The Journal of Immunology



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*J Immunol* published online 3 November 2014
http://www.jimmunol.org/content/early/2014/11/01/jimmunol.1303092



*The Journal of Immunology* is published twice each month by The American Association of Immunologists, Inc., 9650 Rockville Pike, Bethesda, MD 20814-3994.

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Published November 3, 2014, doi:10.4049/jimmunol.1303092



# Glucose Transporter 1–Expressing Proinflammatory Monocytes Are Elevated in Combination Antiretroviral Therapy–Treated and Untreated HIV$^+$ Subjects

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**Monocyte activation during HIV-1 infection is associated with increased plasma levels of inflammatory markers and increased risk for premature development of age-related diseases. Because activated monocytes primarily use glucose to support cellular metabolism, we hypothesized that chronic monocyte activation during HIV-1 infection induces a hypermetabolic response with increased glucose uptake. To test this hypothesis, we evaluated glucose transporter 1 (Glut1) expression and glucose uptake by monocyte subpopulations in HIV-seropositive (HIV$^+$) treatment-naive individuals ($n$ = 17), HIV$^+$ individuals on combination antiretroviral therapy with viral loads below detection ($n$ = 11), and HIV-seronegative (HIV$^-$) individuals ($n$ = 16). Surface expression of Glut1 and cellular uptake of the fluorescent glucose analog 2-($N$-(7-nitrobenz-2-oxa-1, 3-diazol-4-yl) amino)-2 deoxyglucose were analyzed by flow cytometry on monocyte subpopulations. Irrespective of treatment status, monocytes from HIV$^+$ persons had significantly increased surface expression of Glut1 compared with those from HIV$^-$ controls. Nonclassical (CD14$^+$CD16$^{++}$) and intermediate (CD14$^{++}$CD16$^+$) monocyte subpopulations showed higher Glut1 expression than did classical (CD14$^{++}$CD16$^-$) monocytes. Intermediate monocytes from treatment-naive HIV$^+$ individuals also showed increased uptake of 2-($N$-(7-nitrobenz-2-oxa-1, 3-diazol-4-yl) amino)-2 deoxyglucose compared with those from HIV$^-$ controls. Our results show that HIV infection is associated with increased glucose metabolism in monocytes and that Glut1 expression by proinflammatory monocytes is a potential marker of inflammation in HIV-infected subjects. However, the possibility exists whereby other Gluts such as Glut3 and Glut4 may also support the influx of glucose into activated and inflammatory monocyte populations.** ***The Journal of Immunology*****, 2014, 193: 000–000.**

The advent of combination antiretroviral therapy (cART) has greatly reduced the morbidity and mortality associated with HIV infection. Yet, HIV-seropositive (HIV$^+$) persons treated with cART continue to have shorter life spans than age-matched, HIV-uninfected controls (1). Decreased life span is believed to be due, in part, to chronic immune activation that does not completely resolve during cART (2) and that consequently leads to heightened risk for and early development of age-related diseases (3).

Chronic immune activation during HIV infection occurs, in part, from the increased translocation of microbial products across the epithelial lining of the lamina propria into the systemic circulation (4). The loss of integrity of the mucosal barrier of the gastrointestinal tract results from depletion of CD4$^+$ T cells (5), allowing plasma LPS to become elevated during HIV infection regardless of cART (6). Chronic low-level endotoxemia causes sustained activation of monocytes and production of proinflammatory cytokines associated with age-related diseases (7–9). Currently, no proven treatment limits immune activation in HIV$^+$ persons treated with cART.

Human monocytes consist of three subpopulations that can be identified by differential expression of the cell-surface markers, CD14 and CD16 (10). Classical monocytes express high levels of CD14 but lack CD16 (CD14$^{++}$CD16$^-$); intermediate monocytes express high levels of CD14 and variable levels of CD16 (CD14$^{++}$CD16$^+$); and nonclassical monocytes express low levels of CD14 and high levels of CD16 (CD14$^+$CD16$^{++}$). Nonclassical and intermediate monocytes are collectively described as CD16$^+$ monocytes. Compared with classical monocytes, CD16$^+$ monocytes secrete higher levels of proinflammatory cytokines and are

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Received for publication November 18, 2013. Accepted for publication September 29, 2014.

This work was supported by the Australian Centre for HIV and Hepatitis Virology Research; a 2010 developmental grant (Creative and Novel Ideas in HIV Research) from the University of Washington Center for AIDS Research; a National Institutes of Health–funded program under Award AI027757, which is supported by the National Institute of Allergy and Infectious Diseases, the National Cancer Institute, the National Institute of Mental Health, the National Institute on Drug Abuse, the National Institute of Child Health and Human Development, the National Heart, Lung, and Blood Institute, and the National Institute on Aging; Creative and Novel Ideas in HIV Research and the Australian Centre for HIV and Hepatitis Virology Research (to C.S.P); and a National Health and Medical Research Council of Australia Principal Research Fellowship (to S.M.C.).

C.S.P. conceived the project; C.S.P. and S.M.C. designed the study and analyzed data; C.S.P. and J.Z. performed the experiments; C.S.P. and M.G. analyzed data; J.J.A. designed experiments and analyzed data; C.S.P. and J.J.A. wrote the manuscript; S.M.C., A.J., A.L., and J.M.M. interpreted data, provided critical insight, and revised the manuscript.

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The online version of this article contains supplemental material.

Abbreviations used in this article: cART, combination antiretroviral therapy; Glut, glucose transporter; HDL, high-density lipoprotein; HIV$^-$, HIV-seronegative; HIV$^+$, HIV-seropositive; MFI, mean fluorescence intensity; 2-NBDG, 2-($N$-(7-nitrobenz-2-oxa-1, 3-diazol-4-yl) amino)-2-deoxyglucose.

more proficient in presenting Ag (11, 12). Although $CD16^+$ monocytes normally represent ~10% of all monocytes, their numbers increase during inflammatory diseases, including untreated HIV infection (13). Because infection and treatment with M-CSF can result in an increase in the proportion of intermediate monocytes followed by a sequential increase in nonclassical monocytes, intermediate monocytes are thought to represent a transitional phenotype during maturation of classical monocytes (14, 15).

Mononuclear phagocytes primarily derive their energy from glucose and can, when activated, meet their energy demands by upregulating expression of glucose transporters (Gluts) (16, 17). The insulin-responsive transporters Glut3 and Glut4 are expressed on monocytes and play a role in maintaining the basic metabolic functions of these cells (18–20). Glut1 is less responsive to insulin than Glut4 (18, 20) and is expressed at low levels on unstimulated monocytes but is induced in vitro in response to stimulation by mitogens or LPS (21, 22). Given the chronic inflammatory state that exists in $HIV^+$ subjects, we hypothesized that Glut1 would be unregulated on monocytes in response to inflammatory signals. We further speculated that the inflammatory monocyte subpopulations would be enriched with Glut-expressing cells and reflect the levels of systemic inflammation in $HIV^+$ subjects. To our knowledge, the effect of HIV infection on glucose metabolic activity in monocyte subpopulations has not been investigated.

In this study, we examined the effect of HIV infection on the expression of Glut1 and the uptake of glucose by classical, intermediate, and nonclassical monocytes. We show that, irrespective of cART treatment, HIV infection increases the frequency of Glut1-expressing proinflammatory $CD16^+$ monocytes and causes a marked increase of glucose uptake by intermediate monocytes. These results suggest that HIV infection increases glucose metabolic activity in monocytes.

## Materials and Methods

### *Participant recruitment and blood separation*

The study population included 17 untreated $HIV^+$ individuals ($HIV^+$/naive), 11 $HIV^+$ individuals treated with cART with virologic suppression <50 HIV RNA copies/ml ($HIV^+$/cART), and 16 HIV-seronegative ($HIV^-$) controls. Study participants were recruited from the Infectious Diseases Unit at The Alfred Hospital in Melbourne, VIC, Australia, and from the local community. Informed consent was obtained from all participants, and the research was approved by The Alfred Hospital Research and Ethics Committee. Fresh blood samples from study participants were collected in EDTA anticoagulant tubes for blood chemistry analysis or in citrate anticoagulant tubes for immunophenotyping and metabolic analysis. Mostly male subjects were recruited because $HIV^+$ persons in Australia are predominantly of this sex (>90%). The exclusion criteria included coinfection with HCV, vaccinations <3 wk before sampling, and physical trauma or surgery within 3 wk before participation. Because of sample limitations for some experiments, a representative sample of the study group was selected in which the CD4 T cell counts, viral load, sex, and age were similar and not statistically different from the study group.

### *Phenotyping*

Blood samples collected into anticoagulant were processed within 1 h of collection. Blood was mixed with 20 vol of 1× FACS lysing solution (BD Biosciences, North Ryde, NSW, Australia), incubated on ice for 10 min to lyse RBCs, and washed twice at 4°C with FACS wash (0.5% BSA in 1× calcium- and magnesium-free PBS; Invitrogen, Mount Waverley, VIC, Australia). The remaining WBCs were incubated on ice for 30 min in the dark with the following pretitrated Abs: Glut1-FITC/allophycocyanin (R&D Systems), CD3-allophycocyanin, CD4-PerCP, CD8-PE, CD14-allophycocyanin, CD16-PE-Cy7, CD38-PE, and HLA-DR–FITC (BD Biosciences). In parallel, samples stained with appropriate conjugated Ig isotype control Abs were used to set gates to define positive immunofluorescence. Cells were washed twice in FACS wash and fixed in 0.5% formaldehyde before analysis. Analysis was performed using a FACSCalibur flow cytometer (BD Biosciences). At least 200,000 events were acquired within the monocyte gates and analyzed using FlowJo software, version 8.8 (Tree Star, Ashland, OR).

### *Intracellular Glut1 detection*

Intracellular Glut1 ($Glut1_{c\text{-}term}$) was detected as previously described (23), except that the permeabilizing component A of the Intra Stain kit (Dako) was diluted 1:1 with 1× PBS before use, and the secondary Ab was DyLight conjugated.

### *Plasma cytokine and biochemical analysis*

Plasma cytokine levels were quantified using a high-sensitivity Milliplex cytokine panel (Millipore, Macquarie Park, NSW, Australia) on a Luminex 100 instrument. 25-Hydroxy vitamin D was assayed using the IDS iSYS assay (Immunodiagnostic Systems, Scottsdale, AZ), according to the manufacturer's recommendations. Serum insulin, high-sensitivity C-reactive protein, and D-dimer levels were quantified using immunoturbidimetric assays (Kamiya Biomedical Company, Seattle, WA) and analyzed on a Cobas Mira instrument. Fasting glucose, triglyceride, and total and high-density lipoprotein (HDL) cholesterol levels were measured using a Beckman DXi 600 instrument. All cytokine and biochemical analyses were conducted by Cardinal Bio-Research.

### *In vitro activation of monocyte subpopulations*

For monocyte purification, human PBMCs were obtained from buffy coats (purchased from the Australian Red Cross Blood Service, Southbank, VIC, Australia) using Ficoll-Paque density gradient centrifugation. Monocytes were isolated by countercurrent elutriation using a Beckman J-6M/E centrifuge equipped with a JE 5.0 rotor. Preparations were 90–92% pure with viability of >98% determined by flow cytometry and trypan blue staining, respectively. Total monocytes were incubated with CD16 and CD14 Abs (described earlier) to identify monocyte subpopulations, resuspended in 1×PBS buffer, and sorted with a FACSAria flow cytometer. Purified subpopulations were suspended at a concentration of $1 \times 10^6$ cells/ml, from which 200 μl was placed in 5-ml polypropylene FACS tubes. Cells were left untreated or stimulated with 10 ng/ml LPS from *E. coli* 0111:B4 (Sigma-Aldrich) and 5 ng/ml IFN-γ (R&D Systems) at 37°C for 24 h in RPMI 1640 supplemented with 10% human serum, penicillin/streptomycin (Invitrogen), and 2 mM L-glutamine (Invitrogen). Stimulations were stopped by adding 20 vol ice-cold FACS wash, and cells were pelleted by centrifugation. Cell-surface staining was conducted as described earlier. All laboratory glassware, plasticware, and reagents used in this study were endotoxin free.

### *Glucose uptake in cell culture medium*

Glucose concentrations in cell culture medium were measured using a GlucMeter (CESCO Bioengineering).

### *2-(*N*-(7-nitrobenz-2-oxa-1, 3-diazol-4-yl) amino)-2 deoxyglucose uptake assay*

The fluorescently labeled glucose analog, 2-(*N*-(7-nitrobenz-2-oxa-1, 3-diazol-4-yl) amino)-2-deoxyglucose (2-NBDG; Invitrogen), was used to measure glucose transport in monocytes. We developed a whole-blood glucose uptake assay ("Gluco-flow assay") to measure glucose uptake in patients' blood samples. In brief, cells were incubated for the indicated periods with 15 μM 2-NBDG, and RBCs were lysed as described earlier, washed once with PBS, stained for cell-surface markers, washed and resuspended in ice-cold PBS, kept on ice, and analyzed within 15 min using a FACSCalibur.

### *L-Lactate measurement*

Secreted L-lactate concentrations in cell-free culture supernatants were determined at room temperature using the Glycolysis Cell-Based Assay Kit, according to the manufacturer's instructions (Cayman Chemical).

### *Statistical analysis*

The nonparametric Mann–Whitney *U* test was used for comparison tests of unpaired data, and the Wilcoxon matched pairs signed rank test was used for paired data. Measures of central tendency are expressed as median and interquartile range (25th, 75th percentile). Linear regression was applied to assess the relation between Glut1 expression on monocyte subpopulations and other covariates. Markers with a significant value <0.1 in univariate analyses were entered in a multivariate linear regression model, and the final model was derived through a process of backward elimination. The *p* values <0.05 were considered significant. All statistical analyses were performed using GraphPad Prism (version 6.0; GraphPad Software) or Stata (version 11).

Table I. Clinical characteristics of study groups

| Variables | *N* | Groups | | | *p* | | |
|---|---|---|---|---|---|---|---|
| | | HIV$^-$ (A) | HIV$^+$/Naive (B) | HIV$^+$/cART (C) | A versus B | B versus C | A versus C |
| Sex (M/F) | 43 | 14/2 | 16/1 | 11/0 | — | — | — |
| Median BMI (IQR), kg/m$^2$ | 30 | 22.3 (20.2–25.1) | 24.7 (21.9–26.7) | 25.4 (20.6–29.0) | 0.18 | 0.67 | 0.24 |
| Age ± SD, y | 37 | 52.6 ± 24.0 | 43.6 ± 9.2 | 51.50 ± 10.1 | 0.51 | 0.06 | 0.80 |
| MSM (self-reported), *n* | 25 | 6 | 9 | 10 | — | — | — |
| Median CD4$^+$ T cell count (IQR), cells/μl | 20 | — | 425 (400–637) | 666 (363–710) | — | 0.20 | — |
| Median nadir CD4$^+$ T cell count (IQR), cells/μl | 10 | — | — | 389 (77–550) | — | — | — |
| Time on cART ± SD, y | 10 | — | — | 9.3 ± 5.7 | — | — | — |
| Median % CD3$^+$CD4$^+$ T cells (IQR) | 43 | 58.7 (49–64) | 27.2 (17.1–36.1) | 40.3 (27.3–47.6) | **<0.0001** | **0.03** | **0.0008** |
| Median CD4/CD8 ratio (IQR) | 42 | 1.7 (1.2–2.1) | 0.43 (0.20–0.72) | 0.81 (0.45–1.04) | **<0.0001** | **0.03** | **0.001** |
| Median viral load (IQR), copies/ml | 22 | — | 58,800 (39,500–87,000) | <50 | — | — | — |
| Median plasma glucose (IQR), mmol/L | 29 | 4.7 (4.5–5.5) | 4.7 (4.6–4.9) | 4.8 (4.6–6.8) | 0.84 | 0.24 | 0.37 |
| Median plasma insulin (IQR), U/ml | 30 | 8.8 (4.7–22.3) | 4.7 (3.1–7.4) | 5.5 (2.2–34.3) | 0.09 | 0.90 | 0.48 |
| Median plasma triglyceride (IQR), mmol/L | 29 | 0.90 (0.5–1.6) | 1.0 (0.80–1.6) | 1.6 (0.85–2.12) | 0.50 | 0.33 | 0.16 |
| Median plasma HDL cholesterol (IQR), mmol/L | 29 | 1.4 (1.2–1.8) | 0.70 (0.6–1.1) | 0.80 (0.63–0.97) | **0.001** | 0.95 | **0.0002** |
| Median plasma cholesterol (IQR), mmol/L | 29 | 4.9 (3.2–5.8) | 4.2 (3.2–4.8) | 3.2 (2.8–3.8) | 0.36 | **0.027** | 0.07 |
| Median plasma hsCRP (IQR), mg/L | 30 | 0.95 (0.5–2.7) | 1.3 (0.6–2.6) | 0.90 (0.5–2.9) | 0.40 | 0.65 | 0.80 |
| Median plasma D-dimer (IQR), μg/ml | 28 | 0.64 (0.5–1.2) | 1.1 (0.7–1.6) | 0.7 (0.2–1.1) | 0.24 | 0.20 | 0.38 |
| Median plasma vitamin D (IQR), nmol/L | 29 | 46 (25–60) | 79 (47–92) | 37 (34–102) | 0.09 | 0.40 | 0.84 |
| Median plasma TNF (IQR), pg/ml | 30 | 4.4 (3.0–7.5) | 9.7 (6.6–15.7) | 8.7 (5.7–12.9) | **0.01** | 0.50 | 0.06 |

The nonparametric Mann–Whitney *t* test was used to evaluate significant difference between each group. The bold values are of statistical significance, $p < 0.05$.
BMI, body mass index; hsCRP, high-sensitivity C-reactive protein; IQR, interquartile range; MSM, men who have sex with men.

## Results

### *Subject clinical characteristics*

We examined blood monocytes and laboratory biomarkers from 17 HIV$^+$/naive, 11 HIV$^+$/cART, and 16 HIV$^-$ individuals (Table I). The percentage of CD4$^+$ T cells and the CD4/CD8 ratio from HIV-infected individuals were significantly lower than analogous measures found in HIV$^-$ individuals. Compared with HIV$^+$/cART individuals, HIV$^+$/naive individuals had a lower percentage of CD4$^+$ T cells and lower CD4/CD8 ratio. TNF plasma levels were significantly greater in HIV$^+$/naive individuals compared with HIV$^-$ individuals, as shown previously (24). Plasma HDL-cholesterol levels were significantly lower in blood from HIV$^+$ individuals compared with HIV$^-$ individuals, irrespective of cART treatment, also consistent with previous observations (25). Total cholesterol levels were significantly lower in blood from for HIV$^+$/cART individuals when compared with HIV$^+$/naïve individuals. All other clinical characteristics were similar between subject groups.

### *HIV infection is associated with an increased percentage of circulating CD14$^+$ monocytes expressing Glut1*

HIV infection has been associated with activation of monocytes in vivo (26), whereas in vitro, monocyte activation has been observed to increase Glut1 expression (21, 22). We accordingly sought to determine whether the frequency of monocytes expressing Glut1 in vivo is increased in HIV-infected individuals. Monocytes in whole blood were first gated using light scatter properties and then based on CD14 and Glut1 expression using flow cytometry (Fig. 1A). Because of the low percentage of Glut1-expressing monocytes in HIV$^-$ individuals, 200,000–500,000 events within the monocyte gates were acquired. Monocytes from HIV$^+$ persons showed a significant increase in the percentage expressing cell-surface Glut1 compared with monocytes from HIV$^-$ individuals, irrespective of treatment status (Fig. 1B, *left panel*). The levels of cell-surface Glut1 on the total monocyte population were significantly higher in HIV$^+$/cART persons relative to HIV- persons, and there was a trend toward greater Glut1 levels on monocytes from HIV$^+$/naive individuals compared with those from HIV$^-$ individuals (Fig. 1C, *left panel*). We confirmed that metabolic activation of monocytes as measured by cell-surface Glut1 expression was elevated in HIV-infected compared with uninfected persons using a different Ab that recognizes an intracellular C-terminal epitope of Glut1 (Fig. 1C, *right panel*). In contrast with extracellular Glut1, >98% of monocytes from all subjects stained using the C-terminal Ab (Fig. 1B, *right panel*, and Supplemental Fig. 1). Because the percentage of monocytes staining for extracellular Glut1 was substantially lower than staining with the intracellular epitope (compare Fig. 1B, *left* and *right panels*), these data indicate that the majority of Glut1 is sequestered within monocytes. Measurement of Glut1 mRNA from Glut1$^+$ and Glut1$^-$ monocytes from HIV$^+$/cART subjects isolated by FACS showed higher Glut1 mRNA expression in Glut1$^+$ monocytes compared with Glut1$^-$ monocytes (Supplemental Fig. 2), validating the Glut1 staining procedure by flow cytometry. We next compared the activation state of Glut1$^+$ and Glut1$^-$ monocytes for all subject groups. Within each subject group, Glut1$^+$ monocytes coexpressed higher levels of the activation marker CD38 compared with Glut1$^-$ monocytes (Fig. 1D).

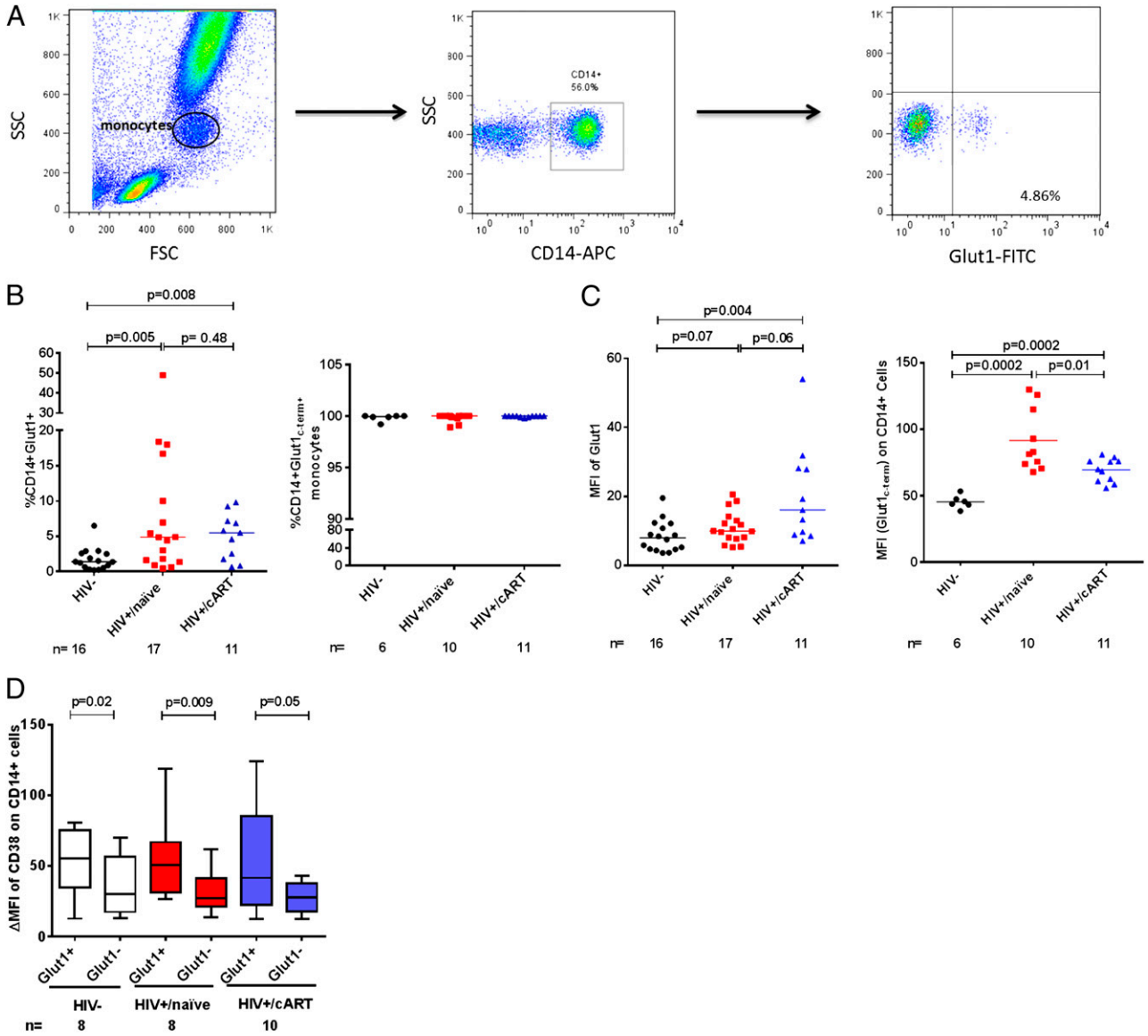


**FIGURE 1.** Monocytes expressing Glut1 are activated and their frequency is increased during HIV infection. Samples of whole blood were analyzed by flow cytometry for Glut1 expression on monocytes within 1 h of collection. (**A**) Monocytes were gated based on forward and side scatter characteristics and expression of CD14, and then examined for expression of Glut1. The dot plot is from a representative HIV$^+$/naive donor. (**B**) Monocytes from HIV$^-$, HIV$^+$/naive, and HIV$^+$/cART individuals were gated to determine the percentage of monocytes expressing Glut1 on their cell surface (*left panel*) and percentage of monocytes expressing intracellular Glut1 (Glut1$_{c\text{-term}}$, *right panel*). (**C**) MFI of cell-surface Glut1 (*left panel*) and intracellular Glut1 (Glut1$_{c\text{-term}}$, *right panel*) on monocytes from HIV$^-$, HIV$^+$/naive, and HIV$^+$/cART individuals. (**D**) Expression of the activation marker CD38 was measured for gated Glut1$^+$ and Glut1$^-$ monocytes for each subject group. The Mann–Whitney *t* test was used to evaluate significant differences between each group, and the Wilcoxon matched-pairs signed rank *t* test was used to analyze changes between paired data. Horizontal lines within histograms represent median value, and whiskers represent minimum and maximum values.

### *Frequencies of Glut1$^+$ cells are higher in the nonclassical and intermediate subpopulations*

Inflammatory monocyte subpopulations are proportionally increased during HIV infection and have been associated with markers of immune activation that may increase cardiovascular risk (26, 27). Given these previous findings, we used flow cytometry to measure Glut1 expression on nonclassical (CD14$^+$CD16$^{++}$), intermediate (CD14$^{++}$CD16$^+$), and classical monocyte (CD14$^{++}$ CD16$^-$) subpopulations. These monocyte subpopulations were discerned using an established gating strategy in which monocytes are gated by forward and side scatter properties, and then examined for the expression of CD14 and/or CD16 (Fig. 2A and Supplemental Fig. 3) (28). Within each subject group, nonclassical monocytes showed the highest percentage and mean fluorescence intensity (MFI) of Glut1 expression, followed by intermediate monocytes, and then classical monocytes (Fig. 2B, 2C). HIV$^+$/naive and HIV$^+$/cART individuals showed a significantly greater percentage of Glut1-expressing intermediate monocytes compared with HIV$^-$ individuals. The percentage of Glut1-expressing nonclassical monocytes was significantly greater in the HIV$^+$/naive subjects compared with HIV$^-$ individuals (Fig. 2B).

### *HIV infection increases glucose uptake in intermediate monocytes*

Glucose is a major energy source for monocytes during times of cellular activation (16), and Glut1 expression is upregulated during these times (21, 22). Because Glut1 was expressed at different levels on individual monocyte subpopulations, we sought to assess

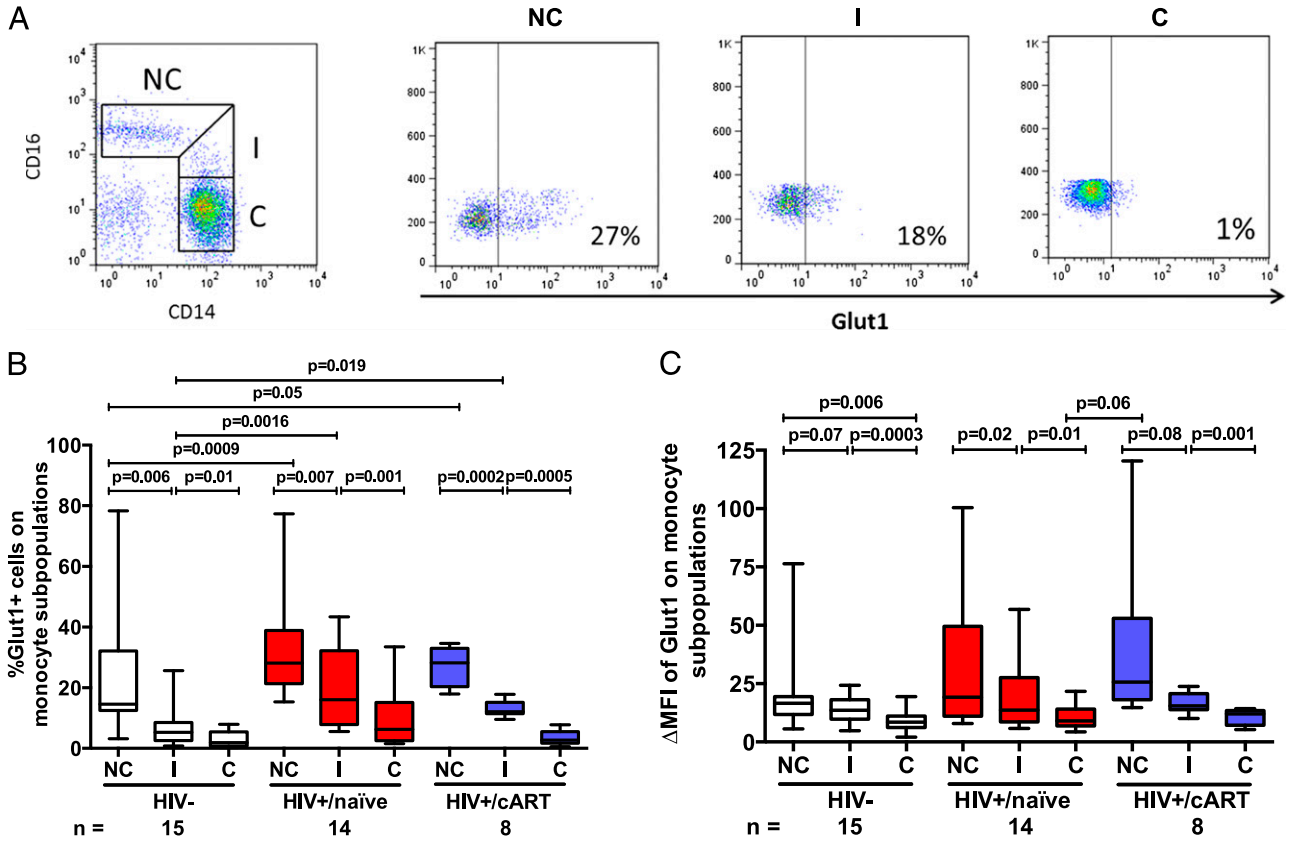


**FIGURE 2.** CD16-expressing proinflammatory monocytes are increased during HIV infection. (**A**) Monocytes were gated on scatter properties as described in Fig. 1, and monocyte subpopulations (classical [C], intermediate [I], and nonclassical [NC]) were defined based on expression of CD14 and CD16. Monocyte subpopulations were then examined for Glut1 expression, shown on the *x*-axis against the side scatter on the *y*-axis. Dot plots are from a representative HIV$^+$ donor. Monocytes from HIV$^-$, HIV$^+$/naive, and HIV$^+$/cART individuals were examined for (**B**) the percentage of each monocyte population expressing Glut1 and (**C**) the Glut1 MFI of Glut1 on each monocyte population.

the level of glucose uptake for each monocyte population. Whole blood was incubated with the fluorescent glucose analog 2-NBDG, and its uptake by monocytes was measured by flow cytometry. The kinetics of 2-NBDG uptake were measured in monocytes from HIV$^-$ individuals who had been incubated with 2-NBDG for periods as long as 120 min (Fig. 3A). Given these results, we chose to incubate monocytes from each subject group with 2-NBDG for 15 min (a time at which there was ~50% uptake) to determine the uptake for each monocyte population (Fig. 3B). Surprisingly, within the HIV-infected group, intermediate monocytes showed significantly greater levels of 2-NBDG uptake than did classical and nonclassical monocytes. Expression of the monocyte activation marker HLA-DR was also significantly higher on intermediate monocytes compared with classical monocytes (Fig. 3C), consistent with previous observations (29). Intermediate monocytes from HIV$^+$/naive and HIV$^+$/cART individuals showed significantly greater 2-NBDG uptake compared with intermediate monocytes from HIV$^-$ individuals. These data show that activation of monocytes during HIV infection is associated with increased uptake of glucose, with intermediate monocytes accounting for the highest per-cell level of glucose uptake.

### *Monocyte activation is associated with increased glucose uptake*

The identification of increased percentages of Glut1-expressing proinflammatory monocytes in HIV$^+$ individuals, together with the observation that these monocyte subpopulations express high levels of HLA-DR, prompted us to examine whether monocyte activation results in increased uptake of glucose and production of TNF. Microbial translocation of LPS and other microbial products across the gut mucosa occurs during HIV infection and, together with HIV viremia, is a source of chronic monocyte activation (4). We therefore assessed whether in vitro LPS stimulation of monocyte subpopulations from HIV$^-$ persons causes increased uptake of glucose. Because monocyte stimulation by LPS is optimal in the presence of IFN-γ (30, 31), monocyte subpopulations were purified by FACS and then incubated in the presence or absence of both LPS and IFN-γ. After 24 h, activated monocytes were then incubated with 2-NBDG (Fig. 4A). Within each monocyte subpopulation, LPS- and IFN-γ–stimulated monocytes showed significantly increased 2-NBDG uptake compared with unstimulated monocytes. We next determined whether stimulation of monocyte subpopulations with LPS and IFN-γ resulted in increased uptake of glucose in the cell culture medium (Fig. 4B). Consistent with the results using 2-NBDG, stimulated cells within each monocyte subpopulation showed greater levels of glucose uptake compared with unstimulated monocytes. When total monocytes from HIV-uninfected individuals were stimulated with LPS and IFN-γ, Glut$^+$ monocytes showed increased expression of TNF compared with Glut1$^-$ cells (Supplemental Fig. 4).

### *Monocyte activation is associated with increased glycolytic metabolism*

During activation, mononuclear phagocytes have increased levels of aerobic glycolytic metabolism, resulting in the generation and secretion of high levels of L-lactate (16). To determine whether glucose metabolism is increased during monocyte activation, we

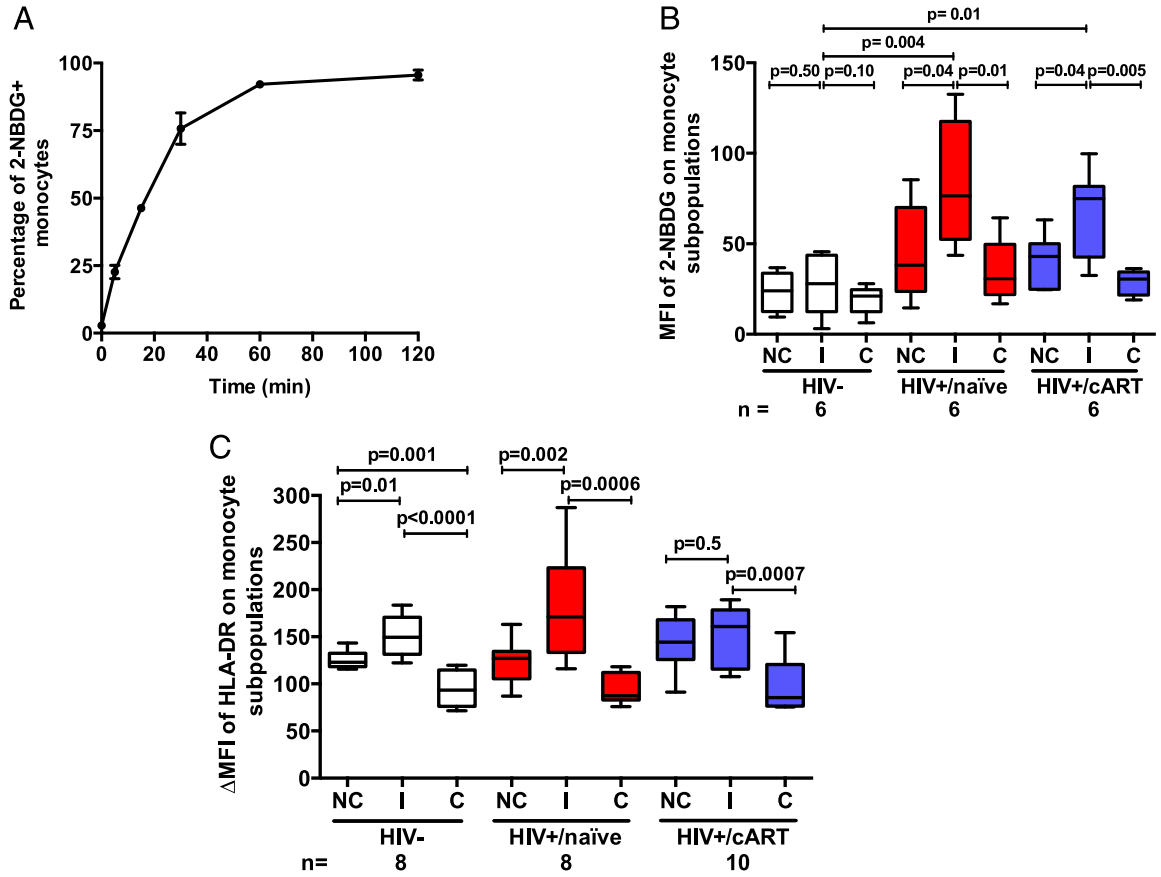


**FIGURE 3.** Intermediate monocytes from HIV$^+$ individuals show high levels of 2-NBDG uptake. (**A**) Monocytes from HIV$^-$ individuals ($n$ = 2) were incubated with the fluorescent glucose analog 2-NBDG (15 μM) up to 120 min. After gating for scatter properties and CD14, the percentage of monocytes that had taken up 2-NBDG was assessed by flow cytometry. (**B**) Monocyte subpopulations from HIV$^-$, HIV$^+$/naive, and HIV$^+$/cART individuals were incubated with 2-NBDG for 15 min and then gated for scatter properties and expression of CD14 and CD16. (**C**) Monocyte subpopulations from HIV$^-$, HIV$^+$/naive, and HIV$^+$/cART individuals were examined for HLA-DR expression by flow cytometry.

assessed L-lactate levels appearing in culture medium for stimulated and unstimulated monocyte subpopulations. The levels of L-lactate in culture medium were significantly increased from basal levels in all of the monocyte subpopulations (Fig. 4C). Although not significant, there was a trend toward higher net levels of L-lactate secretion by intermediate monocytes in response to LPS and IFN-γ stimulation when compared with the other subpopulations (Fig. 4D). Taken together, these results show that intermediate monocytes take up more glucose and are more likely to have greater rates of aerobic glycolysis than nonclassical and classical monocytes.

## Discussion

During HIV infection, chronic innate immune activation with expansion of proinflammatory monocyte subpopulations and related cytokine production is only partially returned to normal levels upon the initiation of cART (2, 28, 32–34). In the studies reported in this article, we show that, irrespective of cART, there is also an increase in the percentage of monocytes expressing Glut1 in HIV$^+$ individuals when compared with monocytes from HIV$^-$ individuals. The increased levels of intracellular Glut1 during HIV infection suggest that Glut1 expression is regulated at the transcriptional, translational, or posttranslational level. Glut1 expression and glucose uptake by CD16$^+$ inflammatory monocytes were both greater than that of classical monocytes. Given that CD16$^+$ monocytes express high levels of Glut1 and L-lactate production, we speculate that increased glycolytic activity by proinflammatory monocytes is associated with the production of proinflammatory molecules. Consistent with this hypothesis, we observed significantly higher glucose uptake in monocytes activated with LPS and IFN-γ.

Per our observations, Glut1 expression (as measured by MFI) was higher in intermediate and nonclassical monocytes than in classical monocytes. Consistent with the high Glut1 expression level, intermediate monocytes showed greater levels of 2-NBDG uptake compared with classical monocytes. Surprisingly, we found no difference in 2-NBDG uptake by classical and nonclassical monocytes, even though both the frequency of Glut1-expressing nonclassical monocytes and expression of Glut1 by nonclassical monocytes were significantly greater than classical monocytes. Although we observed an overall increase in Glut1 expression in monocytes from HIV-infected individuals, we cannot rule out the possibility that other Gluts, such as Glut3 and Glut4 that can be expressed on monocytes (19, 20), might also be regulated during HIV infection. This possibility is supported by the discrepancy between Glut1 expression and 2-NBDG uptake within specific subsets of monocytes. One plausible explanation for this discrepancy is that the increased expression of Glut1 on nonclassical monocytes coincided with downregulation of one or more other Gluts, resulting in reduced glucose uptake compared with intermediate monocytes. It is also possible that other Gluts may be preferentially upregulated on the classical and intermediate monocyte subpopulations during HIV infection. The former explanation is more likely because Glut1 is a major Glut on the hematopoietic cell lineage and is predominantly regulated by inflammatory signals.

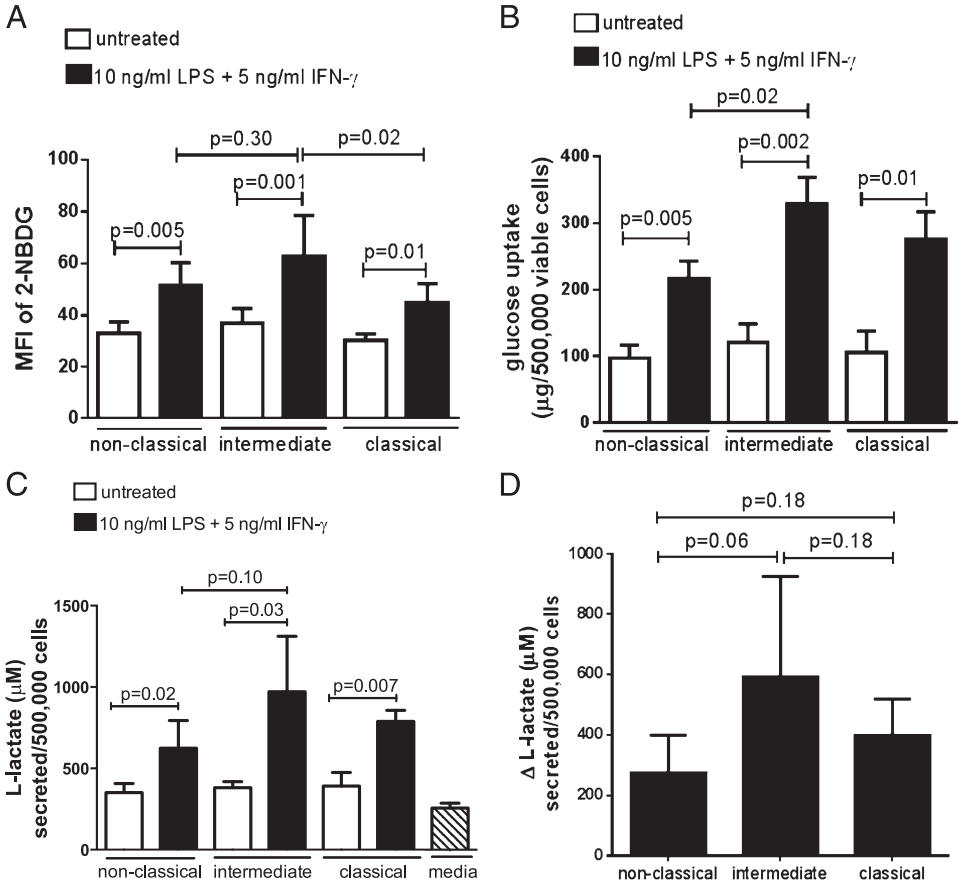


**FIGURE 4.** Monocyte stimulation increases glucose uptake and L-lactate production. Monocyte subpopulations from HIV$^-$ individuals were isolated by FACS and incubated without or with LPS (10 ng/ml) and IFN-γ (5 ng/ml) for 24 h ($n$ = 4 independent experiments). (**A**) To analyze acute glucose uptake by flow cytometry, we incubated monocytes for 15 min with 2-NBDG. (**B**) A GlucMeter was used to measure the remaining glucose in cell-free culture filtrates from cells cultured for 24 h in RPMI 1640 medium. (**C**) Monocytes subpopulations from the same subjects in (B) were incubated without or with LPS (10 ng/ml) and IFN-γ (5 ng/ml) for 24 h ($n$ = 4 independent experiments), cell culture medium was from each condition, and L-lactate concentrations were measured at room temperature. (**D**) Net L-lactate production is highest in activated intermediate monocytes. Experimental data from the same subjects and treatment conditions in (C) were used to calculate the net production of L-lactate by different subpopulations of activated monocytes.

We recently determined that the percentage of CD4$^+$ T cells expressing Glut1 was partially normalized by cART in HIV$^+$ individuals compared with untreated HIV$^+$ individuals (23), consistent with observations that CD4$^+$ T cell activation is partially normalized during cART treatment (35). In this study, by contrast, we found that the percentage of Glut1-expressing total monocytes was similar for HIV$^+$ persons, regardless of whether they were treated with cART. This observation agrees with previously reported findings that markers of innate immune activation can remain elevated after the initiation of cART (26, 36–38). Although HIV infection caused upregulation of cell-surface Glut1 expression on monocytes in this study and on CD4$^+$ T cells in our previous study (23), Glut1 expression on CD8$^+$ T cells (23) and on NK cells (C.S. Palmer, unpublished observations) is unchanged during HIV infection. Thus, Glut1 regulation in the context of HIV infection may vary between immune cells.

In this study, we show that intermediate monocytes have high levels of glycolytic activity and HLA-DR expression. Expansion of intermediate monocytes is correlated with inflammation (39–41), HIV disease progression (42), and clinical cardiovascular events (43). Supporting these reported findings, we observed that Glut1 expression on intermediate monocytes was significantly associated with plasma levels of D-dimer and inversely correlated with plasma HDL-cholesterol levels (C.S. Palmer, S.M. Crowe, and J. Zhou, unpublished observations), both cardiovascular risk factors in HIV$^-$ and HIV$^+$ individuals (44–47). These results are in accord with a recent study by Funderburg et al. (48), who showed a positive correlation between the proportion of intermediate monocytes and D-dimer levels. Our results extend their findings by identifying heightened glucose metabolic activity as a potential mediator of intermediate monocyte activation.

In addition to cardiovascular disease, proinflammatory monocytes are expanded in cancer (49), kidney disease (50), and liver disease (51). An increased risk for each of these age-related diseases is associated with HIV infection. Recently, monocytes from HIV$^+$ persons ≤45 y of age were shown to share characteristics with monocytes from HIV$^-$ persons who were ≥65 y of age (26). Monocytes from these two groups shared characteristics of chronic activation, with HIV viremic persons ≤45 y of age and HIV$^-$ persons ≥65 y of age showing increased percentages of proinflammatory monocytes compared with HIV$^-$ young persons. It is currently unknown whether increased glycolytic activity in proinflammatory monocyte subpopulations is present in people with other inflammatory conditions or in the aged population. Further studies will be required to determine whether increased glucose metabolic activity plays a central role in age-related diseases, and to potentially target this pathway to limit diseases associated with HIV and non–HIV-related inflammatory conditions.

One of the most significant limitations in the management of HIV-infected individuals is the persistently high state of inflammation accompanied by monocyte activation and associated with several non–AIDS-related comorbidities. The mechanisms underlying such monocyte activation during HIV infection are incompletely understood, making it difficult to therapeutically target chronic low-level inflammation in HIV-infected individuals. Several pathways that could modulate glycolytic metabolism in myeloid and lymphoid cell lineages are well established. These include the regulation of Glut1 by PI3K and Akt pathways, and competitive inhibition of hexokinase, a rate-limiting enzyme in glycolysis. Our results support the exploration of antiglycolytic drugs such as 2-deoxy-D-glucose, which was well tolerated in a phase 1 clinical trial (52). Because Glut1 is also necessary for

efficient HIV infection of $CD4^+$ T cells (53), the major cellular target of HIV, antiglycolytic drugs could potentially offer additional protection to $CD4^+$ T cells in addition to limiting monocyte activation.

One limitation of this study is the small sample size. A larger sample size and longitudinal analysis will be required to evaluate the role of Glut1 as a potential marker of inflammation and development of non–AIDS-associated comorbidities. Taken together, our results identify a hypermetabolic response by monocytes, particularly $CD16^+$ proinflammatory subpopulations, during HIV infection. We propose a model in which unactivated proinflammatory monocytes expressing low levels of Glut1 and showing low glucose uptake and metabolism become activated during HIV infection and express high levels of Glut1 and have high glucose uptake and metabolism. The activated $Glut1^+$ proinflammatory monocytes can then express inflammatory mediators that may contribute to serious non-AIDS events. Given our observation of a significant positive association between the levels of Glut1 on inflammatory monocyte subsets and the levels of plasma D-dimer (C.S. Palmer, unpublished observations), it will be of interest to determine in future studies whether Glut1 expression on proinflammatory monocytes identifies individuals at risk for HIV-related comorbidities.

## Acknowledgments

We gratefully acknowledge the contribution to this work by the Victorian Operational Infrastructure Support Program received by the Burnet Institute.

## Disclosures

The authors have no financial conflicts of interest.

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