Monitoring Immune Activation with Whole-Body Fluorodeoxyglucose–Positron-Emission Tomography in Simian Immunodeficiency Virus–Infected Rhesus Macaques

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Sanhita Sinharay,*,† Sharat Srinivasula,‡,† William Schreiber-Stainthorp,* Swati Shah,* Paula Degrange,§ Andrew Bonvillain,‡ Jing Wang,‖ Lori Dodd,‡ Jorge A. Carrasquillo,* Dima A. Hammoud,* and Michele Di Mascio‡

*Center for Infectious Disease Imaging, Radiology and Imaging Sciences, Clinical Center, National Institutes of Health, Bethesda, MD; †AIDS Imaging Research Section, Clinical Monitoring Research Program Directorate, AIDS Imaging Research Section, Frederick National Laboratory for Cancer Research, Frederick, MD; ‡AIDS Imaging Research Section, Integrated Research Facility, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Frederick, MD; §Biostatistics Research Branch, Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD; †Molecular Imaging Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD; and ‡AIDS Imaging Research Section, Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

ABSTRACT

This study aimed to assess immune activation in tissues by measuring glucose metabolism with \textsuperscript{18}F-fluorodeoxyglucose (FDG) and investigate the associations of various peripheral markers of disease progression with initiation and interruption of combination antiretroviral therapy in SIV-infected rhesus macaques (Macaca mulatta). Mixed-effect linear models revealed a significant inverse association of peripheral blood CD4\textsuperscript{+} T cell counts (p < 0.01) and a direct association of plasma viral load (p < 0.01) with the FDG uptake in the spleen, bone marrow, and most clusters of lymph nodes. In contrast, no significant associations were found for the liver and the bowel FDG uptake. We also found no association of the fraction of proliferating peripheral blood T and B lymphocytes with FDG uptake in any analyzed tissues. The bowel FDG uptake of uninfected animals was heterogeneous and reached levels as high as those seen in the bowel or the clusters of lymph nodes or the spleen of high viremic SIV-infected animals, suggesting that factors beyond SIV-induced immune activation dominate the gut FDG uptake. ImmunoHorizons, 2021, 5: 557–567.

INTRODUCTION

Despite the inability of combined antiretroviral therapy (cART) to eradicate HIV (HIV-1) from infected patients, the availability of this lifetime treatment has markedly reduced the global burden of HIV-associated disease over the last decade (1). Although modeling studies show the life expectancy of people living with HIV-1 has been slowly approaching that of age-matched HIV-1–negative individuals (2), even with early treatment and access to care, an approximately eight-year life expectancy gap remains between HIV-infected versus -uninfected individuals (3). Both cART treatment and residual viral...
production in treated patients contribute to poorer quality of life. Some of the most common comorbidities observed in the treated HIV-1 patients include dyslipidemia (4), cardiovascular disease (5, 6), bone density abnormalities (7), and neurocognitive dysfunction (8). A large body of preclinical and clinical research over the past decade focused on the etiological factors behind these comorbidities, with universally accepted evidence for residual immune activation despite curbing plasma viremia <20 copies/ml in cART-treated suppressed patients (9).

The links between residual immune activation and residual viral replication/persistent CD4\(^+\) T cell depletion, although postulated, have not been consistently shown. Some immunologists suggested, for instance, that persistent immune activation of clinical relevance might not be resolved following viral eradication (10). Indeed, lower CD4\(^+\) T cell counts in the peripheral blood (PB) of HIV-uninfected patients diagnosed with idiopathic CD4 lymphocytopenia are also associated with higher lymphocyte activation and proliferation (11). During HIV-1 infection, two main forces independently contribute to immune activation: inflammatory response to HIV infection and the homeostatic response to CD4\(^+\) T cell depletion (12–14). Because of the collinearity of plasma viremia and PB CD4\(^+\) T cell counts, the latter observation could be only visualized within larger populations of untreated and cART-treated patients with variable ranges of immunovirological set points, as demonstrated in a retrospective analysis of ex vivo BrdU incorporation, a thymidine analogue, in PBMCs (13). The immune activation pathway in HIV-1 infection has been attributed to several direct and indirect causes (15), such as the role of viral proteins like nef in direct lymphocyte activation or through infected macrophages (16, 17), microbial translocation due to loss of epithelial integrity in gut mucosa (18), production of viral proteins by defective proviruses (19), and early cytokine storms following adaptive clonal immune cell expansion (20).

In both HIV and the SIV model, determined primarily through mathematical modeling studies of in vivo labeling using BrdU (21–24) or deuterated glucose (25–27), it was recognized that not only T lymphocytes but also other immune cells, such as NK cells, monocytes and macrophages, neutrophils, and B cells (28, 29), accelerate their turnover. The proliferation rates obtained adopting these two experimental approaches showed similar results and strengths of correlations with the Ki-67 proliferation marker, further corroborating the intimate link between lymphocyte turnover and immune activation in HIV/SIV infection.

Fluorodeoxyglucose (FDG), a glucose analogue used extensively in cancer research, concentrates in neoplastic cells (30), activated macrophages (31), lymphocytes (32), and granulocytes (33). Upon activation of resting T cells, several metabolic pathways are activated that increase energy production to support proliferation and effector functions (34). In this regard, CD28 costimulation leads to enhanced glucose uptake by upregulating Glut-1 levels, the main glucose transporter, by ~20-fold over 24 h (34, 35). Palmer et al. (36) have reported direct associations between increased glucose metabolic activity and PB CD4\(^+\) T cell activation and depletion during chronic HIV infection, suggesting that Glut-1 expression on CD4\(^+\) T cells may have prognostic value in HIV-1 disease.

Most of the knowledge on lymphocyte turnover in lentivirally infected hosts was obtained through PB measurements. However, only <1% of lymphocytes reside in the PB at any time (37), with its steady state being highly sensitive to changes in trafficking rates between the periphery and lymphoid tissue compartments (38), adding biological noise to the measurements of turnover following specific perturbation of the immune system's steady state.

FDG positron-emission tomography (PET) imaging, under specific conditions, can noninvasively provide an in vivo map of immune cell turnover in various organs. Albeit experimentally demanding, FDG-PET has been an attractive platform since the beginning of the HIV epidemic and has been used to interrogate putative links between localized immune activation and disease progression, both in human patients and in SIV-infected nonhuman primates. Some questions that could be potentially addressed with this technology are the following: is the level of immune activation similar among the lymphoid organs in chronically infected hosts? How does the localized immune activation correlate with PB markers of disease progression? Does residual immune activation in treated hosts concentrate in specific anatomic compartments?

A few of these questions were partly answered in preclinical (39, 40) and clinical studies (41–44). Overall, these studies have demonstrated an increase in immune activation levels in specific lymphoid tissues of HIV/SIV-infected hosts and its normalization to levels observed in healthy controls following prolonged cART. However, one enduring weakness of these studies has been a lack of longitudinal imaging following perturbations of the quasi–steady state of the immune system (e.g., initiation or interruption of cART). Moreover, specific anatomic compartments like the spleen, bone marrow, and bowels were not systematically evaluated.

In the current study, we aimed to assess metabolic changes that occur over time in multiple organs, including the spleen, bone marrow, bowels, liver, and axillary, inguinal, submandibular, and mesenteric/retroperitoneal clusters of lymph nodes (LNs) in a group of SIV-infected animals that were administered FDG as part of a simian AIDS brain imaging study (45). We imaged infected animals before and up to a median of six months following initiation or interruption of cART and explored the association of in vivo findings to peripheral markers of SIV disease.

**MATERIALS AND METHODS**

**Animals, SIV infection, and antiretroviral therapy**

Fifteen adult Indian rhesus macaques (Macaca mulatta), negative for Mamu-B008 and -B017, were used in this study following the National Institute of Allergy and Infectious Diseases Animal Care and Use Committee–approved protocols. All

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experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals. At recruitment, eight macaques (two males and six females, age [mean ± SD]: 6.9 ± 1.9 y, weight [mean ± SD]: 7.2 ± 2.5 kg) were SIV infected (four with SIVmac251 and four with SIVE660). The macaques were infected intrarectally with either 1 × 10^3 median tissue culture ID_{50} of SIVmac251 or 1 × 10^3.8 to 1 × 10^4 tissue culture ID_{50} of SIVE660. The remaining seven animals were healthy uninfected controls (two males and five females, age [mean ± SD]: 7.6 ± 3.6 y, weight [mean ± SD]: 5.8 ± 1.1 kg).

The seven uninfected controls and one SIVE660-infected animal were imaged only once. The remaining seven SIV-infected animals were imaged multiple times at different phases of infection (Supplemental Fig. 1). Five untreated, SIV-infected macaques (average ~16 mo [range 14–17.5 mo] of untreated chronic infection), following baseline FDG scans, initiated cART (tenofovir [9-(2-phosphonyl-methoxypropyly)adenine] 20 mg/kg + emtricitabine [2',3'-dideoxy-5-fluoro-3'-thiacytidine] 30 mg/kg s.c. once daily and raltegravir 20 mg/kg/bid mixed in food) and imaged longitudinally on treatment. Three animals from the treatment-initiation group (on cART for >6 mo) and two other long-term, cART-treated, SIV-infected animals (on cART for >18.5 mo), following baseline FDG scans and interrupted therapy, and were imaged longitudinally.

^{18}F-FDG–PET imaging. A total of 45 whole-body ^{18}F-FDG–PET static scans were performed. Twenty-eight scans were acquired on the Siemens Biograph PET/CT scanner (Siemens HealthCare), and 17 scans were acquired using GE Advance scanner (GE Healthcare). Animals, fasted for 12 h before imaging, were anesthetized with a restraint dose of ketamine (10 mg/kg) and propofol (0.2 mg/kg) and then maintained on 1.3–2% isoflurane during the scan. A mean dose of 5 mCi ^{18}F-FDG (range 4.3–5.6 mCi) was administered i.v. as a bolus injection via the leg’s saphenous vein. Approximately 72 min after FDG injection, the animals were placed in the prone position, and static images were acquired at 8 min per field of view at four bed positions to capture the whole-body image from the top of the head to midthighs. Respiration, heart rate, and body temperature were monitored throughout the scan. Raw PET data were reconstructed using the ordered subset expectation maximization algorithm (Siemens PET/CT: 3 iterations and 21 subsets; GE Advance: 4 iterations and 28 subsets). Reconstructed images were corrected for attenuation (using computed tomography [200 mA, 0.5 s/rotation, pitch = 1] for scans acquired on Siemens PET/CT and using rotating rod ^{68}Ge transmission source for scans acquired on GE Advance), radioactive decay, deadtime, normalization, random coincidences, and scattering of radiation. Both scanners were cross-calibrated to within ±2% difference using a phantom with known amounts of ^{18}F.

PET images were saved in DICOM format, and quantitative analysis was performed in PMOD 3.7 (PMOD Technologies, Zurich, Switzerland) and repeated independently and blindly by a second operator with MIM 6.5 (Cleveland, OH). Both operators manually drew volume of interest (VOI) on the PET image to obtain tissue standardized uptake value (SUV), calculated using the following formula: SUV = (c/d)*w, where c is decay-corrected tissue concentration (in microcuries per milliliter), d is the injected dose (in microcuries), and w, the body weight (in grams). Maximum SUV (SUVmax) was extracted for clusters of axillary, inguinal, submandibular, and mesenteric/retroperitoneal LNs, spleen, bone marrow, bowel, and liver and confirmed with CT coregistration when available. For the bone marrow, the VOI was placed on L4–L5 lumbar vertebrae. The bowel uptake was obtained by placing a VOI in the entire abdomen field of view and excluding the uptake in the stomach, liver, kidneys, and isolated regions with high FDG uptake that overlapped with mesenteric or retroperitoneal LNs. Given the heterogeneity, both mean and SUVmax levels were obtained for the bowel.

**Virion load, soluble factors, and cell counts**

**Viral RNA and DNA measurements.** Virion-associated SIV-RNA in plasma was measured using quantitative real-time RT-PCR with a minimum detection threshold of two copies per milliliter as previously described (46). Cell-associated SIV-DNA from PBMCs was quantified using the method previously described (47) with a minimum detection threshold of five copies of SIV-DNA per million PBMCs.

**Soluble factors.** IL-2, IL-8, IL-15, IL-1Ra, and MCP-1 cytokine levels in plasma were quantified using Quantikine kits (R&D Systems and Thermo Fisher Scientific, respectively) and validated for rhesus plasma through linearity and parallelism (48).

**Lymphocyte immunophenotyping.** EDTA-treated fresh blood samples were stained with fluorochrome-conjugated mAbs. Briefly, the four-color staining panel used immunophenotyping mAbs (CD3-FITC [20 µl, clone SP34, no. 556611], CD4–allophycocyanin [5 µl, clone SK3, no. 340443], CD8-PerCP [20 µl, clone SK1, no. 347314], and CD20–PE-Cy7 [5 µl, clone L27, no. 335793]; all BD Biosciences) or isotype control mixture (IgG1–PE-Cy7 [5 µl, clone SK3, no. 340443]) and v-dimer levels in plasma were measured using ELISA kits (R&D Systems and Thermo Fisher Scientific, respectively) and validated for rhesus plasma through linearity and parallelism (48).
Mouse Anti-Ki-67 Set (no. 556027; BD Biosciences) was added to the cells, incubated for 30 min at 4°C in the dark, and washed twice with 1 ml of 1× BD Perm/Wash Solution. Cells were then fixed by resuspending in 300 μl of 2% formaldehyde and analyzed by flow cytometry (FACScanto II; BD Biosciences). Data were analyzed using BD FACSDiva software.

Statistical analysis
Linear mixed-effect univariate models were constructed to investigate the associations of CD4+ T cell counts (cells per microliter), CD8+ T cell counts (cells per microliter), log10-transformed plasma viral load (VL; SIV-RNA copies per milliliter), log10-transformed fraction of proliferating Ki-67+ cells, log10-transformed cell-associated SIV-DNA (copies per 1×10^6 cells or copies per milliliter), and soluble factors (in picograms per milliliter) with FDG uptake (SUVmax) in tissues. Multivariate mixed models were constructed to determine the statistically significant biomarkers that are more predictive of SUV changes. Linear mixed-effect regression was used for comparison of quasi–steady state scans. All statistical analyses were performed in R software. Because of multiple comparisons, a p value of <0.01 was considered statistically significant.

Because of brown adipose tissue activation resulting in high uptake of [18F]-FDG in the neck and axilla, one PET scan resulted in uncertainty in only axillary LN SUV assessment and was thus excluded from axillary LN analysis. Because of the complexity involved in identifying and delineating abdominal LNs, mesenteric/retroperitoneal LN SUV data were evaluated in a subgroup of 31 PET scans.

RESULTS

FDG uptake in tissues: quasi–steady state
We first explored the differences in tissue-specific FDG SUV and considered a surrogate marker for tissue level immune activation in a subgroup of 26 FDG scans performed during the slow-changing period (quasi–steady state) of the immune system. For quasi–steady-state analysis, the 26 scans were divided into three groups: healthy control (seven scans from seven healthy uninfected controls), untreated chronic SIV infection (11 scans from eight SIV chronically infected animals followed during untreated infection for >27 wk), and long-term, cART-treated (eight scans from seven SIV-infected animals that received cART for >26 wk) (Supplemental Fig. 1).

As shown in the representative maximum intensity projection image (Fig. 1A), compared with uninfected controls, we observed that an increase in FDG uptake characterizes SIV chronic infection not only in the clusters of LNs but also in the spleen and bone marrow (Fig. 2), although only the clusters of inguinal and submandibular LNs had reached statistical significance (p < 0.01). After prolonged cART, FDG uptake in all these organs appeared similar to healthy uninfected controls and significantly lower than untreated chronic animals (p < 0.01), except the mesenteric/retroperitoneal LNs.

FDG uptake in tissues: dynamic state
The dynamic-state analysis included all the 45 scans obtained in the study (Supplemental Table 1): seven scans from seven healthy uninfected controls, 20 scans from SIV-infected animals off cART, and 18 scans from SIV-infected animals on cART. Changes measured in PB biomarkers of SIV disease (plasma VL, CD4+ T cell counts) paralleled changes in FDG uptake observed upon treatment modification (initiation/interruption) in all organs except the liver and the bowel (Fig. 3). Following cART initiation, the FDG uptake in lymphoid tissues dropped and plateaued by month one. In contrast, upon treatment withdrawal, the FDG uptake continued to increase beyond month one and appeared to plateau by approximately month three.

Following treatment modification, the drop/rise in FDG uptake was rapid in various clusters of LNs, but the change was more gradual for the spleen and the bone marrow. In contrast, the liver and mean levels of bowel FDG uptake remained almost constant throughout the study. Linear mixed models showed highly significant positive associations of FDG uptake among all analyzed tissues (p < 0.005) except with liver and bowel uptake. The FDG uptake in the bowel achieved in localized areas levels as high as those seen in the LNs or the spleen but failed to follow the changes in PB biomarkers of SIV disease progression (Fig. 3). Consistently, the variability in bowel FDG uptake in the group of uninfected animals is the highest among analyzed tissues (Supplemental Fig. 2).

FDG uptake in tissues versus peripheral markers of SIV disease progression
Figs. 1B and 4 show the longitudinal changes in the FDG uptake in axillary LN clusters following initiation of cART. The whole-body PET images of an SIV-infected animal in Fig. 4 and the PET three-dimensional video of the same animal in Supplemental Video 1 show an apparent decrease of FDG uptake following initiation of cART, not only in the LN clusters but also in the spleen and the bone marrow, but not in the bowels. Univariate analysis demonstrated a significant inverse association of SUVmax with PB CD4+ T cell counts and a significant direct association with plasma VL in all tissues except liver and bowels (Table I). Similarly, positive associations were found between SUVmax in all tissues (except the liver and the bowels) and the SIV-DNA levels in the PB CD4+ T cell pool. In multivariate analysis with repeated measurements in which both CD4+ T cell counts and plasma viremia were selected as predictors, only plasma viremia remained associated with the level of FDG uptake in the clusters of axillary, inguinal, and submandibular LNs (Table II). Surprisingly, the percentage of Ki-67+ in PB CD4 or CD8 T cells and CD20 B cells, which are known to correlate with peripheral biomarkers of immune activation, did not reveal any associations with tissue FDG uptake (Table I).

We next explored the associations between the levels of proinflammatory soluble factors in plasma and peripheral markers of SIV disease progression or FDG uptake. IL-2
showed significant positive associations with the levels of SIV-DNA in CD4<sup>+</sup> T cells (p < 0.01), but not with plasma viremia or the levels of PB T cell proliferation or PB CD4<sup>+</sup> T cell count. No other associations were found between soluble factors and PB biomarkers of disease progression. Interestingly, the only soluble factor that showed significant positive associations with SUVmax in some clusters of LNs was the I-FABP (p < 0.01), a biomarker of intestinal permeability. However, after adjusting for plasma viremia, the statistical significance of the association with I-FABP was lost (Table III).

**DISCUSSION**

In this study, we longitudinally imaged SIV-infected rhesus macaques with FDG-PET following perturbation with initiation and interruption of cART. Throughout the study course, we observed statistically significant positive associations between the level of FDG uptake in the lymphoid organs analyzed and plasma viremia or SIV-DNA levels in the PBMC. As referenced in the introduction, several studies have shown that immune activated lymphocytes and myeloid cells preferentially uptake FDG; however, the relative contribution of these cell populations to the FDG uptake per unit mass of lymphoid tissue has not been demonstrated in the settings of lentiviral pathogenesis in vivo. Lentiviral-induced chronic immune activation is now believed to be the major cause of AIDS in patients and SIV nonhuman primate model of HIV pathogenesis (49). Multiple theories compete to explain how chronic immune activation is established in the setting of HIV/SIV pathogenesis, with some of these focusing on injuries to specific organs of the immune system. One theory (50) attributes an irreversible immune system injury to the early damage caused by the virus to the gut mucosa by allowing excess translocation of gut bacteria and bacterial products with resultant systemic inflammation. An alternative view focuses on the early injuries caused by the virus to the lymph nodal niches, preventing the establishment of an effective immune response (51). Regardless of the location of the first significant injury to the immune system, chronic immune activation eventually affects all organs of the immune system and is associated with several biomarkers of disease progression, including low thymic output, lymphoid tissue fibrosis (52), poor immune reconstitution and renewal of CD4<sup>+</sup> T cells, dysfunction of T and B cells (53, 54), and mucosal damage at the gut surface (55). These problems can result in immune suppression, with subsequent poor control of pathogens, even in patients with HIV RNA levels below 50 copies/ml, suggesting that persistent low levels of Ag stimulation from undetectable viral replication or production of viral proteins by defective proviruses can induce immune activation and immune dysfunction (55). In support of this view, there is evidence that some of those clinical or pathological outcomes are also observed in populations with endemic chronic immune activation not caused by HIV (56) and in experimentally induced chronic immune activation (57).

As the majority of preclinical and clinical studies in the past two decades have focused on measurements of chronic immune...
activation obtained in the PB, which may mask differential levels of immune activation in specific lymphoid organs or their associations with disease progression, several teams began to interrogate the use of FDG-PET imaging technology to assess localized (anatomic compartment specific) immune activation in a few preclinical (39, 40) and clinical studies (41–44). Although they all suffered from a limited degree of serial FDG-PET imaging, they described LN tissue activation patterns in varying stages of HIV-1 or SIV infection, but many other organs were mostly overlooked. Earliest studies suggested high FDG uptake in cervical and axillary LNs in untreated, SIV-infected macaques compared with uninfected controls (39) and a similar lymphoid tissue activation in the upper body (axillary, cervical, and mediastinum LNs) in simian HIV89.6PD-infected macaques within the first 2 wk of viral inoculation (40). In addition to the LNs, Scharko et al. (39) also reported higher FDG uptake in the spleen and small intestine of three infected animals in mid- or late-stage disease, although organ uptakes were not quantitated. Similar results of increased LN activation were observed in a clinical FDG-PET study in early untreated and chronically infected HIV-1 patients but without evidence of increased splenic uptake, except in one patient imaged at the peak of acute infection (41). FDG imaging in long-term nonprogressors or cART-treated, HIV-1-infected patients with suppressed plasma VLs had little to no FDG nodal uptake. In contrast, viremic untreated patients showed hypermetabolic nodal accumulation of FDG (43) but without evidence of increased FDG SUV in the spleen or bone.

FIGURE 2. Quasi–steady-state analysis.
Box plots of $^{18}$F-FDG uptake in (A) spleen, (B) submandibular, (C) axillary, (D) inguinal, and (E) mesenteric/retroperitoneal LNs, (F) bone marrow, (G) liver, and (H and I) bowel, quantified as maximum or mean SUV in the uninfected (Healthy Control), untreated chronic SIV infection for >27 wk (Chronic) and long-term cART treated for >26-wk (cART-treated) scans. Only two of seven healthy control scans contributed to mesenteric/retroperitoneal LN SUVmax data. *$0.001 < p < 0.01$, **$p < 0.001$. 

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The gut region of interest was not analyzed in this study. Similarly, in a cross-sectional study in HIV-1–infected patients (44), a normal pattern of FDG uptake was reported in cART-treated patients, whereas the cART-naive group with high viremia (with an average of ~500 PB CD4+ T cell counts) showed increased FDG uptake in LNs (>10-fold compared to cART-treated patients).

**FIGURE 3. Dynamic-state analysis.**
Graphical representation of changes in maximum or mean SUV for all analyzed tissues and log_{10} plasma VL (PVL) (solid black line), CD4+ T cell counts (solid brown line), and the average of all lymphoid tissues analyzed (solid orange line) with (A) initiation or (B) interruption of cART. Each point depicts the mean of \( n = 5 \) animals.

**FIGURE 4. Maximum intensity projection PET images following \([^{18}F]\)-FDG administration in a chronically SIV-infected animal (A10V120) imaged longitudinally after initiation of cART, and 3 mo after therapy interruption.**
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[18F]-FDG SUVmax in all tissues analyzed as outcome advanced HIV-1–infected patients (with was noted in previous FDG-PET studies of HIV-1–infected dence of a significant increase in the bone marrow FDG uptake row following interruption/initiation of cART. Although no evi-

enhancement/reduction of immune activation in the bone mar-

in HIV-1–infected patients. model; however, this was not consistently found or prominent immune activation in the spleen was also provided in the SIV organs analyzed in our study. Some evidence of enhanced compared with healthy controls (58) at 0.05 significance level cART-treated and VL-suppressed HIV-1–infected patients as larger sample size has shown higher FDG uptake in the LNs of longitudinal study (Fig. 2). However, one clinical study with a

in the clusters of LNs, which promptly normalized to healthy

beyond the clusters of LNs. Although the pathologic or in rhesus macaques to image whole-body immune activation PB CD4 pool needs to be much more depleted in humans than

in CD20

in CD8

in CD4

Log SIV-DNA per milliliter 0.27

Log SIV-DNA/1 × 10⁶ CD4⁺ T 0.31

Log SIV-DNA per milliliter 0.27

Log Ki67 in CD4⁺ in CD8⁻ T (%) NS

Log Ki67 in CD4⁺ in CD20⁻ B (%) NS

Log plasma VL 0.06

CD8⁺ T NS

CD4⁺/CD8⁻ 0.31ab

Log Ki67 in CD4⁺ in CD20⁻ B (%) NS

Our preclinical retrospective analysis reveals prompt enhancement/reduction of immune activation in the bone mar-

axillary; BM, bone marrow; Ing, inguinal; Sub, submandibular.

physiological significance of changes in FDG activity in the bone marrow remains unclear, it has been proposed that it likely reflects marrow metabolism, mainly regulated by granulo-

cyte progenitors and stimulated by endogenous hematopoietic growth factors, hence suggesting a direct insult caused by lentiviral infection to this primary lymphoid organ with the extent of its dysregulation possibly associated to an accelerated course of the disease (60).

Overall, those FDG-PET studies in lentivirally infected hosts provided evidence of viral-induced chronic immune activation in the clusters of LNs, which promptly normalized to healthy control levels following initiation of cART, as confirmed in our longitudinal study (Fig. 2). However, one clinical study with a larger sample size has shown higher FDG uptake in the LNs of cART-treated and VL-suppressed HIV-1–infected patients as compared with healthy controls (58) at 0.05 significance level but failed to measure the uptake levels in the other lymphoid organs analyzed in our study. Some evidence of enhanced immune activation in the spleen was also provided in the SIV model; however, this was not consistently found or prominent in HIV-1–infected patients.

with aviremic treated patients), but only a slight increase (~30%) in splenic FDG uptake and no increase in bone marrow FDG uptake. Again, the gut was not analyzed in this study either.

 Previous studies failed to report associations between FDG uptake in the bowel and HIV/SIV disease progression. Murine models of colitis (61) have shown that FDG uptake following parenteral administration is primarily explained by uptake in immune cells rather than epithelial cells in the bowel. However, increases in the percentage of proliferating epithelial cells of the gut mucosa have also been demonstrated in SIV-infected animals (62), which could also contribute to the increase in FDG uptake in the bowel VOI, given that epithelial cells represen the majority of mononuclear cells of the gut mucosa. The lack of a significant association between bowel FDG uptake and markers of SIV disease progression in our study suggests that other factors beyond lentiviral-induced immune activation dominate the overall FDG uptake in this anatomic compartment. However, we also advise caution in drawing conclusions on gut uptake measurements, mainly because it is challenging to delineate for the VOI analysis given its complex anatomy and heterogeneity, and our observations warrant further studies to assess the causes of high variability in bowel FDG uptake in healthy uninfected controls.

Although we have analyzed tissue uptakes in a small group of animals and following two different types of perturbations of the quasi–steady state of the immune system, the longitudinal dataset generated in our study was sufficient to highlight in

TABLE I. Slope estimates from univariate linear mixed-effect analysis between [18F]-FDG SUVmax in all tissues analyzed and PB biomarkers of SIV disease

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Spleen</th>
<th>Ax LN</th>
<th>Ing LN</th>
<th>Sub LN</th>
<th>BM</th>
<th>Bowel</th>
<th>Mesenteric/Retroperitoneal LN</th>
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</tr>
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<tbody>
<tr>
<td>CD4⁺ T</td>
<td>0.6 × 10⁻⁵</td>
<td>0.6 × 10⁻⁶</td>
<td>0.6 × 10⁻⁶</td>
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<tr>
<td>CD4⁺/CD8⁻</td>
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<td>0.44ab</td>
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<td>NS</td>
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<td>0.10a</td>
<td>0.10a</td>
<td>0.07a</td>
<td>0.03a</td>
<td>NS</td>
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<tr>
<td>Log SIV-DNA/1 × 10⁶ CD4⁺ T</td>
<td>0.31a</td>
<td>0.48a</td>
<td>0.43a</td>
<td>0.38a</td>
<td>0.12a</td>
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<td>NS</td>
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<td>Log Ki67 in CD4⁺ in CD20⁻ B (%)</td>
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³P < 0.001.

TABLE II. Slope estimates from multivariate linear mixed-effect analysis with PB CD4⁺ T cells and log₁₀ plasma VL as the covariates and [¹⁸F]-FDG SUVmax in all tissues analyzed as outcome

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<th>Biomarker</th>
<th>Spleen</th>
<th>Ax LN</th>
<th>Ing LN</th>
<th>Sub LN</th>
<th>BM</th>
<th>Bowel</th>
<th>Mesenteric/Retroperitoneal LN</th>
<th>Liver</th>
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<td>NS</td>
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<td>NS</td>
<td>NS</td>
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³P < 0.001.

⁴0.001 < P < 0.01.

Ax, axillary; BM, bone marrow; Ing, inguinal; Sub, submandibular.
multivariate repeated measurement analysis, highly statistically significant associations of localized immune activation, as reflected by FDG uptake, with peripheral biomarkers of SIV disease progression. Although plasma viremia and CD4+ T cell count were both univariately correlated with the FDG uptake in each of the lymphoid organs analyzed, the multivariate analysis suggested that plasma viremia is the stronger predictor of SUVmax in tissues. Association, however, does not imply causation. Moreover, plasma viremia and CD4+ T cell counts are two covariates characterized by differential levels of biological and experimental noise, and the ideal dataset to study the major contribution to FDG uptake in a given organ would require knowledge of viral levels and CD4+ T cells in that organ.

The analysis of proinflammatory soluble factors showed that only plasma levels of I-FABP, a marker of enterocyte damage (65), predicted FDG uptake in the cluster of inguinal and submandibular LNs, yet not with the same strength of plasma viremia. This observation suggests that other cytokines (64) or possibly soluble viral products (e.g., nef) (65) are likely responsible for localized immune activation in lymphoid organs. To our knowledge, this association has not been previously reported and is consistent with a role of loss of intestinal mucosa integrity in sustaining the whole-body immune activation. Alternatively, because the TCR and CD28 synergize to induce the synthesis and surface expression of the main glucose transporter Glut-1 (34), localized levels of FDG uptake can also be explained, in principle, by localized levels of viral replication and presentation, possibly facilitated by incorporation into the virion of ligands, such as B7-2 and MHC class II, that directly activate T cells (66). Several studies demonstrated increased glucose uptake as a functional response to the metabolic requirements of immune activation induced by HIV infection and explained by increased surface expression of Glut-1 on PB T lymphocytes (36, 67). Glut levels were not measured in this study. The rapid FDG uptake dynamics posttreatment modification observed in LNs compared with the spleen, in addition to differential activation levels among the tissues, could be related to the expression level of various glucose transporters, the predominant Glut in the tissue, and the affinity of FDG to that major Glut.

In conclusion, this longitudinal study in an SIV model provides, to our knowledge, novel details regarding the pattern of immune activation within organs such as the spleen, bone marrow, and bowel, in addition to previously studied LN clusters. Immune activation within various lymphoid organs significantly correlated with peripheral markers of disease progression.

### DISCLOSURES

The authors have no financial conflicts of interest.

### ACKNOWLEDGMENTS

We sincerely thank Dr. Irini Sereti and Dr. Cliff Lane for critique and insightful comments in reviewing the manuscript, the National Institute of Allergy and Infectious Diseases veterinary and animal care staff for maintaining the nonhuman primates used in this study, PET Department staff at National Institutes of Health Clinical Center for supporting PET scans, and Dr. David Erickson from the Oregon National Primate Research Center for producing I-FABP and D-Dimer measurements. We also thank Dr. Claire Delage for helpful discussions throughout the study, Merck for providing raltegravir, and Gilead for providing tenofovir and emtricitabine.

### REFERENCES


Suppl. Table 1. Animal characteristics and therapy status for all 45 \(^{18}\)F-FDG scans in 15 rhesus macaques.

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<th>Therapy status</th>
<th>Plasma viral load (copies/mL)</th>
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Suppl. Figure 1. The 26 $^{18}$F-FDG scans performed during the quasi-steady state of the immune system are indicated with circles – 7 scans from 7 healthy uninfected controls (orange), 11 scans from 8 SIV-chronically infected animals followed during untreated infection for >27 weeks (red), and 8 scans from 7 long-term (>26 weeks) combination antiretroviral therapy (cART) treated SIV-infected animals (green).
Suppl. Figure 2. Maximum Intensity Projection (MIP)-PET images following $^{18}$F-FDG administration in 5 of total 7 healthy uninfected controls.
Suppl. Video Legend:
Suppl. Video 1. Maximum Intensity Projection (MIP)-PET 3D video following $^{18}$F-FDG administration in a chronically SIV-infected animal (A10V120) imaged longitudinally after initiation of combination antiretroviral therapy, and three months post therapy interruption.