



**HAL**  
open science

## **Dramatic HIV DNA degradation associated with spontaneous HIV suppression and disease-free outcome in a young seropositive woman following her infection**

Philippe Colson, Catherine Dhiver, Catherine Tamalet, Jeremy Delerce, Olga Glazunova, Maxime Gaudin, Anthony Levasseur, Didier Raoult

► **To cite this version:**

Philippe Colson, Catherine Dhiver, Catherine Tamalet, Jeremy Delerce, Olga Glazunova, et al.. Dramatic HIV DNA degradation associated with spontaneous HIV suppression and disease-free outcome in a young seropositive woman following her infection. *Scientific Reports*, 2020, 10 (1), 10.1038/s41598-020-58969-6 . hal-02481077

**HAL Id: hal-02481077**

**<https://amu.hal.science/hal-02481077v1>**

Submitted on 18 Dec 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

OPEN

# Dramatic HIV DNA degradation associated with spontaneous HIV suppression and disease-free outcome in a young seropositive woman following her infection

Philippe Colson<sup>1,2,3</sup>, Catherine Dhiver<sup>1,3</sup>, Catherine Tamalet<sup>1</sup>, Jeremy Delerce<sup>1</sup>, Olga O. Glazunova<sup>1</sup>, Maxime Gaudin<sup>2</sup>, Anthony Levasseur<sup>1,2</sup> & Didier Raoult<sup>1,2\*</sup>

Strategies to cure HIV-infected patients by virus-targeting drugs have failed to date. We identified a HIV-1-seropositive woman who spontaneously suppressed HIV replication and had normal CD4-cell counts, no HIV-disease, no replication-competent virus and no cell HIV DNA detected with a routine assay. We suspected that dramatic HIV DNA degradation occurred post-infection. We performed multiple nested-PCRs followed by Sanger sequencing and applied a multiplex-PCR approach. Furthermore, we implemented a new technique based on two hybridization steps on beads prior to next-generation sequencing that removed human DNA then retrieved integrated HIV sequences with HIV-specific probes. We assembled  $\approx 45\%$  of the HIV genome and further analyzed the G-to-A mutations putatively generated by cellular APOBEC3 enzymes that can change tryptophan codons into stop codons. We found more G-to-A mutations in the HIV DNA from the woman than in that of her transmitting partner. Moreover, 74% of the tryptophan codons were changed to stop codons (25%) or were deleted as a possible consequence of gene inactivation. Finally, we found that this woman's cells remained HIV-susceptible *in vitro*. Our findings show that she does not exhibit innate HIV-resistance but may have been cured of it by extrinsic factors, a plausible candidate for which is the gut microbiota.

The evolution of vertebrates has included the integration in their genomes of multiple retrovirus sequences<sup>1</sup>. Humans are not the exception to the rule as  $\approx 8\%$  of their genome consist of retroviral DNA. Most integrated retrovirus sequences were inactivated by substantial degradation, and only remain as relics of ancient retrovirus epidemics<sup>2,3</sup>. This general biological phenomenon that consists in the cannibalism of the DNA from viral invaders has been revealed to be on-going in koalas with retroviruses causing an AIDS-like syndrome<sup>4</sup>. Regarding HIV, the problem of prevention and cure of HIV infection has not been solved since its discovery<sup>5,6</sup>. Three cases of HIV-1 remission have been described including two following CCR5 delta32/delta32 hematopoietic stem-cell transplantation<sup>7-9</sup>. However, some pathways could open new therapeutic and preventive avenues. An alternative option to cure patients of HIV might be to strengthen, if identified, innate antiviral defenses. A group of cellular enzymes named Apolipoprotein B mRNA Editing Catalytic Polypeptide-like 3 (APOBEC3) exists whose function is to destroy invading viruses, including retroviruses<sup>10</sup>. The predominant role of APOBEC3 is to deaminate cytosines (Cs) that are changed to thymines (Ts), which leads to guanine (G)-to-adenine (A) mutations in integrated viral DNA. The genomes of HIV and SIV in great apes encode a protein, Vif, that inhibits their action<sup>11</sup>. However, it has been evidenced in gorillas that a single mutation in the APOBEC3G gene can confer resistance to SIV from chimpanzees by counteracting Vif activity<sup>11</sup>. In addition, it was recently shown that a patient experienced a dramatic decrease in peripheral blood mononuclear cell (PBMC) HIV-1 DNA load in response to a release of immunity by monoclonal antibodies targeting PD-1<sup>12</sup>, whose activity is known to be modulated by the gut microbiota<sup>13,14</sup>.

<sup>1</sup>IHU Méditerranée Infection, 19-21 boulevard Jean Moulin, 13005, Marseille, France. <sup>2</sup>Aix-Marseille Univ., IRD, AP-HM, MEPHI, 19-21 boulevard Jean Moulin, 13005, Marseille, France. <sup>3</sup>These authors contributed equally: Philippe Colson and Catherine Dhiver. \*email: [didier.raoult@gmail.com](mailto:didier.raoult@gmail.com)

Moreover, the regulation of immune responses by exogenous factors including the microbiota is an emerging field in cancer immunotherapy<sup>13,14</sup>. Thus, the immune control of HIV infection under the influence of exogenous factors is not a theoretical impossibility.

We previously described two HIV-1-seropositive patients who we believe might have been spontaneously cured of HIV<sup>15,16</sup>. Indeed, although they never received antiretrovirals, they persistently have a suppressed HIV replication, normal CD4 T cell counts, and no HIV-related disease for more than 10 years (one was HIV-diagnosed in 1985). In addition, no replication-competent HIV was retrieved by culture, and HIV DNA was not detected in peripheral blood mononuclear cells (PBMC) by our diagnosis assay. PBMC HIV DNA was only laboriously obtained by performing hundreds of PCR. While searching for other index cases to understand if it is possible to be cured spontaneously of HIV, we investigated a third case.

## Methods

**Sample collection.** Samples were obtained from the case in January 2015, September 2015 and January 2017 in an attempt to obtain the greatest number of HIV sequences from peripheral blood mononuclear cells (PBMCs). Peripheral blood collected in May 2019 was also used for PBMC HIV co-culture with a procedure that included prior PBMC stimulation by phytohemagglutinin and interleukin 2, testing of PBMC susceptibility to HIV strains, and HIV seroneutralization. More invasive specimens representing possible deep HIV reservoirs were neither available nor sampled in this woman. Samples were obtained from the transmitting partner in January 2017 and in June 2019. This study was approved by our institution's ethics committee (ethics committee of IHU Méditerranée Infection) (N°2018–001). All research was performed in accordance with relevant guidelines/regulations. Informed written consent was obtained from the patients.

**PCR amplification, Sanger sequencing and multiplex PCR technique.** HIV-1 DNA Sanger population sequencing was performed as described previously<sup>15</sup>. All HIV genes were targeted by at least one PCR system (see supplementary information), and all PCRs were conducted in quadruplicate. PCR positivity was determined based on obtaining an HIV sequence by Sanger sequencing. A multiplex PCR technique called “Bortsch” was also performed as described previously<sup>15</sup>.

**Human DNA depletion, HIV-1 DNA enrichment procedures and Illumina next-generation sequencing of DNA extracted from the woman PBMCs.** *Human and HIV-1-specific probe design.* Whole human-specific probes (baits) were constructed as described in a previously developed protocol<sup>17</sup> with modifications<sup>18</sup>. A full-length human genome derived from a modern reference individual (HapMap individual NA21732; Coriell Institute for Medical Research, Camden, NJ) was used as a template to generate biotinylated RNA “bait” libraries spanning the entire human genome. For the design of HIV-1-specific probes, full-length HIV-1 genomes or HIV-1 DNA fragments were fenestrated using a Perl script into 120 nucleotide-long fragments with a sliding window of 60 nucleotides. The targeted HIV-1 sequences were genomes from the set of HIV-1 reference genomes of the Los Alamos National Laboratory database, National Institute of Allergy and Infectious Diseases, National Institutes of Health (<https://www.hiv.lanl.gov/content/sequence/NEWALIGN/align.html#comp>), HIV genomes obtained from two patients whose cases were previously described<sup>15</sup>, and HIV-1 DNA that had been recovered from the woman and her transmitting partner. The set of 20,000 probes was synthesized by Arbor Biosciences (Arbor Biosciences, Ann Arbor, MI, USA).

*Library preparation for the next-generation sequencing.* DNA extraction was performed on 200 µL of PBMCs ( $\approx 2 \times 10^5$  cells) collected from the woman, using the EZ1 Virus Mini Kit v2.0 (Qiagen Hilden, Germany) according to the manufacturer's protocol. Five paired-end libraries were prepared using 1 ng of extracted DNA and MiSeq Technology with the paired-end method and the Nextera XT kit (Illumina Inc., San Diego, CA, USA). DNA was fragmented, and adaptors containing the Illumina P5/P7 primer sequences and tags were added.

*Human DNA depletion procedure.* Five depletions of human nucleic acids were performed separately by hybridization of 50 µL of each prepared Illumina library ( $\approx 100$  ng of DNA) with 500 ng of biotinylated human RNA-bait library. Targeted fragment/probe heteroduplexes were captured using magnetic streptavidin-harboring beads (MyOne Streptavidin C1 Dynabeads (Life Technologies, Carlsbad, USA)), as previously described<sup>18</sup>. The unbound fraction (supernatant) was concentrated and cleaned using  $1.8 \times$  AMPure XP beads (Beckman Coulter, Fullerton, CA, USA) according to the manufacturer's protocol with elution into 30 µL of 1X TE buffer. The five purified fractions were then pooled and concentrated using a MinElute PCR Purification Kit (Qiagen) according to the manufacturer's protocol with elution in 10 µL of elution buffer. To generate sufficient material for targeted enrichment, this product was amplified using eight PCR amplification cycles with Illumina P5/P7 primers, before purification using a MinElute PCR Purification Kit (Qiagen) and elution with 10 µL of elution buffer.

*Targeted HIV enrichment through hybridization capture.* A total of 500 ng of the human-depleted library was used to perform the targeted HIV enrichment step involving hybridization with the HIV-specific probes using a myBaits target capture kit (Arbor Biosciences) according to the manufacturer's instructions (Hybridization capture for targeted NGS manual version 4.01). Hybridization-based capture reactions with undiluted HIV-1 probes (500 ng) was performed at 65 °C for 16 h. Streptavidin-coated magnetic beads (myBaits kit) were added to the hybridization mixture, and the sample was additionally incubated for 5 min at 65 °C. After washing steps, beads were resuspended in 30 µL of 10 mM Tris-Cl, 0.05% Tween-20 solution (pH 8.0–8.5). The captured DNA

was dissociated from beads by heating the suspension at 95 °C for 5 min. HIV-1 DNA and human albumin DNA were quantified by a multiplex real-time PCR assay as previously described<sup>15</sup>.

**Next-generation sequencing and sequence read analysis.** The product of the HIV enrichment procedure was normalized according to the Nextera XT protocol for pooling and sequencing on a MiSeq instrument (Illumina). A single run of 39 h generating  $2 \times 250$  base pairs was carried out for paired-end sequencing and cluster generation. Reads were filtered based on their quality, generated paired reads were imported into the CLC software (<https://www.qiagenbioinformatics.com/products/clc-genomics-workbench/>) and then assembled by mapping to the HIV genome GenBank accession no. K03455.1 (HIV-1 strain HXB2) and to sequences obtained from the woman and transmitting partner PBMCs. Reads identified as corresponding to HIV sequences were exported as fasta and SAM files.

### Comparisons of HIV-1 sequences obtained from the woman PBMCs and her transmitting partner PBMCs, and analysis of G-to-A mutations and of substitution of tryptophan codons by stop codons.

A custom script written in Python language was used to analyze the SAM file generated from the mapping of reads obtained by next-generation sequencing, and to count differences in amino acids between HIV sequences recovered from the PBMCs of the woman and her transmitting partner. In addition, alignments of nucleotide sequences obtained by Sanger and next-generation sequencing were performed using the MUSCLE program<sup>19</sup>. The phylogenetic analysis was performed using the MEGA6 software ([www.megasoftware.net](http://www.megasoftware.net)) with the neighbor-joining method. Moreover, nucleotide sequences were incorporated into a Microsoft Excel file (one nucleotide per cell) for their analysis using sequences from the HIV-1 K03455.1 genome (HXB2 strain) or those obtained from the transmitting partner PBMCs as references. An alignment was also performed using the MUSCLE program<sup>19</sup> for amino acid sequences obtained by translation of the nucleotide sequences from the three open reading frames using the Transeq online tool ([https://www.ebi.ac.uk/Tools/st/emboss\\_transeq/](https://www.ebi.ac.uk/Tools/st/emboss_transeq/)) and amino acid sequences from the proteins of the HXB2 strain as references. These sequences were added into the Microsoft Excel file (one amino acid per cell) alongside the corresponding nucleotide sequences. G-to-A mutations including those associated with substitution of tryptophan codons (TGG) by stop codons (TGA, TAG, or TAA) and generated by APOBEC3 enzymes<sup>10</sup> were searched using the Microsoft Excel software. In addition, we used the Hypermut tool ([www.hiv.lanl.gov/HYPERMUT/hypermut.html](http://www.hiv.lanl.gov/HYPERMUT/hypermut.html)) to count G-to-A mutations in HIV DNA sequences obtained from the PBMCs of the woman with, as reference, HIV DNA sequences obtained from the PBMCs of her transmitting partner<sup>20</sup>.

**HIV culture assay.** HIV co-culture using PBMC from the woman and those from her transmitting partner, and testing for woman PBMC resistance to a laboratory HIV strain (NL4.3) and to the HIV strain cultured from the transmitting partner PBMCs were conducted as previously described<sup>15,21</sup> (see Supplementary information). HIV seroneutralization experiments were also conducted as previously described<sup>15</sup>, with plasma from the woman added undiluted or at a 1:100 dilution to the culture medium, and using PBMCs from the woman and the HIV strain cultured from the transmitting partner (see Supplementary information).

**APOBEC3 gene sequencing and expression level assessment.** APOBEC3G DNA sequence was obtained by Sanger sequencing using in house protocols (see Supplementary information). The expression of the APOBEC3G and APOBEC3F genes was measured by quantifying messenger RNA (mRNA) in the PBMCs using quantitative real-time reverse transcriptase (RT)-PCR assays (APOBEC3G: previously described primers<sup>22</sup> (see Supplementary information) and hydrolysis probe 5'-FAM-TGTTATGAGGTGGAGCGCATGCA-TAMRA; APOBEC3F: primers A3F\_MBF: 5'-GGCAATGTATCCACACATATCTACTT and A3F\_MBR: 5'-CAGGAGATA GGTGAGTGGTGCT, probe A3F\_MBP: FAM-5'-GCAAAGCCTATGGTCGGAACGAAA). DNA/RNA extraction from the PBMCs of the woman, of her transmitting partner, and of two HIV-seronegative individuals used as controls was performed using the EZ1 Virus Mini kit with a EZ1 Advanced XL instrument (Qiagen, Courtaboeuf, France), according to the manufacturer's recommendations. Then, this extract was treated with the TURBO DNA-free DNase kit (Ambion, Austin, TX, USA). Real-time reverse transcriptase (RT)-PCR was performed by an in house real-time PCR protocol with the SuperScript<sup>®</sup> III Platinum<sup>®</sup> One-Step qRT-PCR Kit (Invitrogen, Carlsbad, CA, USA) on a CFX96 Touch real-time PCR detection system (Bio-Rad, Hercules, CA, USA). PCR amplifications were performed using the following conditions: 50 °C for 30 min, 95 °C for 2 min, then 45 cycles including 20 s at 95 °C and 1 min at 60 °C. Numbers of PBMCs were measured using a plasmid containing a sequence of human albumin gene<sup>15</sup> (see Supplementary information). Tests were performed in duplicates. Quantitative RT-PCR results were normalized using albumine amplification levels and calculated by the  $2^{-\Delta\Delta CT}$  comparative method.

**Supplementary material.** HIV sequences obtained in the present study are available at <https://www.mediterranee-infection.com/acces-ressources/donnees-pour-articles/hiv/> or have been submitted to GenBank (submission ID: 2225641).

## Results

The patient is a 37-year-old woman sexually infected with HIV-1 between 2002 and 2004 by a single partner and diagnosed as seropositive in July 2006 (see Supplementary Fig. S1). She never received antiretrovirals except in 2012 during the third trimester of her pregnancy (zidovudine, 300 mg/d). Nevertheless, to date, she has persistently had normal CD4 T lymphocyte counts (mean value ( $\pm$ standard deviation) between 2006 and 2018,  $1,221 \pm 172/\text{mm}^3$ ) and remained free of HIV-related disease. In addition, HIV RNA was not detected in plasma using commercialized PCR assays on ten occasions during follow-up (using most recently Abbott RealTime HIV-1 and Beckman-Coulter Veris HIV-1 assays (detection limits: 40 and 35 copies/mL, respectively)), and no

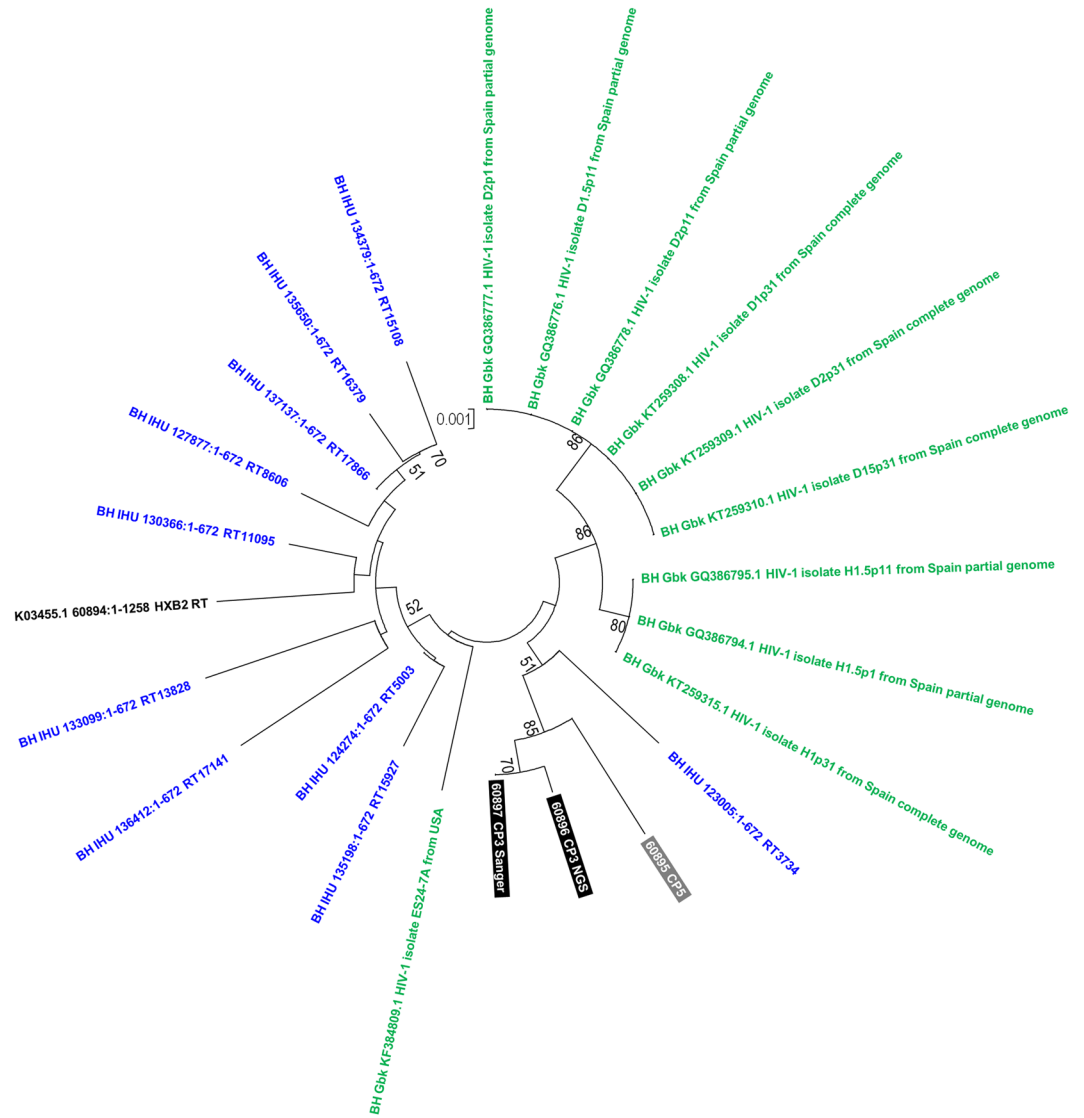
Patients	PCR testing		P
	Number performed	Number of positives (%)	
Present case: woman	392	26 (7)	$<10^{-5}$ $<10^{-5}$ 0.006
Transmitting partner	59	25 (42)	
Case No. 1 <sup>15</sup>	218	44 (20)	
Case No. 2 <sup>15</sup>	253	34 (13)	

**Table 1.** Proportions of positive HIV DNA test results for PCR systems used on DNA of peripheral blood mononuclear cells from HIV seropositive cases and controls. Cases No. 1 and No. 2 are two patients whose cases were previously reported<sup>15</sup> and from whom HIV could neither be detected by standard PCR assays nor cultured, whereas very small amounts of HIV DNA could be laboriously retrieved from peripheral blood mononuclear cell DNA.

replication-competent HIV was retrieved by co-culture on two occasions, including using PBMCs collected in 2019 as determined by HIV-1 RNA or DNA undetectability after 28 days of culture, whereas the same culture procedure allowed growing the virus from the PBMCs of the transmitting partner. Moreover, no PBMC HIV-1 DNA was detected by routine diagnostic tests<sup>15</sup> (detection limit: 20 copies/ $10^6$  PBMCs) on seven occasions between 2010 and 2019. However, the woman PBMCs were found to be susceptible to the HIV-1 NL4-3 strain and to the HIV strain cultured from the PBMCs of her transmitting partner (see Supplementary Figs. S3 and S4). In addition, her plasma did not neutralize infection of her PBMCs by the HIV-1 strain obtained from the PBMCs of her transmitting partner (see Supplementary Fig. S5). These findings indicate that this woman acquired the capability to combat HIV after her infection and suggests the role of an extrinsic factor. Moreover, she was not infected with a defective HIV strain as in her transmitting partner, a 30-year-old man HIV-diagnosed in 1990, the HIV DNA load was 350 copies/million PBMCs, and the CD4 T cell count fell to  $<200/\text{mm}^3$ , which required antiretroviral therapy.

Based on these findings and our previous work<sup>15</sup>, we suspected that the HIV genome in this young woman had been drastically degraded after its integration. We attempted to obtain fragments of HIV DNA from her PBMCs and assess their degradation by using thorough molecular procedures and the transmitting partner's HIV sequences as a reference. We used different strategies to retrieve the maximum number of HIV sequences from the PBMCs of this woman in whom standard assays had failed to detect HIV DNA. First, we performed nested PCR targeting HIV sequences from the literature, including with a multiplex PCR technique<sup>15</sup>. During these steps, HIV sequences were obtained by Sanger sequencing from 26 (7%) of 392 nested PCRs performed on the woman PBMCs (Table 1). The mean PCR product size was  $251 \pm 201$  nucleotides, 54% being shorter than 200 nucleotides, and they assembled into 3,254 nucleotide-long noncontiguous fragments classified as of HIV-1 subtype B (Fig. 1). HIV sequences obtained from the woman matched those of the near full-length HIV genome (9,337 nucleotides) recovered from the PBMCs of her partner, confirming that this patient was the transmitting partner and that the young woman was infected with a nondefective viral strain and eliminating a contamination. Second, for the first time, we implemented a technique using two successive steps of uptake on probe-coated beads. The two steps consisted of human DNA depletion with human DNA-targeting probes followed by HIV DNA enrichment with a set of HIV probes complementary to the transmitting partner HIV sequences. This enriched HIV DNA was thereafter nonspecifically amplified and sequenced by Illumina next-generation sequencing. We obtained 73 reads through this procedure that increased the length of the assembled HIV genome by 17% (1,133 nucleotides) (Fig. 2a; see Supplementary information). Overall, by these three approaches and carrying out hundreds of manipulations, we obtained a set of noncontiguous fragments covering 4,387 nucleotides of integrated HIV DNA of this young woman.

Then, as the genome of the HIV strain that infected this young woman had been obtained from her transmitting partner, we were able to determine the number of G-to-A mutations attributed to APOBEC3G activity in her HIV DNA, and specifically the number of tryptophan codons eliminated either by their change to stop codons or because fragments of DNA containing the tryptophan codons were lost. G-to-A mutations were observed in the woman's HIV DNA at 152 (16%) positions of the HIV DNA from the transmitting partner that only harbored G, and a G-to-A excess was detected in the woman compared to her transmitting partner, notably for the genes encoding HIV reverse transcriptase; integrase; Vif, which counteracts APOBEC3G; Vpr; Vpu; and Env gp120 (Table 2; see Supplementary Table S1). We thereafter determined that HIV sequences of the woman only covered 47 (52%) of the 91 tryptophan codons of the HIV genome; however, these codons were all present in sequences retrieved from the transmitting partner (Table 3; Fig. 2b). In the woman's HIV DNA, changes from a tryptophan codon to a stop codon were observed 5 times in *vif* and *gp120* envelope genes, 4 times in integrase gene, and twice in *gag*, reverse transcriptase, *vpr* and *vpu* genes, contrasting with no such changes in HIV DNA from the transmitting partner. With regard to HIV sequences generated by next-generation sequencing of DNA from the woman PBMCs, 67 (74%) of the 91 tryptophan codons present in the transmitting partner HIV DNA were either retrieved but changed to stop codons (as observed for 17 (63%) of 27 tryptophan codons on average (Table 4)) or located in HIV-1 DNA regions that we did not retrieve as a possible consequence of their degradation or loss. Thus, G-to-A mutations, the absence of codon coverage, or both states combined were significantly more frequent at tryptophan codons than at any other codon. Overall, we found that G-to-A mutations generating stop codons occurred in at least one sequence at 23 (49%) of the 47 covered tryptophan codons (at



**Figure 1.** Phylogenetic analysis of HIV reverse transcriptase sequences obtained from the PBMC DNA of the woman and her transmitting partner. The HIV-1 genome fragment analyzed here corresponds to a 671-nucleotide alignment generated from sequences of the reverse transcriptase-encoding gene and corresponding to nucleotides 2,596–3,266 of the HIV-1 genome GenBank accession no. K03455.1. Sequences obtained from the present cases are indicated by a bold white font and a black (CP3, woman) or a gray (CP5, transmitting partner) background. The 10 sequences with the highest BLAST score recovered from the NCBI GenBank nucleotide sequence database (<http://www.ncbi.nlm.nih.gov/nucleotide/>), labeled with BH Gbk (for best BLAST hit GenBank) and indicated by a green font, and from our local sequence database, labeled with BH IHU (for best BLAST hit IHU-Méditerranée Infection) and indicated by a blue font, were incorporated in the phylogeny reconstruction. Nucleotide alignments were performed using the MUSCLE software (<http://www.ebi.ac.uk/Tools/msa/muscle/>). The evolutionary history was inferred in the MEGA6 software (<http://www.megasoftware.net/>) using the neighbor-joining method and the Kimura 2-parameter method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree; the scale bars indicate the number of nucleotide substitutions per site. Bootstrap values >50% are labeled on the tree. NGS, next-generation sequencing.

9/35 (26%) and 15/23 (65%) of those covered by Sanger and next-generation sequencing, respectively ( $p = 0.002$ ; Mantel-Haenszel Chi-square test)) in viral sequences recovered from the woman, whereas no such change was detected in HIV sequences obtained from the transmitting partner at any of the 91 covered tryptophan codons. Mutations changing tryptophan codons into stop codons were hence more numerous in sequences obtained by next-generation than Sanger sequencing. The Hypermut tool<sup>20</sup> also detected the presence of numerous G-to-A mutations attributable to APOBEC3 enzymes in 73 of the 99 HIV sequences retrieved from the woman PBMCs either by next-generation or by Sanger sequencing, using as reference HIV DNA from her transmitting partner. In addition, with respect to the dinucleotide context, GG-to-AG and GA-to-AA mutations were observed in



HIV-1 gene	Number of positions harboring G-to-A mutations	
	Number	%
<i>gag</i>	15	12
<i>pol protease</i>	6	15
<i>pol reverse transcriptase</i>	20	9
<i>pol RNase H</i>	6	29
<i>pol integrase</i>	45	26
<i>vif</i>	30	23
<i>vpr</i>	13	17
<i>tat</i>	4	9
<i>vpu</i>	17	33
<i>rev</i>	4	11
<i>env gp120</i>	17	19
<i>env gp41</i>	1	3
<i>nef</i>	2	13

**Table 2.** G-to-A mutations detected in HIV DNA fragments obtained by Sanger or next-generation sequencing from woman PBMCs at G-harboring positions in the transmitting partner's HIV DNA.

HIV-1 gene	Number (%) of sequences harboring mutations generating tryptophan codon-to-stop codon changes	
	Woman PBMCs (Sanger/NGS)	Transmitting partner PBMCs
<i>gag</i>	–/2 (50%)	0
<i>pol protease</i>	–/1 (100%)	0
<i>pol reverse transcriptase</i>	0/2 (50%)	0
<i>pol integrase</i>	0/4 (67%)	0
<i>vif</i>	1 (11%)/5 (71%) (1 in common)	0
<i>vpr</i>	1 (17%)/2 (100%) (1 in common)	0
<i>tat</i>	0/–	0
<i>vpu</i>	2 (50%)/0	0
<i>rev</i>	–/–	0
<i>env gp120</i>	6 (86%)/0	0
<i>env gp41</i>	–/–	0
<i>nef</i>	–/–	0

**Table 3.** Tryptophan-to-stop codon mutations detected in HIV DNA fragments obtained from the woman and transmitting partner PBMCs by Sanger and next-generation sequencing. –, no sequence; NGS, next-generation sequencing.

which is the signature of APOBEC3 cellular enzymes, and led to gene inactivation by changing tryptophan codons into stop codons. Clinically, the woman never developed immunodeficiency or HIV-related symptoms, which suggests that she was cured of HIV<sup>16</sup>. The fate of HIV infection was totally different in her transmitting partner although both individuals were infected with a same HIV strain. These findings highlight that the different outcome relied on host response to infection, not on the viral strain. Although we cannot ascertain that this woman was not infected with an already partially defective virus, the woman harbored far less abundant and more degraded HIV DNA than her transmitting partner, revealing a more extensive action of APOBEC3 enzymes. In humans, greater APOBEC3G amounts in blood resting memory CD4 cells were associated with lower PBMC HIV DNA levels<sup>23</sup>. In addition, increased rates of G-to-A mutations were observed among HIV-seropositive individuals who spontaneously suppress HIV replication<sup>15,24</sup>.

The present case is critical because it shows that some individuals are likely to inactivate integrated HIV after infection. The investigation of such outcomes of HIV infection has been neglected. Possibly, this is in part because they contradict the current dogma that people cannot be cured of HIV neither by antiretroviral drugs and still less spontaneously. We cannot exhaust all the questions raised by the present case. Nonetheless, as this woman's cells were found to be, at least currently, susceptible to HIV infection by a laboratory strain and the strain from her transmitting partner, this phenomenon could be linked to extrinsic factors. A possible explanation is that the microbiota of this woman hyperactivated APOBEC3 enzymes. Preliminary work has highlighted that bacterial components could stimulate the expression of APOBEC3G and enhance its activity on HIV genomes, which is certainly a path worthy of exploration<sup>22</sup>. The modulation of the immune response by the digestive microbiota, in particular in the ileum where lymphocytes pass several times per day, is one of the keys that may open the



HIV-1 codons *	Numbers				P			
	Total in the HIV genome	Covered by NGS reads	Not mutated (mean)	Mutated (mean)	Uncovered vs covered by NGS reads	Mutated vs not mutated	Lost vs not mutated	Mutated or lost vs not mutated
A	188	59	36	23	—	—	—	—
C	55	14	12	2	—	—	—	—
D	125	35	29	6	—	—	0.0388	—
E	227	75	45	30	—	—	—	—
F	86	26	19	7	—	—	—	—
G	226	80	57	23	—	—	—	—
H	72	22	16	7	—	—	—	—
I	212	77	48	29	—	—	—	—
K	211	77	56	21	—	—	—	—
L	260	74	55	20	—	—	—	—
M	62	24	15	9	—	—	—	—
N	147	42	34	8	—	—	—	—
P	176	62	41	21	—	—	—	—
Q	180	65	45	20	—	—	—	—
R	192	52	33	19	—	—	—	—
S	168	56	39	17	—	—	—	—
T	170	46	36	10	—	—	—	—
V	184	65	40	25	—	—	—	—
<b>W</b>	<b>88</b>	<b>27</b>	<b>10</b>	<b>17</b>	—	<b>0.0189</b>	<b>0.0009</b>	<b>0.0051</b>
Y	80	33	23	10	—	—	—	—

**Table 4.** G-to-A mutations detected in HIV DNA fragments obtained from the woman PBMCs by next-generation sequencing in reference to HIV DNA obtained from the transmitting partner PBMCs. —, > 0.05; \*Named according to the IUPAC amino acid notation (<https://iupac.org/>; W = tryptophan); NGS, next-generation sequencing.

way to new therapeutic strategies to fight HIV, as has been reported in the oncology field for which specific gut microbes are shown to drastically modulate responses to cancer immunotherapies<sup>14</sup>. This alternative approach could involve bacteria specifically associated with spontaneous HIV suppression and may consist in fecal transplants and biotherapies.

Received: 5 June 2019; Accepted: 6 December 2019;  
Published online: 13 February 2020

## References

- Feschotte, C. & Gilbert, C. Endogenous viruses: insights into viral evolution and impact on host biology. *Nat. Rev. Genet.* **13**, 283–96 (2012).
- Gifford, R. & Tristem, M. The evolution, distribution and diversity of endogenous retroviruses. *Virus Genes* **26**, 291–315 (2003).
- Katzourakis, A., Rambaut, A. & Pybus, O. G. The evolutionary dynamics of endogenous retroviruses. *Trends Microbiol.* **13**, 463–8 (2005).
- Tarlinton, R. E., Meers, J. & Young, P. R. Retroviral invasion of the koala genome. *Nature* **442**, 79–81 (2006).
- Barre-Sinoussi, F., Ross, A. L. & Delfraissy, J. F. Past, present and future: 30 years of HIV research. *Nat. Rev. Microbiol.* **11**, 877–83 (2013).
- Sengupta, S. & Siliciano, R. F. Targeting the latent reservoir for HIV-1. *Immunity* **48**, 872–95 (2018).
- Gupta, R. K. *et al.* HIV-1 remission following CCR5Delta32/Delta32 haematopoietic stem-cell transplantation. *Nature* **568**, 244–248 (2019).
- Hutter, G. *et al.* Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. *N. Engl. J. Med.* **360**, 692–8 (2009).
- Zaunders, J. *et al.* Possible clearance of transfusion-acquired nef/LTR-deleted attenuated HIV-1 infection by an elite controller with CCR5 Δ32 heterozygous and HLA-B57 genotype. *J. Virus Erad.* **5**, 73–83 (2019).
- Albin, J. S. & Harris, R. S. Interactions of host APOBEC3 restriction factors with HIV-1 *in vivo*: implications for therapeutics. *Expert Rev. Mol. Med.* **12**, e4 (2010).
- Darc, M. *et al.* Origin of the HIV-1 group O epidemic in western lowland gorillas. *Proc. Natl. Acad. Sci. USA* **112**, E1343–52 (2015).
- Guihot, A. *et al.* Drastic decrease of the HIV reservoir in a patient treated with nivolumab for lung cancer. *Ann. Oncol.* **29**, 517–518 (2017).
- Routy, B. *et al.* Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science* **359**, 91–7 (2018).
- Zitvogel, L., Ma, Y., Raoult, D., Kroemer, G. & Gajewski, T. F. The microbiome in cancer immunotherapy: Diagnostic tools and therapeutic strategies. *Science* **359**, 1366–70 (2018).
- Colson, P. *et al.* HIV infection en route to endogenization: two cases. *Clin. Microbiol. Infect.* **20**, 1280–8 (2014).
- Tamalet, C. *et al.* Reevaluation of possible outcomes of infections with human immunodeficiency virus. *Clin. Microbiol. Infect.* **22**, 299–311 (2016).
- Carpenter, M. L. *et al.* Pulling out the 1%: whole-genome capture for the targeted enrichment of ancient DNA sequencing libraries. *Am. J. Hum. Genet.* **93**, 852–64 (2013).

18. Gaudin, M. and Denués, C. Application of human whole-genome in-solution capture (WISC) for viral metagenomics. *Submitted* (2019).
19. Edgar, R. C. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **32**, 1792–7 (2004).
20. Rose, P. P. & Korber, B. T. Detecting hypermutations in viral sequences with an emphasis on G→A hypermutation. *Bioinformatics.* **16**, 400 (2000).
21. Tamalet, C. *et al.* Short-term evaluation of zidovudine-treated patients: decrease in plasma and cellular viraemia titres. *AIDS* **6**, 1403–4 (1992).
22. Wang, Z. *et al.* Heat-stable molecule derived from *Streptococcus cristatus* induces APOBEC3 expression and inhibits HIV-1 replication. *PLoS One* **9**, e106078 (2014).
23. De Pasquale, M., Kourteva, Y., Allos, T. & D'Aquila, R. T. Lower HIV provirus levels are associated with more APOBEC3G protein in blood resting memory CD4+T lymphocytes of controllers *in vivo*. *PLoS One* **8**, e76002 (2013).
24. Eyzaguirre, L. M. *et al.* Elevated hypermutation levels in HIV-1 natural viral suppressors. *Virology* **443**, 306–12 (2013).

## Acknowledgements

We thank Annick Abeille, Emilie Doudon, Emeline Baptiste, Nathalie Duclos and Gwilherm Penant for their technical assistance. This work was supported by the French Government under the “Investments for the Future” program managed by the National Agency for Research (ANR), Méditerranée-Infection 10-IAHU-03 and was also supported by Région Provence Alpes Côte d’Azur and European funding FEDER PRIMMI (Fonds Européen de Développement Régional - Plateformes de Recherche et d’Innovation Mutualisées Méditerranée Infection). The funders of the study had no role in study design, data collection, data analysis, data interpretation, and writing of the article. All authors had full access to the data in the study and the corresponding author had final responsibility for the decision to submit for publication.

## Author contributions

D.R. and P.C. designed the study. C.D. provided clinical data. P.C., J.D., O.O.G., M.G. and A.L. performed the molecular biology or bioinformatic analyses. P.C., C.D., C.T., A.L. and D.R. analysed the study data. D.R. and P.C. wrote the manuscript. All authors revised or reviewed the manuscript critically and approved the final version.

## Competing interests

The authors declare no competing interests.

## Additional information

**Supplementary information** is available for this paper at <https://doi.org/10.1038/s41598-020-58969-6>.

**Correspondence** and requests for materials should be addressed to D.R.

**Reprints and permissions information** is available at [www.nature.com/reprints](http://www.nature.com/reprints).

**Publisher’s note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2020