A Murine Viral Outgrowth Assay to Detect Residual HIV Type 1 in Patients With Undetectable Viral Loads

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Background. Sensitive assays are needed for detection of residual human immunodeficiency virus (HIV) in patients with undetectable plasma viral loads to determine whether eradication strategies are effective. The gold standard quantitative viral outgrowth assay (QVOA) underestimates the magnitude of the viral reservoir. We sought to determine whether xenograft of leukocytes from HIV type 1 (HIV)–infected patients with undetectable plasma viral loads into immunocompromised mice would result in viral amplification.

Methods. Peripheral blood mononuclear cells or purified CD4+ T cells from HIV or simian immunodeficiency virus (SIV)–infected subjects with undetectable plasma viral loads were adoptively transferred into NOD.Cg-PrkdcscidIl2rgtm1Wjl/SzJ (NSG) mice. The mice were monitored for viremia following depletion of human CD8+ T cells to minimize antiviral activity. In some cases, humanized mice were also treated with activating anti-CD3 antibody.

Results. With this murine viral outgrowth assay (MVOA), we successfully amplified replication-competent HIV or SIV from all subjects tested, including 5 HIV-positive patients receiving suppressive antiretroviral therapy (ART) and 6 elite controllers or suppressors who were maintaining undetectable viral loads without ART, including an elite suppressor from whom we were unable to recover virus by QVOA.

Conclusions. Our results suggest that the MVOA has the potential to serve as a powerful tool to identify residual HIV in patients with undetectable viral loads.

Keywords. HIV; SIV; quantitative viral outgrowth assay (QVOA); humanized mouse; cure.
humans and chimpanzees, humanized mice can be infected with HIV [13] and have been used to study HIV persistence and latency [14, 15]. We hypothesized that xenograft of leukocytes from HIV-infected patients with undetectable plasma viral loads into severely immunocompromised mice would result in viral amplification within the aberrant murine host. Such amplification could potentially allow us to detect viral reservoirs within the peripheral blood of HIV-infected humans and simian immunodeficiency virus (SIV)-infected pigtailed macaques with plasma viral loads that are undetectable by standard quantitative reverse transcription (qRT)–PCR methods as a result of long-term antiretroviral therapy (ART) or elite control.

MATERIALS AND METHODS

HIV-Infected Patient Donors

Whole-blood specimens were obtained from 5 HIV-positive patients receiving suppressive ART regimens and 6 HIV-positive elite suppressors for this study. Patients receiving ART had been infected for an average of 10 years (range, 2–22 years) and had been receiving ART for an average of 3 years (range, 1–6 years). These patients had an average CD4+ T-cell count of 620 cells/µL (range, 409–1001 cells/µL) at the time of this study. Elite suppressors had been infected for an average of 15 years (range, 5–29 years). Elite suppressors had always had plasma viral loads of <50 copies/mL, had a median of 0.06 infectious units per million (IUPM; range, 0.04 to 4.57 IUPM) detected by QVOA, and had an average CD4+ T-cell count of 1026 cells/µL (range, 630–1902 cells/µL). Patients receiving ART had a median proviral HIV DNA load of 1775 copies/million CD4+ T cells and a median HIV messenger RNA level of 258 copies/million CD4+ T cells. Elite suppressors had a median proviral HIV DNA load of 1097 copies/million CD4+ T cells and a median HIV mRNA level of <10 copies/million CD4+ T cells (Pohlmeier et al, unpublished data).

SIV Infection and ART Treatment of Macaques

Male pigtailed macaques aged 2–3 years were dual inoculated by the intravenous route with the neurovirulent clone 17E/Fr and immunosuppressive swarm ΔB670 of SIV as previously described [16]. Starting on day 12 after inoculation, macaques were treated with antiretroviral drugs at the following doses with the combination of drugs indicated in Table 1: 10 mg/kg tenofovir (Gilead) once daily by subcutaneous injection and 10 mg/kg integrase inhibitor L000870812 (Merck), 24 mg/kg ritonavir (AbbVie), and either 270 mg/kg atazanavir (Bristol-Myers Squibb) or 480 mg/kg darunavir (Janssen) twice daily by mouth in a food treat [17]. Macaques were sedated weekly until day 42 after inoculation and then semiweekly thereafter with 10 mg/kg ketamine, underwent a full physical examination by a veterinarian, and had blood specimens collected for determination of viral load. Macaques were sedated 205 days after inoculation and then semiweekly thereafter with 10 mg/kg ketamine, underwent a full physical examination by a veterinarian, and had blood specimens collected for determination of viral load. Macaques were sedated 205 days after inoculation, and blood specimens were collected into acid citrate dextrose (Sigma) for peripheral blood mononuclear cell (PBMC) purification.

Xenograft of Human or Macaque PBMCs or Purified Resting CD4+ T Cells Into NOD.Cg-Pkrdcs-Id2gamt1Wjt/SzJ (NSG) Mice

Human and macaque PBMCs and resting CD4+ T cells were purified as previously described [17, 18]. NSG mice (Jackson Laboratories) were briefly manually restrained and injected intraperitoneally with the indicated number of PBMCs or purified resting CD4+ T cells resuspended in <0.5 mL of sterile saline (Tables 1–3). To allow for monitoring of engraftment and viral load, blood specimens were collected from manually restrained mice by facial sinus bleed no more than once weekly. CD8+ T cells were empirically depleted from humanized mice by intraperitoneal injection with 200–400 μg of anti-human CD8 monoclonal antibody (mAb; EBiosciences) and, in some cases, T cells activated with 100 µg anti-human CD3 mAb (OKT3; EBiosciences). Mice were monitored daily and promptly euthanized if they appeared ill. All mice were euthanized with

Table 1. Macaques Mice Amplify Simian Immunodeficiency Virus (SIV) From Peripheral Blood Mononuclear Cells (PBMCs) From SIV-Infected Macaques Receiving Antiretroviral Therapy (ART) With or Without a Detectable Plasma Viral Load

<table>
<thead>
<tr>
<th>Macaque</th>
<th>Days Infected</th>
<th>Donor PBMC Viral Load, Copies/mL</th>
<th>CD4+ T-Cell Count, Cells/µL</th>
<th>ART Regimen</th>
<th>SIV-Positive Mice, Total No.</th>
<th>SIV-Positive Mice, %</th>
<th>PBMCs Xenograft, No.</th>
<th>Time to Viremia in Mouse, d, Median</th>
<th>Peak Viremia in Mouse, Copies/mL, Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1</td>
<td>205</td>
<td>6.36 × 10^2</td>
<td>1284</td>
<td>TDF, INSTI, RTV, ATV</td>
<td>2</td>
<td>2.00</td>
<td>100</td>
<td>4.0 × 10^7</td>
<td>10.5</td>
</tr>
<tr>
<td>V2</td>
<td>205</td>
<td>4.59 × 10^5</td>
<td>480</td>
<td>TDF, INSTI, RTV, ATV</td>
<td>1</td>
<td>1.00</td>
<td>100</td>
<td>4.0 × 10^7</td>
<td>7</td>
</tr>
<tr>
<td>V3</td>
<td>205</td>
<td>9.50 × 10^2</td>
<td>533</td>
<td>TDF, INSTI, RTV, DRV</td>
<td>1</td>
<td>1.00</td>
<td>100</td>
<td>4.0 × 10^7</td>
<td>7</td>
</tr>
<tr>
<td>V4</td>
<td>205</td>
<td>6.01 × 10^5</td>
<td>792</td>
<td>TDF, INSTI, RTV, ATV</td>
<td>1</td>
<td>1.00</td>
<td>100</td>
<td>4.0 × 10^7</td>
<td>7</td>
</tr>
<tr>
<td>U1</td>
<td>205</td>
<td>0.00</td>
<td>NM</td>
<td>Uninfected</td>
<td>0</td>
<td>1.00</td>
<td>0</td>
<td>4.0 × 10^7</td>
<td>ND</td>
</tr>
<tr>
<td>S1</td>
<td>&lt;1.00</td>
<td>1141</td>
<td>TDF, INSTI, RTV, DRV</td>
<td>3</td>
<td>3</td>
<td>100</td>
<td>4.0 × 10^7</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ATV, atazanavir; DRV, darunavir; INSTI, integrase inhibitor L-000870812; ND, not detectible; NM, not measured; RTV, ritonavir; TDF, tenofovir.
inhaled CO₂ according to the 2013 AVMA Guidelines on Euthanasia at the last time point indicated in the figures, and blood specimens, peritoneal wash fluid, spleens, and other organs were collected. Throughout the study, mice were pair or group housed in shoebox cages on a ventilated rack (Allen-town), provided with free access to nutritionally complete rodent chow (Harlan), provided with a nestlet for enrichment, and handled in a biosafety cabinet.

**Ethics Statement**

All animal work was approved by the Johns Hopkins University Institutional Animal Care and Use Committee and determined to be in accordance with the guidelines outlined in the Animal Welfare Act and Regulations (US Department of Agriculture) and the *Guide for the Care and Use of Laboratory Animals, Eighth Edition* (National Institutes of Health).

**Flow Cytometry Detection of Macaque and Human Cells in Mice**

To quantify the level of engraftment, flow cytometry was completed on blood specimens obtained from xenografted mice every 7 days following xenograft until necropsy; at necropsy, flow cytometry was performed blood specimens, spleens, and peritoneal wash fluid. For humanized mice, cells were stained with anti-mouse CD45 (clone 30F11; BV605), anti-human CD45 (clone H130; PE-Cy7), CD3 (clone UCHT1; PacificBlue), CD4 (clone RPA-T4; PE), CD8 (clone SK1; APC-H7), CD56 (clone B159; FITC), CD16 (clone 3G8; PerCP-Cy5.5), CD25 (clone MA-251; APC), CD69 (clone FN50; APC), and HLA-DR (clone G46-6; APC; BD Biosciences) and were evaluated with a BD FACSCanto II. For macaques, blood specimens were stained with anti-mouse CD45 (clone 30F11; BV605), anti-human CD45 (clone HI30; PE-Cy7), CD3 (clone UCHT1; PacificBlue), CD4 (clone RPA-T4; PE), CD8 (clone SK1; APC-H7), CD56 (clone B159; FITC), CD16 (clone 3G8; PerCP-Cy5.5), CD25 (clone MA-251; APC), CD69 (clone FN50; APC), and HLA-DR (clone G46-6; APC; BD Biosciences) and were evaluated with a BD FACSCanto II. For macaques, blood specimens were stained with anti-mouse CD45 (clone 30F11; BV605), anti-human CD45 (clone HI30; PE-Cy7), CD3 (clone UCHT1; PacificBlue), CD4 (clone RPA-T4; PE), CD8 (clone SK1; APC-H7), CD56 (clone B159; FITC), CD16 (clone 3G8; PerCP-Cy5.5), CD25 (clone MA-251; APC), CD69 (clone FN50; APC), and HLA-DR (clone G46-6; APC; BD Biosciences) and were evaluated with a BD FACSCanto II. For macaques, blood specimens were stained with anti-mouse CD45 (clone 30F11; BV605), anti-human CD45 (clone HI30; PE-Cy7), CD3 (clone UCHT1; PacificBlue), CD4 (clone RPA-T4; PE), CD8 (clone SK1; APC-H7), CD56 (clone B159; FITC), CD16 (clone 3G8; PerCP-Cy5.5), CD25 (clone MA-251; APC), CD69 (clone FN50; APC), and HLA-DR (clone G46-6; APC; BD Biosciences) and were evaluated with a BD FACSCanto II. For macaques, blood specimens were stained with anti-mouse CD45 (clone 30F11; BV605), anti-human CD45 (clone HI30; PE-Cy7), CD3 (clone UCHT1; PacificBlue), CD4 (clone RPA-T4; PE), CD8 (clone SK1; APC-H7), CD56 (clone B159; FITC), CD16 (clone 3G8; PerCP-Cy5.5), CD25 (clone MA-251; APC), CD69 (clone FN50; APC), and HLA-DR (clone G46-6; APC; BD Biosciences) and were evaluated with a BD FACSCanto II.

**Quantification of SIV and HIV in Plasma, Using qRT-PCR**

RNA was isolated from plasma from human and macaque donors and xenografted mice, and viral RNA was quantified using qRT-PCR as previously described [19, 20]. Viral load was normalized to the original plasma input volume to determine copy equivalents per milliliter of plasma. Thus, the limit of detection varies between qRT-PCR runs.

**Quantitation of Viral Outgrowth**

The QVOA was performed on CD4⁺ T cells as previously described [9, 21–23]. CD4⁺ T cells were purified by negative selection, using Miltenyi beads, and were then stimulated with PHA (0.5 µg/mL) and a 5–10-fold excess of irradiated PBMCs from HIV-seronegative donors in medium containing 100 U/mL of interleukin 2. After 1–2 days of culture, the medium containing PHA was removed, and activated CD4⁺ T cell lymphoblasts from HIV-seronegative donors were added to amplify the virus. The cultures were split on day 7, and more CD4⁺ T-cell lymphoblasts were added. Virus was detected using a p24 enzyme-linked immunosorbent assay on days 14 and 21 of culture.

**Quantitation of Proviral DNA and Cell-Associated RNA**

For human samples, cell-associated DNA and mRNA levels were quantified by qPCR as previously described [19, 24]. For macaque samples, nucleic acids were isolated from 5 × 10⁶ macaque PBMCs, using the AllPrep DNA/RNA isolation minikit (Qiagen). Level of intracellular SIV RNA and DNA were quantitated by digital-droplet PCR (BioRad), using the same SIV gag primers and probe and cycle conditions as described above for qRT-PCR.

**Immunohistochemistry for the Detection of CD45⁺ Human and Macaque Cells in Tissues From Xenografted Mice**

Mouse tissues were fixed in 10% neutral buffered formalin for a week and then embedded in paraffin. Antigen retrieval was performed by heating the slides in a microwave at 780 W for 8
minutes in 10 mM sodium citrate buffer at pH 6.0. The remainder of the protocol was performed using the Vector M.O.M. Immunodetection Kit (Vector Labs PK-2200) with anti-CD45 antibody (AbD Serotec MCA1921 Clone 2B11 + PD7/26/16) at a 1:100 dilution for 1 hour at room temperature. Slides were stained with DAB (Vector Labs SK-4100) and counterstained with hematoxylin (BioGenex HK100).

Data Management and Analysis
All data were organized using Microsoft Excel 2013, and statistical analyses were completed with GraphPad Prism. Nonparametric (Spearman) correlative analyses were used to compare the percentage of PBMCs or plasma viral RNA in the mouse with the number of cells or viral load, respectively, in the macaque or human donors.

RESULTS

Virus Can Be Detected in Plasma of NSG Mice Following Xenograft of PBMCs From SIV-Infected Macaques

We aimed to xenograft immunocompromised mice with PBMCs from HIV-infected patients with undetectable viral loads to determine whether we could detect virus with better sensitivity than the QVOA. To validate our xenograft technique, NSG mice were humanized with 8.8–25.2 million human PBMCs, control mice received no PBMCs, and whole-blood specimens were collected 7 days later for flow cytometry analysis. Intraperitoneal injection of >8.8 million PBMCs resulted in humanization of 8 of 9 mice, with CD45+ human cells detectable in the peripheral blood and colonizing the bone marrow, spleen, and peritoneal cavity (Supplementary Figure 1). Circulating CD45+ human cells consisted predominantly of activated CD4+ T cells (Supplementary Figure 1A), and the percentage of human cells circulating in the mouse’s peripheral blood 7 days after humanization correlated with the number of xenografted PBMCs (Spearman $R^2 = 0.87; P = .0005$).

To verify that we could detect lentivirus in plasma specimens from mice xenografted with leukocytes from infected individuals, we initially worked with an SIV-infected pigtailed macaque model of HIV infection [16]. We macaquized 5 of 5 NSG mice with 40 million PBMCs harvested from SIV-infected pigtailed macaques receiving ART, all with measurable plasma viral loads (median, 230 182 copies/mL; macaques V1–4 in Table 1). SIV RNA was detected in the plasma of all mice by 14 days after xenograft, with a median peak SIV load of 10 184 copies/mL (Table 1); the magnitude of the plasma viral load in the xenografted mouse did not correlate with the magnitude of the plasma viral load in the donor macaque (Spearman $R^2 = −0.02; P = .60$). We did not detect any virus in a control mouse xenografted with 40 million PBMCs from an uninfected macaque (macaque U1 in Table 1) or in a control mouse that was not injected with macaque PBMCs (data not shown).
Replication-Competent HIV and SIV Can Be Detected by a Murine Viral Outgrowth Assay (MVOA) in NSG Mice Following Xenograft of PBMCs From Individuals Receiving Suppressive ART With a History of Undetectable Plasma Viral Loads

SIV-infected pigtailed macaques receiving ART are a valuable model for the study of latent reservoirs of HIV [17]. We sought to determine whether the MVOA could detect replication-competent virus in an SIV-infected pigtailed macaque receiving ART that had no plasma viral load detected by qRT-PCR for 78 days prior to PBMC donation, 312 copies per million PBMCs of proviral SIV gag DNA, and 18 copies of cell-associated SIV gag RNA per million PBMCs (macaque S1 in Table 1). We detected SIV 7 days after xenograft in plasma specimens from 3 of 3 mice, each injected with 40 million PBMCs from this donor (peak median MVOA finding, 13 032 copies/mL; Figure 1A). Resting CD4+ T cells are a resilient reservoir for latent virus [25], and xenografting 6.8 million resting CD4+ T cells from this macaque permitted detection of virus 7 days after xenograft (794 copies/mL; Figure 1B).

The MVOA was similarly able to detect replication-competent virus in 5 of 5 HIV-infected patients receiving long-term ART who had undetectable plasma viral loads for at least 1 year (average, 3 years; Table 2). One mouse per patient was engrafted with PBMCs, and CD8+ T cells were depleted with anti-human CD8 mAb 7 days after xenograft because of the concern that these cells may have antiviral activity. CD8+ T-cell depletion also mimics the conditions typically followed in the QVOA, [9, 21]. CD4+ T-cell activation was present in all engrafted mice (Supplementary Figure 1C). We detected HIV in the murine plasma an average of 20 days after xenograft (range, 13–26 days), with a median peak viral load of $4.6 \times 10^3$ copies/mL at an average of 25 days (Figure 2 and Supplementary Figure 2A).

Replication-Competent HIV Can Be Detected by MVOA in NSG Mice Following Xenografting of CD4+ T Cells From HIV-Positive Elite Suppressors With Plasma Viral Loads Below the Limit of Detection

To further challenge the sensitivity of the MVOA, we engrafted mice with cells from elite suppressors. These patients control the replication of HIV to levels below the clinical limit of detection (<50 copies/mL) without ART [26–28] and have very low frequencies of latently infected CD4+ T cells [22]. We have previously shown that purified viral isolates from these patients are...
We demonstrated that the MVOA has the potential to serve as a powerful tool to identify reservoirs of HIV. By xenografting as few as 25 million PBMCs or 10 million CD4\(^+\) T cells into mice (M34 in Figure 3 and M27 in Supplementary Figure 2B); in contrast, we were unable to recover virus when 25 million CD4\(^+\) T cells were tested in the QVOA. The magnitude of the plasma viral load in the xenografted mouse did not correlate with the IUPM as measured by QVOA in the donors (Spearman \(R^2 = -0.10; P = .83\)). Elite suppressors’ CD8\(^+\) T cells are very effective at controlling viral replication in vitro [30–35] and in humanized mice [36], and the presence of residual CD8\(^+\) T cells may have contributed to the transient nature of the viremia in some mice (Supplementary Figure 3).

DISCUSSION

We were able to amplify virus from elite suppressor 5 following engraftment of either 10 million or 20 million CD4\(^+\) T cells into mice (M34 in Figure 3 and M27 in Supplementary Figure 2B); in contrast, we were unable to recover virus when 25 million CD4\(^+\) T cells were tested in the QVOA. The magnitude of the plasma viral load in the xenografted mouse did not correlate with the IUPM as measured by QVOA in the donors (Spearman \(R^2 = -0.10; P = .83\)). Elite suppressors’ CD8\(^+\) T cells are very effective at controlling viral replication in vitro [30–35] and in humanized mice [36], and the presence of residual CD8\(^+\) T cells may have contributed to the transient nature of the viremia in some mice (Supplementary Figure 3).

DISCUSSION

We demonstrated that the MVOA has the potential to serve as a powerful tool to identify reservoirs of HIV. By xenografting as few as 25 million PBMCs or 10 million CD4\(^+\) T cells, we amplified virus from HIV-positive patients and SIV-positive macaques with undetectable viral loads due to either long-term receipt of ART or natural control, including 1 elite suppressor from whom we were unable to recover virus with the QVOA. This assay, although not quantitative, may be more sensitive than the QVOA. Additionally, at a ratio of 1 mouse to every 10–50
Figure 3. Adoptive transfer of peripheral blood mononuclear cells (PBMCs) or CD4+ T cells from human immunodeficiency virus (HIV)–infected elite suppressors with undetectable viral loads into NSG mice results in amplification of HIV type 1. Shown are viral loads and percentages of circulating human CD4+ T cells over time, following adoptive transfer, of CD4+ T cells (dashed lines) or PBMCs (solid lines) in representative mice with xenografted mouse plasma viral loads (red circles) and circulating human CD4+ T cells in mouse blood (blue diamonds). Human CD8+ T cells were depleted by the murine viral outgrowth assay as needed (black arrows). Three of 9 mice from which we were able to amplify virus from elite suppressors required anti-CD3 monoclonal antibody stimulation (yellow arrows) to amplify virus to detectable levels. Open circles represent the limit of detection for each sample in which no virus was detected. Abbreviations: IUPM, infectious units per million cells; QVOA, quantitative viral outgrowth assay.

Figure 4. The murine viral outgrowth assay (MVOA) amplifies replication-competent human immunodeficiency virus (HIV) from peripheral blood mononuclear cells (PBMCs) or CD4+ T cells from HIV-positive patients with undetectable plasma viral loads. Schematic representation of xenograft of NOD.Cg-PrkdcskI2rgtm1Wjl/SzJ (NSG) mice with patient cells and subsequent detection of HIV in the murine plasma. Abbreviation: qRT-PCR, quantitative reverse transcription–polymerase chain reaction.
million CD4+ T cells from patients, the MVOA can more efficiently be used to sample very large numbers of cells, compared with the 10:1 ratio of feeders to patient cells required in the QVOA (Figure 4) [9, 21]. The MVOA is promising not only as a simple and sensitive diagnostic assay for evaluating putative curative and preventive therapies in patients with otherwise undetectable plasma viral loads, but also because it may be adapted for future studies into the pathogenesis of the control of viral replication.

The sensitivity of the MVOA is likely due to a number of factors, including the ability to screen large numbers of host cells (up to 60 million per mouse), xenogeneic human anti-mouse responses that lead to the activation of human CD4+ T cells, and the use of qRT-PCR to detect virus once amplified (similar to the QVOA modifications reported by Laird et al [21]). The efficacy of the MVOA may potentially be enhanced by exogenous activation of T cells. While the anti-CD3 mAb OKT3 alone was effective in flushing out virus in cells from 2 patients, this antibody eventually leads to T-cell depletion in human recipients [37, 38], and therefore a combination of anti-CD3 and anti-CD28 mAbs may be more effective in inducing prolonged T-cell activation.

The MVOA presented in this article is a sensitive assay of residual virus in the peripheral blood with a binary output, rather than a quantitative assay. However, the MVOA could potentially be adapted to produce a quantitative result, similar to the IUPM produced by QVOA [9]; multiple mice could be xenografted with serial dilutions of an HIV-positive patient’s cells, thus allowing a similar calculation to be completed to determine an IUPM through the MVOA. Additionally, although the MVOA as described detected reservoirs in the peripheral blood, we anticipate that it may be able to similarly detect virus from cells isolated from tissue biopsy specimens from HIV-positive patients or animal models. The location of latent viral reservoirs within the tissues is an area of active investigation [17, 39], and therefore the MVOA may be used to elucidate the location of latent viral reservoirs in the tissues.

The MVOA has several limitations. The sensitivity of the assay is directly related to the volume of plasma obtained from the xenografted mouse at each time point. The level of engraftment is variable, and this affects the percentage of the adoptively transferred cells that are actually assayed. Finally, the time frame between engraftment and virus detection is also variable, and this affects the percentage of the adoptively transferred cells that are actually assayed. Therefore, the MVOA may be needed to distinguish between these 2 scenarios.

Despite these limitations, the MVOA is unique in that it can determine whether viral rebound will occur when ART is discontinued in an in vivo system without any undue effect on the patient. Interestingly, the adoptive transfer of PBMCs from SIV-infected monkeys receiving ART into uninfected monkeys has been shown to be a sensitive method for detecting residual virus [40]. In our study, we evaluated the effect of discontinuation of common suppressive ART regimens on both HIV and SIV replication in PBMCs. We detected virus production by PBMCs isolated from these patients by MVOA within a time frame (<4 weeks) consistent with that reported following interruption of ART in patients who initiated treatment during the chronic phase of HIV infection [41]. Time to virus production in mice xenografted with cells from patients undergoing different antiretroviral regimens (ie, initiation of ART during primary infection and intensification of ART) could be compared to previous reports of the kinetics of viral recrudescence following cessation of these therapies [42, 43] to determine whether time to outgrowth in the MVOA is reflective of the efficacy of a therapy targeted to provide lasting control of viral replication in the patient.

The question of whether HIV has truly been eradicated from a patient is paramount following treatment with experimental curative regimens. Larger studies are needed to definitively determine whether the MVOA is more sensitive than the QVOA. However, the MVOA provides a novel simple and sensitive strategy for screening very large numbers of cells prior to the discontinuation of ART.

**Supplementary Data**

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary materials are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

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Potential conflicts of interest. All authors: No reported conflicts.

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