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Plasma Viremia and Cellular HIV-1 DNA Persist Despite Autologous Hematopoietic Stem Cell Transplantation for HIV-Related Lymphoma

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Abstract

A cure of HIV-1 has been achieved in one individual through allogeneic stem cell transplantation with a CCR5delta32 homozygous donor. Whether myeloablation and autologous stem cell transplantation for lymphoma in patients on suppressive ART can eliminate HIV-1 reservoirs is unknown. Low-level plasma viremia and total HIV-1 DNA and 2-LTR circles in blood mononuclear cells were quantified after autologous transplantation in 10 patients on suppressive ART using quantitative PCR assays capable of single copy nucleic acid detection. Plasma viremia was detectable in 9 patients and HIV-1 DNA was detectable in all 10 patients, indicating that HIV-1 had not been eliminated.

Keywords

HIV-1 pathogenesis; HIV-1 persistence; AIDS-related lymphoma

Introduction

Current antiretroviral therapy (ART) is effective in suppressing plasma viremia and preventing HIV-1 disease progression, but is not curative because of the persistence of HIV-1 in long-lived cells, primarily CD4+ memory T-cells.^{1–3} In addition, most patients on

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Authorship Contributions

J.J.R., J.A.Z., and J.W.M. designed the study. A.R.C. performed the experiments, and A.R.C. and J.W.M. analyzed the resulting data. A.K., J.A.Z., S.L., R.T.M., and D.K.M. provided samples for the study. A.R.C. and J.W.M. wrote the manuscript and all authors provided critical input.

effective ART have residual low-level viremia, derived from an unknown source, that can persist for at least 7 years.^{1–5} Allogeneic hematopoietic stem cell transplantation for relapsed acute myelogenous leukemia (AML) with a CCR5 $\Delta 32$ homozygous donor has achieved a drug-free cure of HIV-1 in one individual.^{6,7} Although this case report has generated excitement that a cure for HIV-1 is possible, the specific components of treatment for AML that were necessary to achieve a cure of HIV-1 are undefined. In particular, whether intensive myeloablative chemotherapy followed by autologous stem cell transplantation (ASCT) in patients on suppressive ART has an impact on reservoirs of HIV-1 is unknown.

In the era of suppressive ART, cancer remains a significant cause of HIV-related mortality in both developed and developing countries.^{8–10} ART has led to a significant decrease in AIDS-defining cancers, but the incidence and mortality of non-Hodgkin's lymphoma (NHL) remains high.^{8,11} Furthermore, the incidence of non-AIDS defining cancers including Hodgkin's lymphoma (HL) has increased in the era of ART.^{12,13} Many studies have demonstrated the feasibility of ASCT in HIV-1 patients with both NHL and HL, but the impact of chemotherapy and ASCT on reservoirs have not been evaluated.^{14–17} Given that intensive myeloablative chemotherapy causes depletion of CD4⁺ T-cells,^{18,19} HIV-1 infected cells might be expected to be similarly depleted. To date, only two studies have examined HIV-1 DNA in PBMC following ASCT^{20,21}. However, neither study examined low-level viremia or 2-LTR circles with highly-sensitive quantitative PCR (qPCR) assays in well-characterized patients with viremia suppressed by ART. We therefore evaluated low-level viremia, total HIV-1 DNA and 2-LTR DNA following myeloablative chemotherapy and ASCT in patients on effective ART with plasma HIV-1 RNA suppressed to <50 copies/mL before and after transplantation.

Material and Methods

We conducted a multicenter, cross-sectional study of patients with HIV-related lymphoma who underwent ASCT, had survival of at least 6 months following ASCT, and had plasma HIV-1 RNA suppressed on ART to <50 copies/mL. This study was approved by local institutional review boards and written informed consent was obtained from all study participants.

HIV-1 RNA from plasma was initially quantified using a RT-qPCR assay with single copy sensitivity targeting the *gag* region (gSCA) of the HIV-1 genome.²² If HIV-1 RNA was undetectable with gSCA, an alternative assay with single copy sensitivity (iSCA) targeting the p31 (integrase) region of the HIV-1 *pol* gene was performed. Total HIV-1 DNA and 2-LTR circles (2-LTRs) from PBMC were quantified using qPCR assays capable of single copy detection.²³ Total HIV-1 DNA and 2-LTRs were normalized for the number of cells assayed by qPCR for the *CCR5* gene.²⁴ Complete descriptions of these assays are available in the supplementary information. Pearson's correlation was used to determine if there was a correlation between time from transplant and HIV-1 DNA levels.

Results

The relevant clinical characteristics of the 10 patients studied are summarized in Table 1. The median age was 51 (range: 24–60) years old, patients were either Caucasian (N=7) or Hispanic (N=3), and were diagnosed with Burkitt's (N=3), Hodgkin's (N=3) or Non-Hodgkin's (N=4) lymphoma. All patients received a myeloablative chemotherapy conditioning regimen consisting of either CBV (cyclophosphamide, carmustine, etoposide; N=7) or BEAM (carmustine, etoposide, cytarabine, melphan; N=3), followed by ASCT with

mobilized hematopoietic progenitor cells obtained from peripheral blood as treatment for HIV-related lymphoma.

The duration of time from ASCT to sample collection for HIV-1 qPCR assays was variable, with a median of 686 days post-transplant (range: +28 to +4009 days). All patients had plasma viremia suppressed to <50 copies/mL on ART prior to ASCT. ART was given as tolerated during conditioning chemotherapy in 6 of 9 patients, was interrupted during chemotherapy in 3 of 9 patients, and information about ART during conditioning was unavailable for 1 patient. ART regimens consisted of 2–3 nucleoside reverse transcriptase inhibitors and either a non-nucleoside reverse transcriptase inhibitor (N=7) or a ritonavir-boosted protease inhibitor (N=3). Additional clinical information is available in the supplementary information.

As shown in Table 2, 6 of 10 patients had HIV-1 RNA detectable in plasma by gSCA, whereas in 4 patients no HIV-1 RNA was detected. Samples from the 4 patients with undetectable HIV-1 RNA were retested by qPCR targeting integrase (iSCA), and 3 of these patients were found to have detectable HIV-1 RNA (Table 2). The median plasma HIV-1 RNA was 2 copies/mL (range of <0.2 copies/mL to 26 copies/mL), which is consistent with levels previously reported after long-term suppressive ART in non-transplanted patients.^{4,5} One patient (PID 7) had no HIV-1 RNA detected (<0.2 copies/mL) by either the gSCA or iSCA assays performed on a total volume of 25 mL of plasma.

Total HIV-1 DNA and 2-LTRs were measured by qPCR on PBMC extracts and normalized for the number of cells assayed. Total HIV-1 DNA was detected in 9 of 10 patients using primers and probe targeting integrase. The patient (PID 9) with undetectable HIV-1 DNA by qPCR targeting integrase had 340 HIV-1 DNA copies/10⁶ PBMC detected using qPCR targeting HIV-1 *gag*. Taken together, all 10 patients had detectable HIV-1 DNA with a median of 208 copies/10⁶ PBMC (range: 12 to 415 copies/10⁶ PBMC). Of note, the patient (PID 7) with the highest level of HIV-1 DNA (415 cps/10⁶ PBMC) had undetectable plasma viremia by both RT-qPCR assays. There was no correlation between time post-ASCT and total HIV-1 DNA levels ($p=0.18$, $p=0.625$). 2-LTRs were not detectable in any of the patients in this study, but 2-LTRs were readily detected in positive controls run in parallel with the patient samples.

Discussion

We found that plasma viremia and HIV-1 DNA in PBMC persist despite myeloablation and ASCT for HIV-related lymphoma and despite suppression of plasma viremia to <50 copies/mL by ART prior to and following transplant. Our study is novel in that the patient group studied had undetectable plasma HIV-1 RNA by standard detection methods and that sensitive qPCR assays were required to reveal HIV-1 persistence, obviating the need for ART interruption to determine whether cure had been achieved. Simonelli et al. reported a significant decay in HIV-1 DNA 24 months following ASCT.²¹ In our study, HIV-1 DNA levels in blood did not correlate with time after ASCT, including follow-up of >4000 days after transplantation. The absence of detectable 2-LTRs in our patients is consistent with long-term suppressive ART and argues against ongoing viral replication in the patients studied.²³

One possible explanation for persistence of HIV-1 following ASCT is the presence of HIV-1 infected resting CD4⁺ T-cells that may be resistant to the chemotherapeutic agents used for myeloablation. Chemotherapy-mediated cell death favors metabolically active cells through a variety of mechanisms. The primary reservoir of HIV-1 infection is thought to be resting memory CD4⁺ T-cells, which are metabolically quiescent. Thus, although chemotherapy

reduces lymphocyte counts,^{18,19} HIV-1 infected resting CD4⁺ cells may not be profoundly affected. Along these lines, a recent report describes the survival of CD161^{hi} CD8⁺ central and effector memory T-cells despite myeloablative chemotherapy in AML patients.²⁵

Another cause of persistent HIV-1 infection may be the reinfusion of HIV-1 infected cells in the autologous transplant. If an average of 10^{11} mononuclear cells are reinfused after conditioning chemotherapy, approximately 15% of these cells (1.5×10^{10}) will be CD4⁺ T-cells. The estimated frequency of HIV-1 infected CD4⁺ T-cells in blood ranges from 1 in 100 (1.5×10^8) to 1 in 1000 (1.5×10^7), and approximately 1 in 1000 (1.5×10^4 to 1.5×10^5) HIV-1 infected CD4⁺ T-cells will harbor replication competent provirus.³ Consequently, the autologous graft is likely to contain 1×10^4 to 1×10^5 cells with replication competent proviruses that could serve as a source of persistent HIV-1.

In addition, IL-7 levels have been shown to increase in response to lymphopenia induced by chemotherapy,^{26,27} and IL-7 induced T-cell proliferation has been reported to contribute to the size and persistence of HIV-1 reservoirs in patients on ART.²⁸ Hence, homeostatic proliferation of CD4⁺ T-cells may have contributed to the persistence of HIV-1 following ASCT through expansion of the reinfused HIV-infected CD4⁺ T-cells. HIV-1 infected cells in autologous grafts could be reduced or eliminated by using purified CD34⁺ hematopoietic progenitor cells, but this approach is not favored because of delayed lymphocyte recovery following transplantation.²⁹

An important distinction between autologous and allogeneic hematopoietic stem cell transplantation is the occurrence of graft versus tumor response and graft versus host response following allogeneic transplantation.³⁰ Graft versus host response may have played a key role in eliminating HIV-1 infected host cells from the one individual cured of HIV-1 infection, and transplantation of CCR5^{-32/-32} donor cells was likely to have prevented infection of the allograft. Similarly, Henrich et al., recently reported the clearance of HIV-1 DNA from PBMC in 2 HIV-1 infected patients with lymphoma who received continuous ART and allogeneic stem cell transplantation from CCR5 wildtype donors.³¹ This report suggests that allogeneic transplantation and continuous ART to block HIV-1 infection of the allograft may have been sufficient to eliminate HIV-1 reservoirs, although proof of cure in these patients will require cessation of ART. Prior studies of allogeneic transplantation with wildtype CCR5 donor cells without continuous ART have resulted in HIV-1 rebound after cessation of ART probably because of infection of the allograft.³²

In summary, our findings show that myeloablation and autologous transplantation do not deplete HIV-1 reservoirs or cure HIV-1 infection. Genetic approaches targeting CCR5 in autologous cells have been explored,³³ but whether a safe, efficient and practical method can be achieved to knock out CCR5 in most cells targeted by HIV-1 is not known.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Clinical Characteristics of Patients Studied

PID	Age	Gender	Ethnicity	Lymphoma Diagnosis	Conditioning Regimen	Days Post-Transplant*	CD4+ T-cell count post-transplant (cells/ μ L)	ART regimen
1	48	M	Caucasian	BL	CBV	+ 637	752	EFV/TNF/FTC
2	49	M	Caucasian	NHL	CBV	+ 734	1015	EFV/TNF/FTC
3	39	M	Hispanic	BL	CBV	+ 2194	480	FPV/r/TNF/FTC
4	52	M	Caucasian	BL	CBV	+ 28	132	DRV/r/TNF/FTC
5	51	M	Caucasian	HL	BEAM	+ 210	398	EFV/TNF/FTC
6	55	M	Caucasian	HL	BEAM	+ 1288	422	LPV/r/TNF/FTC
7	53	M	Caucasian	NHL	CBV	+ 3970	697	ABC/3TC/NVP
8	24	M	Hispanic	HL	CBV	+ 92	210	TNF/FTC/NVP
9	60	M	Hispanic	NHL	CBV	+ 182	808	EFV/TNF/FTC
10	50	M	Caucasian	NHL	BEAM	+ 4009	920	ABC/AZT/3TC/NVP

* Samples obtained for current study

Abbreviations: PID, patient identifier; ART, antiretroviral therapy; M, male; BL, Burkitt's lymphoma; NHL, non-Hodgkin's lymphoma; HL, Hodgkin's lymphoma; CBV: cyclophosphamide, carmustine, etoposide; BEAM: carmustine, etoposide, cytarabine, melphalan; EFV, efavirenz; TNV, tenofovir; FTC, emtricitabine; FPV/r, ritonavir-boosted fosamprenavir; DRV/r, ritonavir-boosted darunavir, LPV/r, ritonavir-boosted lopinavir; ABC, abacavir; 3TC, lamivudine; NVP, nevirapine; AZT, zidovudine

Table 2

Plasma HIV-1 RNA and Cellular HIV-1 DNA after Autologous Stem Cell Transplantation

PID	gSCA HIV-1 Plasma RNA (cps/mL)	iSCA HIV-1 Plasma RNA (cps/mL)	Total PBMC Assayed by CCRS5	Total HIV-1 DNA (cps/10 ⁶ PBMC)	2-LTR Circles (cps/10 ⁶ PBMC)
1	1.9	NA	1.2 × 10 ⁶	12	<7.5
2	0.6	NA	1.3 × 10 ⁶	48	<7.5
3	<0.6	26	7.9 × 10 ⁵	253	<7.5
4	0.9	NA	1.0 × 10 ⁶	186	<7.5
5	<0.3	25	9.6 × 10 ⁵	156	<7.5
6	<0.4	2.0	5.7 × 10 ⁵	230	<7.5
7	<0.2	<0.2	6.0 × 10 ⁵	415	<7.5
8	7.6	NA	8.4 × 10 ⁵	359	<7.5
9	12.0	NA	1.2 × 10 ⁶	340 *	<7.5
10	0.7	NA	3.8 × 10 ⁵	120	<7.5

Abbreviations: PID, patient identifier; gSCA, gag-specific single copy assay; iSCA, integrase-specific single copy assay; cps, copies; PBMC, peripheral blood mononuclear cells; NA, not assessed

* Patient 9 had undetectable HIV-1 DNA using a primer/probe pair targeting integrase, but had detectable HIV-1 DNA using a primer/probe pair targeting *gag*