+$ T cell decline, chronic activation of $CD4^+$ and $CD8^+$ T cells remains problematic. Future strategies to reverse $CD4^+$ T cell depletion and hyperactivation in HIV-infected patients may include targeting cell metabolism. How HIV infection regulates T cell metabolism and the mechanistic basis for the association between enhanced glycolysis and $CD4^+$ T cell activation and depletion are important questions to be addressed by future studies.

Role of monocyte metabolism in HIV-associated comorbidities

Glycolytic metabolism in monocytes and macrophages drives inflammatory responses. Successful cART has shifted the cause of death for many HIV-infected individuals, including those in developing countries, from opportunistic diseases associated with AIDS to chronic noncommunicable diseases or SNAEs (35, 36). HIV-induced inflammation is a major contributing factor (37). Inflammation persists even during effective cART as the result of ongoing immune dysregulation. Although opportunistic infections and mortality in untreated HIV infection are associated with activated T cells (38–40), SNAEs

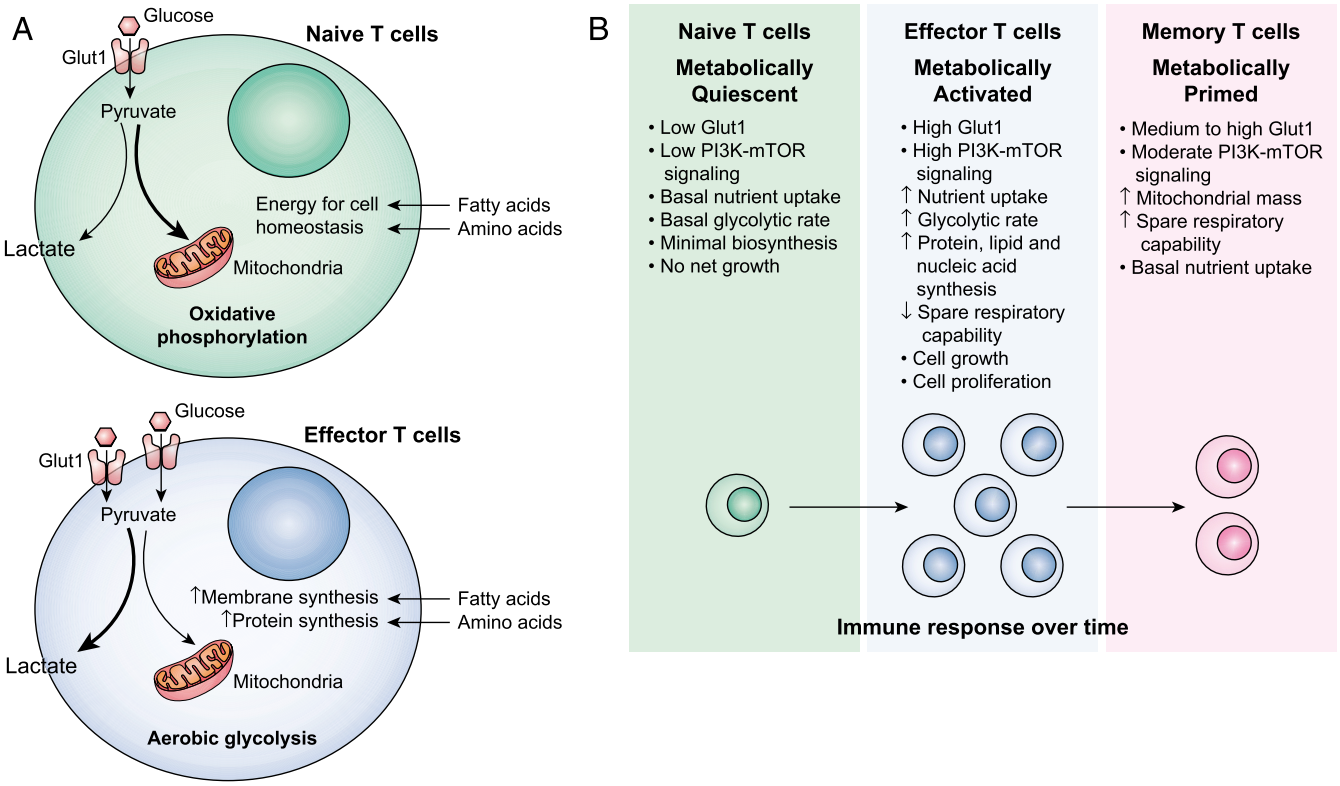

FIGURE 1. Metabolic shifts in glucose metabolism during an immune response. (**A**) Naive T cells predominantly use glucose via OxPhos, whereas effector T cells exhibit high glycolytic metabolism. Precursors of aerobic glycolysis fuel biosynthetic pathways in activated cells required for protein and membrane synthesis. (**B**) Increased PI3K-mTOR signaling, nutrient uptake, and glycolysis are signature features of metabolically activated effector T cells. Memory T cells revert to low nutrient uptake, but are metabolically primed to respond rapidly to inflammatory growth signals or to Ag re-exposure.

in cART-treated HIV infection are associated with activated monocytes (41–44).

In contrast to resting T cells that derive the vast majority of energy from OxPhos, the metabolic phenotype of resting monocytes is less distinct, appearing to obtain energy from oxidative and glycolytic metabolism (45). Activated monocytes and inflammatory M1 macrophages show increased cell surface Glut1 and glucose uptake and a marked increase in glycolysis associated with the production of inflammatory cytokines (3, 46–48).

We recently showed that Glut1-expressing monocytes are increased during HIV infection, regardless of cART status, compared with uninfected controls (47). Examination of monocyte subpopulations from HIV-infected adults showed that intermediate monocytes ($CD14^{++}CD16^{+}$) have higher levels of activation ($HLA\text{-}DR^{+}$) than do classical ($CD14^{++}CD16^{-}$) and nonclassical ($CD14^{+}CD16^{++}$) monocytes. Intermediate monocytes from HIV-infected individuals also take up more of the glucose analog 2-(*N*-(7-nitrobenz-2-oxa-1, 3-diazol-4-yl) amino)-2 deoxyglucose (NBDG) compared with cells from uninfected donors. However, stimulation of uninfected intermediate monocytes in vitro with LPS increased their 2-NBDG uptake, glucose uptake, and L-lactate production, suggesting that persistent innate immune stimuli during HIV infection enhance monocyte Glut1 expression and glycolytic metabolism.

There are no mechanistic links between increased glucose metabolism in monocytes/macrophages and inflammation in HIV-infected individuals. Glut1 is the primary glucose transporter on proinflammatory M1 macrophages. In vitro, HIV infection drives human monocyte-derived macrophages toward an M1-like phenotype (49). M1 macrophages are characterized by elevated Glut1 expression and glycolytic metabolism, which facilitate high production of proinflammatory mediators, including IL-6, TNF, and IL-1RA (48). In contrast, anti-inflammatory M2 macrophages have high mitochondrial OxPhos (50). Interestingly, although it is acknowledged that the Akt-mTORC1 pathway regulates glycolytic metabolism in T cells and contributes to their inflammatory responses (51), the Akt-mTORC1 axis was recently shown to regulate a subset of M2 genes via Acyl-CoA production and histone acetylation (52). It appears that HIV potently induces hexokinase expression in infected macrophages via viral protein R. Hexokinase may play a nonmetabolic role by binding to mitochondria, thereby maintaining mitochondrial integrity and viability of HIV-infected macrophages (32). Disrupting this association between hexokinase and mitochondria to induce apoptosis in persistently infected macrophages may prove valuable in reducing the macrophage HIV reservoir.

A possible association between increased monocyte metabolism and HIV-associated comorbidities

Markers of monocyte activation are associated with morbidity and mortality in cART-treated HIV infection (41–44). We hypothesize that chronic monocyte glycolytic metabolism may be a critical determinant of monocyte activation, contributing to inflammation and the development of SNAEs. Several factors that can stimulate monocyte activation are present during cART-treated HIV infection. Low systemic levels of microbial products (e.g., LPS) resulting from a damaged

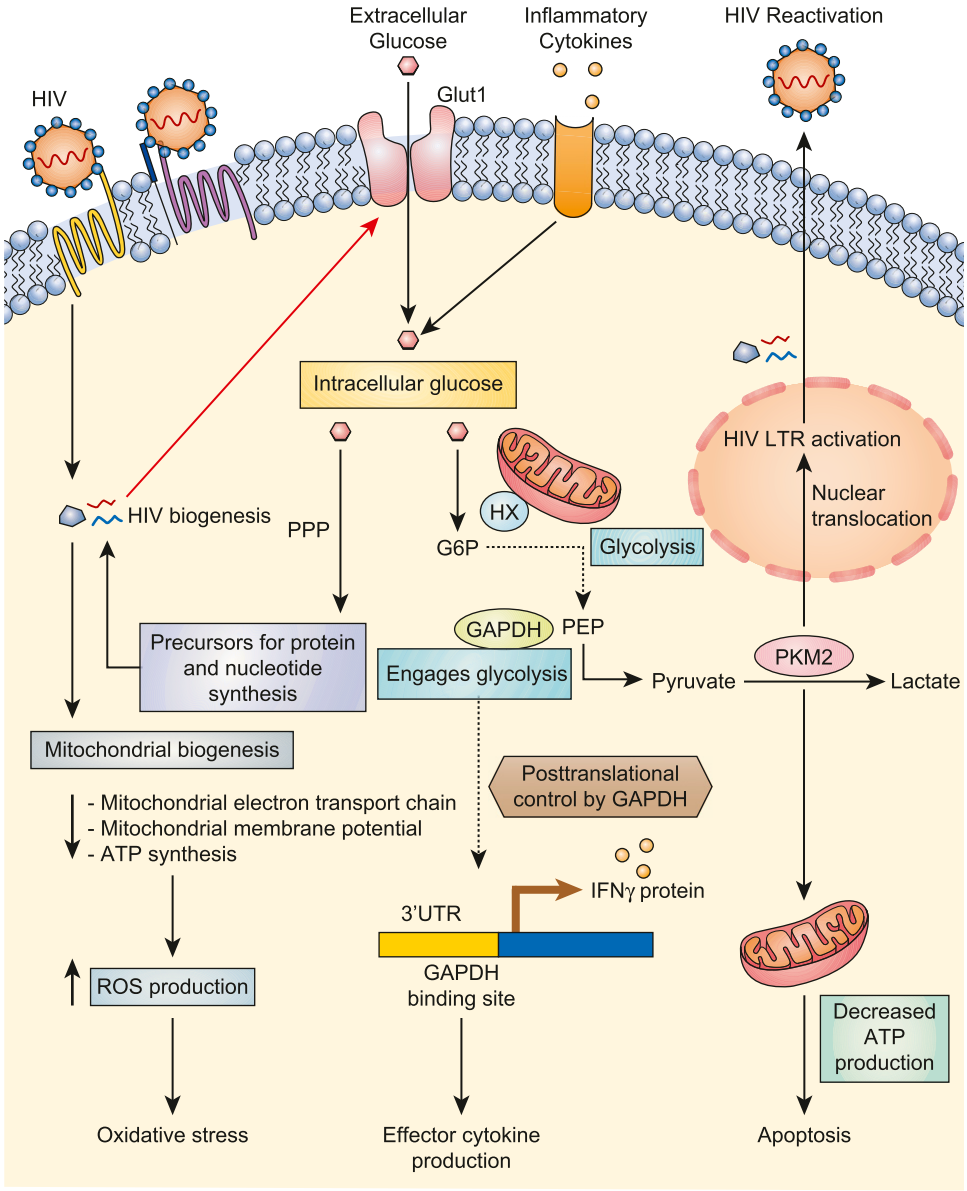

FIGURE 2. HIV-associated metabolic dysregulation influences immune cell responses, HIV biogenesis, and cell survival. Increased cell surface expression of Glut1 on $CD4^+$ T cells and macrophages enhances glucose uptake. Upregulation of hexokinase (HX) converts glucose into G6P and forward feed to produce phosphoenolpyruvate (PEP), which is converted into pyruvate by pyruvate kinase muscle type 2 (PKM2). Nuclear translocation of PKM2 can induce activation of HIV LTR. Conversely, HX can bind to the outer mitochondrial membrane to maintain mitochondrial membrane potential and confer an antiapoptotic phenotype of macrophages. Increased glycolysis disengages GAPDH from its regulatory control ("moonlighting") on inflammatory cytokines. Elevated aerobic glycolysis fuels the pentose-phosphate pathway (PPP) to synthesize amino acids and ribose-5-phosphate required for HIV protein and nucleotide synthesis. Upregulation of these metabolic pathways deprives the tricarboxylic cycle precursors for OxPhos, causing decreased mitochondrial ATP production and apoptosis in some effector $CD4^+$ T cells. HIV infection may dysregulate mitochondrial biogenesis, resulting in oxidative stress.

gut mucosa are a potent source of monocyte stimulation, and in vitro monocyte exposure to LPS increases glycolytic metabolism (47). Residual HIV viremia may also heighten monocyte glycolytic metabolism because HIV Ags can directly stimulate monocytes (53). Recent studies suggest latent coinfections, such as CMV, may not be optimally controlled and could be a source of stimulation for monocytes (54). Future work will establish whether chronic SNAEs occurring in cART-treated HIV infection are associated with monocyte Glut1 expression and glycolytic metabolism.

Regulation of immune cell metabolism by oxidative stress and redox potential

In addition to antigenic stimulation and proinflammatory cytokines, immune cell metabolism may be regulated by oxidative stress and intracellular redox signaling. Redox signals are critical regulators of HIV replication, immune dysfunction, and disease progression (55, 56). Altered energy balance is consistent with studies showing loss of mitochondrial membrane potential, increased production of reactive oxygen species (ROS), and depletion of major intracellular antioxidants, glutathione, and thioredoxin in HIV infection (57).

In the context of HIV infection, viremia is associated with diminished levels of glutathione and increased ROS levels in plasma and lymphocytes (55). Many studies independently confirmed the presence of oxidative stress and loss of immune function during HIV infection, and Glut1 expression on immune cells (a major controller of glucose metabolism, although the precise mechanisms remain unclear) is induced by oxidative stress (58). Thus, ROS may be a physiological inducer of glucose metabolism in HIV-infected cells.

Methods relevant to studying immunometabolism in HIV and other diseases

As set out above, HIV infection provides a useful example of how immune cells need to adapt their metabolism to elicit an appropriate immune response at each stage of an immune challenge. To characterize the genetic, proteomic, and metabolic factors that govern these processes and to understand how they are compromised in disease settings, such as HIV infection, the appropriate use of existing and new techniques is

necessary. We describe some of the methods currently available to characterize metabolism in immune cells.

Glucose uptake. One of the first steps in ensuring that energy supply and demand are matched is control of glucose entry into the cell. Radio-labeled glucose can be used to measure rates of glucose uptake into immune cells with a glucose transport assay. Briefly, this technique involves exposing cells to the tracer 2-deoxy-D-[^{3}H] glucose, which is taken up into cells and trapped. The reaction is stopped after a set period of time, and the cells are solubilized before radioactivity is determined by scintillation counting, normalized to cell number (59). Measurements of glucose uptake in cells with radioactive glucose isotopes are favorable for kinetic studies of glucose transport; however, the inconvenience and expense associated with radioactive waste disposal are significant (60). Another way to measure glucose uptake in immune cells is via flow cytometry. The fluorescently labeled glucose analog 2-NBDG is a fluorescent analog of glucose that can give an accurate estimation of cellular glucose uptake. This analog, developed by Yoshioka et al. (61), was shown to be rapidly taken up into tumor cells monitored by fluorescence imaging analysis (62), and it showed potential in other cell-based systems (63). This analog was also used to study immunometabolism (9, 64). The advantage of using 2-NBDG is that it can be multiplexed with Abs to study low-frequency cell subsets from a small volume of blood (65). However, because 2-NBDG is not structurally identical to glucose, confirmation of a robust finding is important. For example, detected differences in 2-NBDG can be confirmed via analysis of glucose-6-phosphate (G6P) levels. Hexokinase catalyzes the phosphorylation of glucose to G6P; thus, intracellular G6P can indicate the state of intracellular retention of glucose (9). Radio-labeled sugar transport into the brain was correlated with local Glut density, but glucose uptake may also be dependent on Glut1 activation rather than translocation from intracellular membranes (66). Barros et al. (67) showed that 6-NBDG, another analog of glucose, binds to Glut1 with 300-fold greater affinity than glucose, which explains why its uptake is not efficiently displaced by glucose. However, they concluded that 6-NBDG, used at low concentrations, is transported into astrocytes mainly through Glut. Thus, caution is warranted when designing experiments and interpreting results from studies using these glucose analogs, especially when used at higher concentrations than physiological levels. Because a difference in glucose uptake can reflect alterations in glycolytic capacity or flux, as well as the pharmacodynamics properties of these NBDG analogs (66), additional metabolic investigations are warranted (discussed below).

Glucose transporter expression. The Glut family of glucose transporters is primarily responsible for the uptake of glucose into cells. Slc2a1 (Glut1) has been of particular interest in the immunometabolism field. In animal studies, Glut1^{myc}-knockin mice (in which a tandem-Myc tag is knocked into exon 3, which encodes for the exofacial loop of Glut1) allow sensitive and accurate flow cytometric measurements of endogenous Glut1 at the cell surface using Abs that target Myc (1, 15). These studies showed that Glut1 is required for glucose uptake, T cell activation, and effector T cell functions. Moreover, Glut1 deficiency decreased the ability of effector cells to induce inflammatory disease in vivo (1). Researchers performing studies in human T cells do not have the luxury of Myc tagging and must rely on binding of Glut1 Abs to Glut1. Questions have been raised about the specificity of commercially available Glut1 Abs. Recently, using lentiviral constructs to overexpress Glut1, we (9, 47) validated the use of an R&D Systems Glut1 Ab MAB1418 clone to detect cell surface Glut1 on human T cells and monocytes. However, Glut1 expression did not reflect the levels of glucose uptake in some monocyte subpopulations. Furthermore, Kinet et al. (68) demonstrated a lack of correlation between Glut1 expression and MAB1418 clone staining in different cell types, as well as following Glut1 overexpression. The intracellular pool of Glut1 may be examined using an unconjugated mAb against the C-terminal portion of Glut1 (Glut1$_{c\text{-}term}$; Abcam) and a goat anti-mouse FITC-conjugated secondary Ab. Cells can be surfaced stained and permeabilized using the IntraStain Kit (Dako). Using this protocol, Glut1 expression was shown to be increased on CD4$^+$ T cells in HIV$^+$ persons (9). Other investigators also used Glut1-GFP receptor binding domain (METAFORA biosystems) to detect cell surface Glut1 on immune cells. This method is based on the direct binding of the receptor binding domain of human T cell lymphotropic virus to the 7 aa of the extracellular loop 6 of Glut1 (8, 68, 69).

Mitochondrial function: oxygen consumption and glycolysis. Traditionally, oxygen consumption/respiration measurements were made in cells using equipment such as the Clark electrode. Because these assays require a large biomass, they are not feasible to study the limited quantity of immune cells available from human volunteers. The recent development of the Seahorse XF Bioanalyzer has allowed mitochondrial function to be studied in intact cells. This enables higher-throughput work with lower cell numbers. The primary readouts of the bioanalyzer are oxygen consumption rate (OCR; an estimate of mitochondrial functionality) and extracellular acidification rate (ECAR; an estimate of glycolysis). Two standard assays are commonly performed using this platform. The first, termed a mitochondrial stress test, measures various aspects of mitochondrial function. Following basal respiration measurements, cells are sequentially treated with oligomycin (ATP synthase inhibitor), carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP; proton ionophore) or an alternate uncoupler, such as 2,4-dinitrophenol (DNP), and antimycin A (complex III inhibitor) with or without rotenone (complex I inhibitor), and changes in respiration are recorded in the software. This allows for analysis of various parameters of mitochondrial function. These parameters include basal respiration (difference in OCR before any treatment of mitochondrial inhibitors and after antimycin A or antimycin A and rotenone treatment), uncoupled respiration (difference in OCR following treatment of oligomycin and antimycin A or antimycin A and rotenone), spare respiratory capacity [difference in OCR following treatment of FCCP/DNP and before any drug injection], ATP turnover (difference in OCR following oligomycin treatment and before any injection), and maximal respiratory capacity (OCR following treatment of FCCP minus the rate after antimycin A or antimycin A plus rotenone treatment) (70).

The second commonly performed assay, termed a glycolysis stress test, relies on measurement of the proton production rate, which estimates the rate of glycolysis. During glycolysis,

glucose is converted to lactate, which results in the net production and extrusion of protons into the extracellular medium, which is measured as ECAR. A typical assay involves measurement of ECAR in glucose-naive conditions (non-glycolytic acidification rate), following glucose injection (glycolysis rate), following inhibition of ATP synthase (also referred to as complex V) with oligomycin to shut down OxPhos (the glycolytic capacity of the cell), and after 2-deoxy-D-glucose administration (a glycolytic inhibitor to act as a negative control). The difference in ECAR between glucose and oligomycin injections is calculated as the glycolytic reserve of the cell. Glycolysis, as measured by ECAR on the Seahorse XF Bioanalyzer, should be supported by other confirmatory measurements. For example, assaying L-lactate secreted into culture media is one such method. It was demonstrated in a T cell model that reduced glycolytic capacity corresponded to less extracellular L-lactate in the media (64). More recently, a multiparametric flow cytometry method was developed using a fluorometric assay kit to measure intracellular lactate levels in $CD4^+$ T cells from HIV^+ individuals (9). Together, these assays provide an overall picture of cellular metabolism and are useful in determining the checkpoints and mechanisms via which metabolic processes may be altered.

Metabolite flux via mass spectrometry. Ex vivo approaches, such as the use of mass spectrometry–based metabolomics, can be used to gather important information on individual metabolites and flux through the various metabolic pathways. Wang et al. (71) used this system to determine the concentration of metabolites at different stages of primary T cell activation and used isotopically labeled tracers to determine metabolic flux. These measurements included the generation of ^3H_2O from [3-^{3}H]glucose (a measure of glycolysis) or from [9,10-^{3}H] palmitic acid (mitochondrial-dependent FAO measure). They also investigated the generation of $^{14}CO_2$ from [2-^{14}C]pyruvate (measure of pyruvate oxidation through the tricarboxylic acid cycle and [U-^{14}C]glutamine (glutaminolysis; glutamine consumption through oxidative catabolism) or from [1-^{14}C]-glucose (glucose consumption via the pentose phosphate pathway) (71). Consumption and release metabolomics, a technique incorporating liquid chromatography coupled to mass spectrometry, was used recently to identify alanine as an endogenous substrate for the amino acid transporter SNAT1 in HIV-infected $CD4^+$ T cells. Therefore, this technique can be used to provide insights into how HIV interferes with substrate metabolism (72). Improvements in the resolution of mass spectrometry machines and data acquisition rates have improved accuracy and processing times for such analyses. Further refinement will ensure that such techniques are more readily accessible in the future.

Metabolic inhibitors. Numerous chemical inhibitors can be used to target different metabolic pathways and study their impact on processes such as proliferation, survival, mitochondrial function, and gene expression. For example, FAO can be inhibited using etomoxir, a carnitine palmitoyltransferase-1 inhibitor. Carnitine palmitoyltransferase-1 is an outer mitochondrial membrane enzyme responsible for transportation of long-chain fatty acids from the cytosol into the mitochondria for β-oxidation. Likewise 2-deoxy-D-glucose is a glycolysis inhibitor because of its actions on hexokinase, the rate-limiting step of glycolysis, and is commonly used in glycolytic-capacity assays on the Seahorse Bioanalyzer as a negative control. 6-Diazo-5-oxo-L-norleucine is a glutaminase inhibitor and, thus, can be used to test levels of glutamine catabolism. Studies using these inhibitors, in combination with readouts of mitochondrial function, can provide useful information regarding the metabolic status of immune cells.

Other measures of mitochondrial function. Investigation of mitochondrial function is an evolving field. Other measures that can be taken into account when assessing mitochondrial function or dysfunction include Ca^{2+} buffering capacity, Ca^{2+} uptake (73, 74) by mitochondria, mitochondrial biogenesis (75), ATP production (76), generation of mitochondrial ROS (77), mitochondria-specific autophagy (mitophagy) (78), activity of the respiratory enzyme complexes (79), and changes in mitochondrial membrane potential (80).

Conclusions

Mitochondrial function and metabolism in immune cells are critical to the pathogenesis of HIV disease (including the SNAEs that underlie much of the morbidity in cART-treated cohorts) and many other conditions. Much has been learned in this area in recent years, but there is much still to learn. Unraveling the precise mechanisms and modes of action of metabolic pathways will be a critical step in developing novel therapeutics to improve immune activation and function and in opening new opportunities to treat chronic inflammatory diseases.

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