

Aviremia 10 Years Postdiscontinuation of Antiretroviral Therapy Initiated During Primary Human Immunodeficiency Virus-1 Infection and Association With Gag-Specific T-Cell Responses

Sabine Kinloch-de Loes,^{1,2,3,a} Lucy Dorrell,^{4,5,a} Hongbing Yang,^{4,5} Gareth A. D. Hardy,^{6,7} Sabine Yerly,⁸ Cristina Cellerai,^{1,9} Linos Vandekerckhove,^{10,11} Ward De Spiegelaere,^{10,11} Eva Malatinkova,^{10,11} Willie Wee Lee Koh,^{12,b} and Margaret A. Johnson^{1,2}

¹Division of Infection and Immunity, Royal Free Campus, University College London, ²Royal Free Hospital, London, ³Department of Immunology, University College London Medical School, Royal Free Campus, ⁴Nuffield Department of Medicine, ⁵Oxford National Institute of Health Research Biomedical Research Centre, University of Oxford, ⁶Centre for Immunology and Virology, Imperial College London, and ⁷Chelsea and Westminster Hospital, London, United Kingdom; ⁸Laboratory of Virology, Geneva University Hospital, and ⁹Division of Immunology and Allergy, Department of Medicine, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland; ¹⁰HIV Translational Research Unit, Ghent University, ¹¹University Hospital Ghent, Belgium; and ¹²Department of Immunology and Molecular Pathology, University College London Medical School, University College London Campus, United Kingdom

Combination antiretroviral therapy during primary human immunodeficiency virus-1 infection may enable long-term drug-free virological control in rare individuals. We describe a female who maintained aviremia and a normal CD4⁺/CD8⁺ T cell ratio for 10 years after stopping therapy, despite a persistent viral reservoir. Cellular immune responses may have contributed to this outcome.

Keywords. antiretroviral therapy; CD8 T cell; HIV-1 reservoir; primary HIV-1 infection; viral inhibition assay.

Achieving prolonged control of human immunodeficiency virus (HIV)-1 replication after antiretroviral therapy (ART)

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^aS. K.-d. L. and L. D. are co-first authors.

^bPresent Affiliation: Khoo Teck Puat Hospital, Clinical Research Unit, Singapore.

Correspondence: Lucy Dorrell, BM, DM, FRCP, Nuffield Department of Medicine Research Building, Old Road Campus, Headington, Oxford OX3 7FZ, UK (lucy.dorrell@ndm.ox.ac.uk).

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interruption is one of the aims of present research efforts towards eradication, to alleviate HIV-1 burden, drug toxicity, and costs. Initiation of ART during primary HIV-1 infection (PHI) is associated with reduced size and diversity of viral reservoirs and enhanced immune preservation, relative to chronic infection. This is reflected in prolonged aviremia in a minority of early treated seroconverters who discontinue ART [1–3]. Elucidating the underlying mechanisms may facilitate the development of curative therapy. In this study, we describe the favorable outcome of a woman who initiated ART during a severe acute retroviral syndrome. She stopped therapy 6 years later and has since experienced a decade of apparent spontaneous control. We report the virological and immunological features of this unusual case.

METHODS

Quantification of the Human Immunodeficiency Virus-1 Reservoir

Written permission was obtained from the patient for reporting of her case. The patient was infected with a clade C virus. During 17 years of follow-up, HIV-1 viremia was quantified with several validated assays, which included non-B primers in accordance with laboratory and clinical practice at the time. Low copy viremia was determined as described in [Supplementary Methods](#). Quantification of cell-associated HIV-1 DNA in blood sampled during 2007–2013 was performed as described previously [4, 5]. Integrated HIV-1 DNA was determined in samples from 2013 onwards by Alu-HIV polymerase chain reaction (PCR), in addition to quantification of total and episomal 2-long terminal repeat (LTR) circles [6, 7] ([Supplementary Methods](#)).

Human Leukocyte Antigen Typing

The patient's human leukocyte antigen (HLA) type was determined by ARMS-PCR using sequence-specific primers as follows: HLA A*0101, *3001, B*4901, Cw*0701, DRB1 *0804, DQB1 *0301 (homozygous at HLA-B, Cw, DR, and DQ loci).

Assessment of Human Immunodeficiency Virus-1-Specific Cellular Immune Responses

T-cell responses to the entire HIV-1 proteome were assessed initially using pools of clade B 15-mer peptides overlapping by 11 amino acids and, subsequently, corresponding overlapping clade C 18-mers, (NIH AIDS Reagent Program, final concentration 2 µg/mL) in ex vivo interferon (IFN)-γ enzyme-linked immunospot (ELISPOT) assays, as described previously [8]. Further details are given in [Supplementary Methods](#).

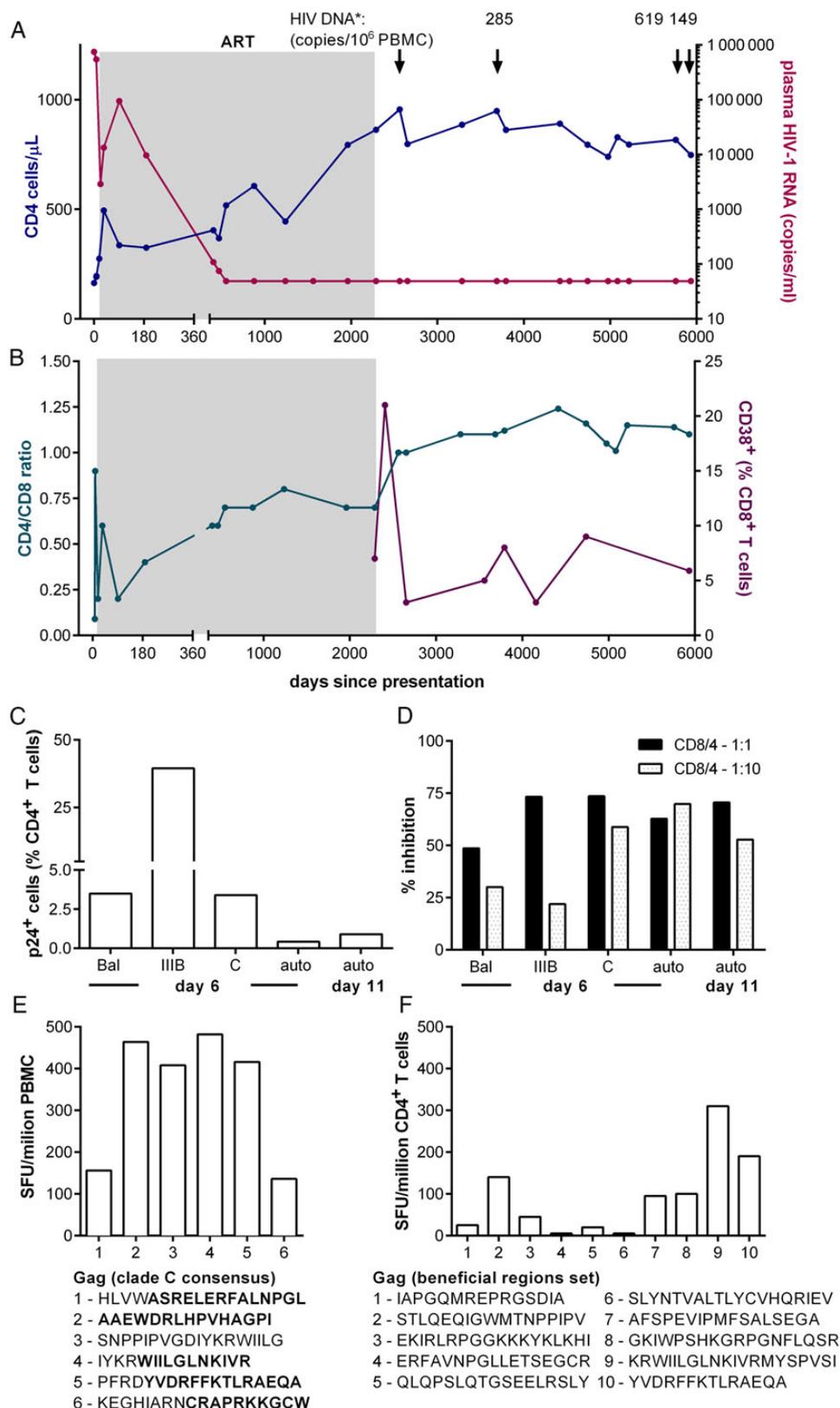


Figure 1. CD4 cell count and plasma viral load changes (A), CD4/CD8 cell ratios, and percentage of activated (CD38⁺) CD8 T cells (B) during primary infection, antiretroviral therapy, and posttreatment discontinuation are shown. Antiretroviral therapy regimen: nucleoside reverse-transcriptase inhibitors zidovudine/lamivudine days 12–2121 and tenofovir/lamivudine days 2122–2289; protease inhibitors indinavir days 12–22, ritonavir (full dose) days 23–203, and ritonavir/saquinavir days 204–2289. The assays used to quantify human immunodeficiency virus (HIV)-1 RNA were as follows, in chronological order: Roche Amplicor HIV-1 Monitor version 1.0 assay with non-clade B primers added (first 2 samples in 1997); Roche COBAS Amplicor HIV-1 Monitor, replaced

Unfractionated and CD8⁺-depleted peripheral blood mononuclear cells (PBMCs) were also tested in IFN- γ ELISPOT assays with pools of peptides representing “beneficial regions” that are preferentially targeted by individuals with reduced viral loads [9] (Supplementary Table 1).

Ex vivo CD8⁺ T-cell viral inhibitory activity was determined using a viral inhibition assay as described previously [8]. Primary CD4⁺ T cells were superinfected with laboratory-adapted or primary virus isolates (Programme EVA Centre for AIDS Reagents, National Institute for Biological Standards and Control) at a multiplicity of infection of 0.01: HIV-1BaL (CCR5-tropic, clade B), HIV-1IIIB (CXCR4-tropic, clade B), and HIV-1C (CCR5-tropic, clade C).

RESULTS

Case Report

A 23-year-old woman of Ethiopian origin presented in 1997 with a 3-week history of fever, sore throat, rash, lymphadenopathy, myalgia, and arthralgia. Her HIV-1 antibody test was positive with incomplete reactivity on HIV Western blot. She had tested HIV-1 seronegative in 1996. Three consecutive CD4⁺ T-cell counts were <200 cells/mm³, and her viral load was initially >750 000 HIV-1 copies (c)/mL. The patient started zidovudine 250 mg twice daily, lamivudine 150 mg twice daily, and indinavir 800 mg 3 times a day. The latter was subsequently switched to ritonavir 600 mg twice daily because of intolerance. She remained viremic (up to 94 400 HIV-1 c/mL) while on treatment and required intensification (twice daily saquinavir 400 mg and ritonavir 400 mg), which was initiated in May 1998. Suppression of viremia <50 HIV-1 c/mL was not achieved until approximately 1 year later, but it was sustained for nearly 5 years thereafter, at which point ART was stopped at the patient's request. The pre-ART discontinuation CD4⁺ T-cell count was 863 cells/mm³ (30%), with a CD4⁺/CD8⁺ T-cell ratio of 0.7. The patient has since remained asymptomatic with undetectable viremia, normal CD4⁺ T-cell counts, and a CD4⁺/CD8⁺ T-cell ratio >1 during 10 years of follow-up off ART (Figure 1). A comprehensive screen for antiviral agents by therapeutic drug monitoring on a blood sample from 2008 was negative (Delphic Laboratories Ltd, Merseybio Liverpool, UK). Furthermore, the

frequency of activated (CD38⁺) CD8⁺ T cells, which was measured during 2004–2010, remained within normal limits (3%–21%) (Figure 1B). Human leukocyte antigen typing revealed that the patient did not express any HLA class I alleles associated with long-term control of HIV.

Evidence of a Detectable Human Immunodeficiency Virus-1 Reservoir During Posttreatment Control

During the posttreatment period with aviremia, HIV-1 DNA was detected on 4 occasions: 2004 (not quantified), 2007, 2013, and 2014. Total HIV-1 DNA was 285, 619, and 149 copies/10⁶ PBMCs in 2007, 2013, and 2014, respectively. At 15 years of aviremia (2014), integrated HIV-1 DNA and 2-LTR circles were 134.3 (95% confidence interval [CI], 56.5–304.4) and 3.9 (95% CI, 0–9.15) copies/10⁶ PBMCs, respectively (Figure 1A). Viremia was undetectable by an ultrasensitive assay (<2 HIV-1 copies/mL).

CD8⁺ T Cells Sampled After 10 Years of Posttreatment Control Suppress Human Immunodeficiency Virus-1 Replication In Vitro

Ex vivo CD8⁺ T cells were tested for their capacity to inhibit the replication of clade B and C HIV-1 isolates and of endogenous HIV-1 in autologous CD4⁺ T cells in vitro. At a CD8⁺/CD4⁺ ratio of 1:1, CD8⁺ T cells demonstrated moderate antiviral activity against clade-matched (C, 73.5%) and mismatched (IIIB, 73.3%; BaL, 49%) virus isolates (Figure 1C and D). Efficient suppression of the clade C isolate was also observed at a lower CD8⁺/CD4⁺ T-cell ratio (1:10%–59% vs 30% for IIIB and 22% for BaL). Antiviral activity against the patient's endogenous virus was equivalent to HIV-1C at both CD8⁺/CD4⁺ T cell ratios (70.5% and 52.3% for 1:1 and 1:10 ratios, respectively) (Figure 1C and D).

Broad CD4⁺ T-Cell Responses Target Vulnerable Regions Within Gag

Interferon- γ -secreting CD8⁺ and CD4⁺ T cells were predominantly targeted to 14 regions within HIV-1 Gag, Pol, and Nef (Figure 1E, Supplementary Figure 1A–C). T-cell responses were also targeted to 7 vulnerable (beneficial) regions within the clade B and C viral proteomes (Supplementary Table 1) and were predominantly CD4⁺ T cell-mediated (Figure 1E and 1F, Supplementary Figure 1D).

Figure 1 continued. by version 1.5 (Ultrasensitive assay) in October 2004; Abbott LCx HIV-1 assay (January 2005–May 2005); Abbott Real-Time HIV-1 assay (May 2005 onwards). Antiretroviral agents included in therapeutic drug monitoring in 2008 were as follows: lamivudine, efavirenz, atazanavir, amprenavir, indinavir, nelfinavir, lopinavir, ritonavir, saquinavir, darunavir, tipranavir. (C) Susceptibility of the patient's CD4⁺ T cells to superinfection with clade (B) and (C) virus isolates and to induction of endogenous HIV-1: frequencies of p24 Ag⁺ CD4⁺ T cells after in vitro activation of CD8-depleted peripheral blood mononuclear cells (PBMCs), followed by superinfection and culture for 6 days, or culture without superinfection (autologous virus) for up to 11 days. (D) Inhibition of replication of superinfecting or autologous viruses by patient's CD8⁺ T cells after 6 days' coculture at CD8/CD4 cell ratios indicated. (E) Responses to individual clade C consensus Gag peptides identified in the peptide matrix assays were confirmed by interferon (IFN)- γ enzyme-linked immunospot (ELISPOT) assays with unfractionated PBMCs. Negative control values have been subtracted. Some of these peptides overlapped “beneficial” regions within clade B and C Gag that were previously associated with virological control—shown in bold [9]. (F) CD4⁺ T-cell responses to peptides representing beneficial regions within clade B/C Gag (listed in Supplementary Table 1) were determined in IFN- γ ELISPOT assays with CD8-depleted PBMCs.

DISCUSSION

This case shows that long-term control of viremia and normalization of immune parameters may follow discontinuation of prolonged ART initiated at PHI, even in the context of persistent viremia of nearly $5 \log_{10}$ HIV-1 c/mL during initial ART and the absence of favorable HLA alleles. Such features would typically preclude long-term nonprogressor status [10].

Salgado et al and Sáez-Ciri3n et al have reported post-ART virological control after treatment during PHI, despite initial viremia of up to $7 \log_{10}$ c/mL. The majority of these subjects had an inducible viral reservoir, indicating that they were infected with replication-competent viruses [2, 3]. We confirmed that our patient's CD4⁺ T cells were susceptible to superinfection with R5- and X4-tropic viruses, with similar frequencies of infected cells to those we have observed following in vitro infection of healthy donor PBMCs [11]. Furthermore, we were able to detect outgrowth of endogenous HIV-1 in CD4⁺ T cells after in vitro activation, albeit at a low level, indicating a persistent inducible reservoir. Although posttreatment controllers thus show some virological similarities with patients with spontaneous elite control of HIV-1, an intriguing difference is the lower activation status in the former [12]. Our patient also showed posttreatment control of immune activation: the frequency of CD38⁺ CD8⁺ T cells has remained within normal limits throughout follow up.

With regard to HIV-specific immune responses, this case is distinct from previously described posttreatment controllers [2, 3]. CD8⁺ T-cell inhibition of both heterologous and autologous HIV-1 replication was detected at a low CD8⁺/CD4⁺ cell ratio. Although these responses were less potent than was observed in HIV controllers (median 85%, $n = 20$) [8, 13], they nevertheless surpassed that of chronic ART-treated individuals who were virologically suppressed for ≥ 1 year (median 24%, $n = 42$; our unpublished observations). This result suggests that our patient's CD8⁺ T-cell antiviral responses were likely to be a contributing factor to her virological control, rather than a consequence of it. The preserved and broad HIV-1-specific CD4⁺ T-cell responses observed in this patient are also particularly surprising when taking into account the profound CD4⁺ T-cell depletion observed before ART initiation. It is noteworthy that this patient had detectable CD4⁺ T cell responses (polyfunctional and proliferative) in 2004 and 2008 (Supplementary Figures 2–4). Furthermore, we were able to show that CD4⁺ T cells were targeted to multiple regions of vulnerability within Gag. Such responses have been associated with spontaneous control of viremia [9, 14]. It is not known whether this association is mediated by direct cytolytic mechanisms or indirectly, through provision of effective help to CD8⁺ T cells. This deserves further exploration. We also considered the possibility that humoral responses contributed to this patient's posttreatment control, because broadly neutralizing antibodies may play a role in controlling viremia in rare cases [15]. However, we found

only low levels of neutralizing antibodies to heterologous virus in this case, when tested during the early period of aviremia in 2006–2007 (data not shown).

CONCLUSIONS

In conclusion, we have identified an atypical case of posttreatment control, which may have been achieved through very early ART during PHI combined with effective cell-mediated immune responses. Our data suggest that strategies aiming to induce such immune responses during early ART might influence posttreatment virological control.

Supplementary Material

Supplementary material is available online at Open Forum Infectious Diseases (<http://OpenForumInfectiousDiseases.oxfordjournals.org/>).

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Potential conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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