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Research Paper

What Is the most Important for Elite Control: Genetic Background of Patient, Genetic Background of Partner, both or neither? Description of Complete Natural History within a Couple of MSM

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ABSTRACT

Background: We describe a homosexual man who strongly controlled HIV-1 for ten years despite lack of protective genetic background.
Methods: HIV-1 DNA was measured in blood and other tissues. Cell susceptibility was evaluated with various strains. HIV-1-specific (CD4 and CD8 activation markers and immune check points) and NK cells responses were assessed; KIRs haplotypes and HLA alleles were determined.
Findings: Two HIV-1 RNA copies/mL of plasma were detected in 2009, using an ultra-sensitive assay. HIV-DNA was detected at 1.1 and 2 copies/10^6 PBMCs in 2009 and 2015 respectively, at 1.2 copies/10^6 cells in rectal cells in 2011. WBs showed weak reactivity with antibodies to gp160, p55 and p25 from 2007 to 2014, remaining incomplete in 2017. CD4 T cells were susceptible to various strains including HIV_{KON}, a primary isolate of his own CRF02_AG variant. CD8 T cells showed a strong poly-functional response against HIV-Gag, producing mainly IFN-γ; a robust capacity of antibody-dependant cell cytotoxicity (ADCC) was observed in NK cells. Case patient was group B KIR haplotype. Neutralizing antibodies were not detected. CD4 and CD8 blood T cells showed normal proportions without increased activation markers. Phylogenetic analyses identified the same CRF02_AG variant in his partner. The patient and his partner were heterozygous for the CCR5ΔD32 deletion and shared HLA-B*07, C*07 non-protective alleles.
Interpretation: This thorough description of the natural history of an individual controlling HIV-1 in various compartments for ten years despite lack of protective alleles, and of his partner, may have implications for strategies to cure HIV-1 infection.

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1. Introduction

Without antiretroviral therapy (ARV), most individuals infected by human immunodeficiency virus type 1 (HIV-1) experience persistent viral replication and a declining CD4 + T cell count, leading to the acquired immunodeficiency syndrome within 10 years.

So-called elite controllers (EC) spontaneously control HIV replication, with low or undetectable HIV-RNA load, strong HIV-1-specific CD8 + T cell responses, and normal CD4 + T cell counts. These individuals represent about 1% of all HIV-infected patients. Their viral load can only be detected by using ultrasensitive assays (<1 copy/ml). Viral sequencing usually reveals no consistent gene deletions or signatures associated with reduced replicative capacity. This spontaneous viral control seems to be driven by host gene polymorphisms such as the CCR5Δ32 deletion or specific HLA class I alleles (B*27 or B*57) (Antoni et al., 2013), and by robust immune responses (Pereyra et al., 2008), consistent with predominant linkage between the HLA class I locus and EC status (Xie et al., 2010).

Here we report the complete natural history of a very rare elite controller during a 10-year period of untreated infection. The patient was a homosexual man with a history of repeated unprotected receptive anal intercourse for ten months with his first and only (HIV-1-infected) partner. He differs from elite controllers reported in other studies by his lack of a (homozygous or heterozygous) protective HLA-class I allele (Bailey et al., 2008; Mendoza et al., 2012; Buckheit et al., 2012). We were also able to study the immunological and virological outcomes of his partner during the same period.

2. Case Report

A 25-year-old man was tested for HIV in November 2007, after reporting unprotected receptive anal sex with pre-ejaculatory fluid exposure from October 2006 to July 2007 with his first and only sexual partner. Both men were uncircumcised. Our case report declared that his first HIV test, in July 2006, had been negative. He was found to be HIV-1-positive by ELISA (HIV-2- and HTLV I/II-negative). HIV-1 WB showed first weak reactivity with antibodies to gp160, p55 and p25 from 2007 to 2014, then positive reactivity with antibodies to gp160, p55, gp41, p40, p24 and p18 and weak reactivity with antibodies to gp110 from three sera sampled between January and November 2015 retested simultaneously (Fig.1a). In April 2017, HIV-1 WB remained incomplete with positive reactivity with antibodies to gp160, p55, gp41, p40, p25, p18, weak reactivity with antibodies to gp110 and no reactivity with antibodies to p68, p34 (Fig. 1a). Dates of serological, T-cell and genotyping assays are indicated below the charts. Plasma IgA, IgG and IgM levels were normal from 2007 to 2017, ruling out impaired global antibody production.

3. Methods

Serum was tested with the following assays: Genscreen Ultra Ag-Ab (Bio-Rad®), Biotest (DiaSorin®), Determine HIV-1/2 (Abbott®), Vidas HIV Duo (bioMérieux®), Vitros HIV1/2 assay (OrthoDiagnostics®), Vironostika HIV Uniform II Ag/Ab (Organon Teknika®), and HIV Architect Combo (Abbott®). Discrimination between HIV-1 and HIV-2 was achieved by using the Immunocomb II HIV1/2 BiSpot (Orgenics®). HIV-1 Western blot was performed using the New Lav Blot 1 (Biorad®). The partner's genotype was analyzed with the ANRS-AC11 algorithm.

HLA sequences were read with a LABScan 200 (Luminex Technology) and computer-assisted HLA Fusion software. KIR genotyping was performed by PCR using standard primers, and internal controls (Vilches et al., 2007).

HIV-1-specific responses were evaluated first by using an IFN-γ ELISpot assay on PBMC and lymph node (LN) cells stimulated with 18 pools of 15-mer synthetic peptides targeting Gag, Reverse Transcriptase and Nef (Samri et al., 2006). Second multi-parametric flow cytometry was performed to further assess HIV-specific CD8 T cells by intracellular cytokine staining (ICS) of IFN-γ, IL2, TNFα and MIP1β, and CD40L expression after incubation with the HIV-1 peptide pools that had induced an IFN-γ ELISpot response (Cardinaud et al., 2011). A CMV peptide and staphylococcal enterotoxin B (SEB) were used as positive controls. Cells were stained with anti-CD3/PB, anti-CD8/APC-CY7, anti-IFN-γ/Alexa-700, anti-TNF α/PCy7, anti-IL2/APC {BD Bioscience®) and anti-CD4/ECD (Beckman Coulter®) (Supplementary Fig. 1 Strategy of staining for poly-functional assays). Poly-functional NK was performed to simultaneously detect degranulation (anti-CD107a mAb, BD Biosciences) and cytokine production (intracellular expression IFN-γ (BD Biosciences) and TNF-α (E-Biosciences) (Béziat et al., 2012).

Finally, multi-parametric analysis of CD4 and CD8 blood T cells was performed for studying activation markers (HLA-DR, CD38, CD25, CD69) and immune check points (PD-1, TIM-3, CTLA-4) combined to anti-CD3, -CD4, -CD45RA, -CD27, -CCR7, -CXCR5, and -CD32. Data were acquired with a Gallios flow cytometer and analyzed with Kaluza-1.2 software (Beckman Coulter®).

Cell-surface expression of CD107a and intracellular expression of TNF-a and IFN-g were assessed on PBMC from the case report (in November 2010 and May 2016) or his partner (in August 2011) in the absence of target (alone), and in the presence of MHC class-I negative K562 cells or CD20 + RAJI cells treated by 1 μg/mL of anti-CD20 mAb (αCD20; Rituximab, Roche), or an Isotype control (Ig Ctl). Effector and target are used at a 1/1 ratio. The values were analyzed with a Boolean gate algorithm (FlowJo; Tree Star). Data are presented as pie charts created with Pestle and Spice software. Colored arcs represent the frequencies of cells producing CD107a, TNF-α and IFN-γ. Pie fractions represent cells performing 0, 1, 2, or 3 functions simultaneously.

HIV-1 RNA was measured by an ultrasensitive assay. Total cell-associated HIV-1 DNA in collected PBMC was detected using an ultrasensitive assay (Descours et al., 2012, Avettand-Fenoel et al., 2008). The technics to isolate the virus from *in vitro*-stimulated total and CD4 + FACS-sorted T cells from blood and tissues and ultrasensitive assays have been previously described (Avettand-Fenoel et al., 2009).

The heat-inactivated serum was tested for neutralizing activity, starting the dilutions at 1:10, using the TZM-bl assay as previously described (Bouvin-Pley et al., 2013) and MN, BX08 and NL4-3 as a highly sensitive (tier 1) indicator strains (Simek et al., 2009).

Informed written consent for participation in this research, including genetic analysis, was obtained from both men, in accordance with French ethical requirements and the Helsinki Declaration.

4. Results

Until May 2017, HIV-1 RNA was strictly undetectable in blood with a routine assay (<20 copies/ml) (Fig. 1b) and HIV-1 WB remained incomplete from November 2007 to April 2017 (middle panel) (Fig. 1a).

Two copies of HIV-1 RNA was detected in plasma with an ultra-sensitive assay in September 2009 (detection limit <1 copy/mL), (Fig. 1b) but not in May 2015 (detection limit <1 copy/mL). Six other samples collected between February 2008 and April 2017 were assessed by ultrasensitive PCR: HIV-RNA was not detected (threshold varying between 1.1 log and 1.6 log according to the volume available) in February 2008, November 2009, September 2012, June 2013, November 2015, and April 2017. The two copies of HIV-1 RNA detected in plasma with an ultra-sensitive assay in September 2009 (detection limit <-1 copy/mL) was not confirmed two month later, in November 2009 with a detection limit <8 copies/mL. In addition no HIV-RNA was detected in cerebrospinal fluid or semen in 2009 (<20 copies/ml). The patient's CD4 + T cell count remained normal throughout follow-up (median 1230 cells/mm^3, IQR: 1053-1324) (Fig. 1c).

No antiretroviral therapy was prescribed at any time during follow-up and we ruled out the possibility that the observed control of HIV-1 replication or weak reactivity of HIV-1 WB pattern were related to

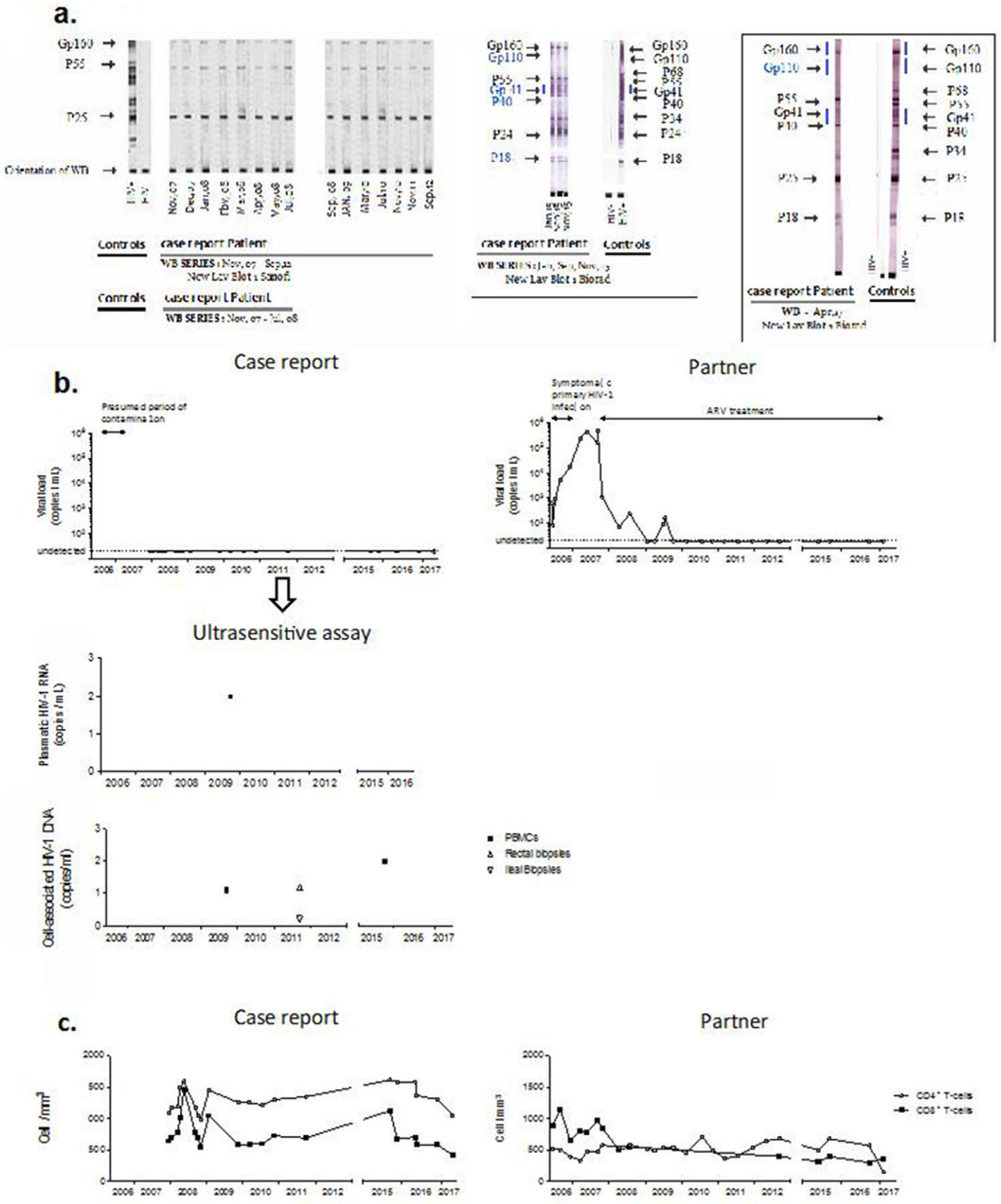


Fig. 1. Immunological and virological characteristics of the case patient (left panel) and his sexual partner (right panel), and HIV-1 phylogenetic analysis. (a) Fifteen HIV-1 WB assays were performed from November 2007 to September 2012. Eight serial samples from November 2007 to July 2008 were retested simultaneously in July 2008 (middle panel) and three samples from January 2015 to November 2015 were retested simultaneously in May 2016. The last HIV-1 WB was performed in April 2017. Dates of serological assays are indicated below the charts. (b) HIV-1-RNA was measured in blood with routine assays. The dotted line indicates the detection limit of 40 or 20 copies/ml (top panel). Ultrasensitive HIV-1-RNA assays were performed from plasma in 2009 and 2015 and HIV-DNA quantification from PBMC, rectal and ileal biopsies (bottom panel) in 2011. Two copies of HIV-1-RNA/ml were detected in 2009 (detection limit <1 copy/ml), and in 2015 (detection limit <8 copies/ml). (c) CD4+ and CD8+ T cell counts were determined in blood by flow cytometry. Dates of T-cell counts are indicated below the charts.

hidden taking of antiretroviral drugs, as no drug was detected in plasma in February 2008 and September 2013.

We detected total cell-associated HIV-DNA in PBMC collected in 2008. Cell-associated HIV-DNA was detected in PBMC at 1.1 copies/10^6 cells in 2009 and 2 copies/10^6 cells (0.4 log) in 2015. Cell-associated HIV-DNA was detected at 1.2 copies/10^6 cells in rectal but was not detected in ileal biopsies in 2011 (Fig. 1b). A 241-bp fragment of the integrase coding region (pol-IN) was amplified by PCR (Fig. 3b). Subsequent amplification attempts on the same sample and on other samples collected in 2009, with targets including highly conserved regions of the env gene (gp41), were all negative.

Finally, attempts to isolate the virus from *in vitro*-stimulated total and CD4+ FACS-sorted T cells from blood, bone marrow, lymph nodes and rectal biopsy specimens were negative.

Given the low HIV-1-RNA and HIV-1-DNA levels and the patient's heterozygosity for the CCR5Δ32 haplotype, we analyzed in 2009 his CD4+ T cells' susceptibility to the following viral strains: HIVYU2b (R5-tropic), HIVNL4-3 (X4-tropic) and HIV_{KON}, the latter being a primary isolate belonging to the same clade as his own CRF02_AG variant. Activated CD4+ T cells from blood were challenged *in vitro*, and p24 Gag release was monitored by ELISA. The cells were clearly susceptible to all the strains, including HIV_{KON} (Fig. 2a).

We then analyzed extensively his immune status. The patient was HLA-A*03 - A*31 and was homozygous for the pejorative allele B*07*07 as well as for HLA-C*07 and DQB1 06. A CD4 and CD8 T cell multi-parametric phenotypic analysis showed in 2010 and 2016 (Supplementary Table 1) normal proportions of naïve (TN) and of the various memory T cell subsets (central-memory (TCM), transitional-(TTM), effector (TEM)) and terminally-differentiated effectors (TEMRA), as well as of TfH (Follicular helper CD4+ T cells) and PD1+ TfH cells, without increased T cell activation. The PD-1, Tim-3 and CTLA-4 immune check-points (ICP) expression was also limited both on CD4 and CD8 T cells while <0.1% display high CD32 expression. The IFN-γ producing HIV-specific CD8+ T cells were first investigated in an ELISpot assay detecting in 2009 significant responses to p17Gag (1–55) in PBMC and lymph node cells (110 SFC/10^6 cells) and to p24Gag (265–319) in PBMC alone (680 SFC/10^6 cells, (Fig. 2b). The p24-specific CD8 T cells decreased to 80 SFC/10^6 PBMC in 2010 and moderately re-increased to 255 SFC/10^6 PBMC in 2016. Then multifunctional assays of PBMC from 2009 and 2010 (Fig. 2c) showed that anti-HIV-1-p24Gag (265–319) IFN-γ-producing T cells were mainly CD8+ and co-produced TNFα. From 2009 to 2015, the CD3-CD56+ NK population was found to be normal.

Patient's NK cells from case report and partner were not constitutively activated, and showed no clonal NK-cell population. Poly-functional analysis revealed no major functional defects, in the presence of K562 targets, but in contrast, a robust capacity of antibody-dependant cell cytotoxicity (ADCC) was observed in NK cells from the case patient, at two different time-points (March 2010 and November 2016), in the presence of Raji cells treated by anti-CD20 mAb (Fig. 4). Case report was of group B KIR haplotype (characterized by one or more of the following genes: KIR2DL5, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS5 and KIR3DS1).

We checked the putative presence of a neutralizing activity in the serum of our patient. Serum collected in November 2010 was tested first. Starting at a dilution of 1:10, no neutralizing activity towards the highly neutralization-sensitive strains MN and BX08 (tier 1) was detected. No neutralizing activity was detected in November 2015, several years later after the initial HIV diagnosis, even towards a very sensitive strain such as NL4-3, (another highly neutralization-sensitive indicator strain).

The case report had HLA-A*03 - A*31, with both HLA-B*07 and HLA-C*07 homozygous alleles, and was homozygous for DQB1 06 (Supplementary Table 1) and had a heterozygous CCR5Δ32 haplotype.

We monitored the patient's only sexual partner from October 2006 to July 2007 who had developed a highly symptomatic primary HIV-1 infection (with lymph nodes enlargement and a rash) in June 2006, despite low viral load (630 copies/ml); 86 copies/ml two weeks later; 970 copies/ml at week 4). Viral load increased progressively: 5300 copies/ml in August 2006 (M2), 17,000 copies/ml in December 2006 (M7), to reach 479,000 copies/ml in September 2007, justifying to start ARV (emtricitabine, tenofovir, lopinavir/ritonavir) (Fig. 1b and c). He was HLA-A*01 - A*11, shared HLA-B*07 and HLA-C*07 with the case, and was HLA-B*52, a protective allele, as well as homozygous for DQB1 06 and HLA-C*12 (Supplementary Table 1). Finally, he had also a heterozygous CCR5Δ32 haplotype. The partner's T cell phenotypic analysis showed no major abnormalities as expected under cART but a slightly increased immune activation and an imbalance towards less naïve and less TfH cells but more differentiated CD4 and CD8 T cells. The NK cell analysis showed in March 2011 characteristics of an HIV progressor, including down-modulation of NKp30 and high frequency of CD57+ NKG2C+ NK cells, despite lack of constitutive NK cell activation and of clonal NK-cell population. Poly-functional analysis revealed no major defects, in the presence of K562 targets but no significant antibody-dependant cell cytotoxicity (ADCC) in the presence of Raji cells treated by anti-CD20 mAb, contrasting with the patient. A KIR genotyping analysis revealed that the partner was positive for KIR3DL1 in a context of HLA-Bw4 epitope (Supplementary Table 2). Partner was of group A with fewer activating genes, and low diverse repertoire (Supplementary Table 2). Contrary to the case patient, the partner had no detectable HIV-1-specific IFN-γ-producing T cells among his PBMC in March 2011, as expected in patients on effective ARV therapy.

Phylogenetic analysis of viral sequences obtained from the case patient's PBMC DNA collected in February 2008, and from plasma samples collected from his only sexual partner in June 2006 and May 2007, before ARV initiation (HIV-1 RNA), based on the analysis of the 241-bp IN coding sequences, showed 100% homology (Fig. 3a). Any laboratory contamination was excluded since PCR assays were performed several months apart and included two different runs for each partner's sample. Thus, the phylogenic analyses clearly demonstrated that case patient was infected by the same CRF02_AG variant present in his unique sex partner.

5. Discussion

We report a very rare profile of HIV-1 infection of our case report in which 1) WB was incomplete but consistently reactive during the ten years of follow-up, 2) an ultra-sensitive assay was necessary to detect plasma HIV-1 RNA, 3) HIV-1 specific CD8+ T cells were poly-functional despite at least one pejorative homozygous allele HLA-C*07, 4) a NK response close to that of a healthy individual 5) a robust capacity of producing ADCC in NK cells and 6) neutralizing antibodies were not found.

Both subjects had a heterozygous CCR5Δ32 haplotype, and shared HLA-B*07, C*07, and DQB1 *06.

Phylogenetic analyses demonstrated that the case patient was infected by the same CRF02_AG strain as his only sexual partner. The impossibility to obtain this virus to replicate could be indicative of a reduced fitness; but, as the partner's virus appeared to have a full replicative capacity in 2007, in spite of a heterozygous CCR5Δ32 haplotype, we ruled out the possibility. The virus had to pass two bottlenecks in the partner and in the case patient, as both subjects had a heterozygous CCR5Δ32 haplotype. The CCR5Δ32 haplotype deletion in our patient, even heterozygous, could have participated to the observed HIV control, as protective factors could have additive effects (Salgado et al., 2011, Casado et al., 2010).

The possibility that the case patient's CD4+ T cells expressed factors restricting HIV-1 replication was also ruled out by *in vitro* experiments showing his CD4+ T cells were clearly susceptible to various HIV-1 strains, including HIV_{KON}, a primary isolate of the same clade as his own CRF02_AG variant (Fig. 2a). Thus the different outcomes of the case patient and his partner, despite infection by the same HIV strain, strongly suggest that host/genetic factors rather than viral factors were responsible.

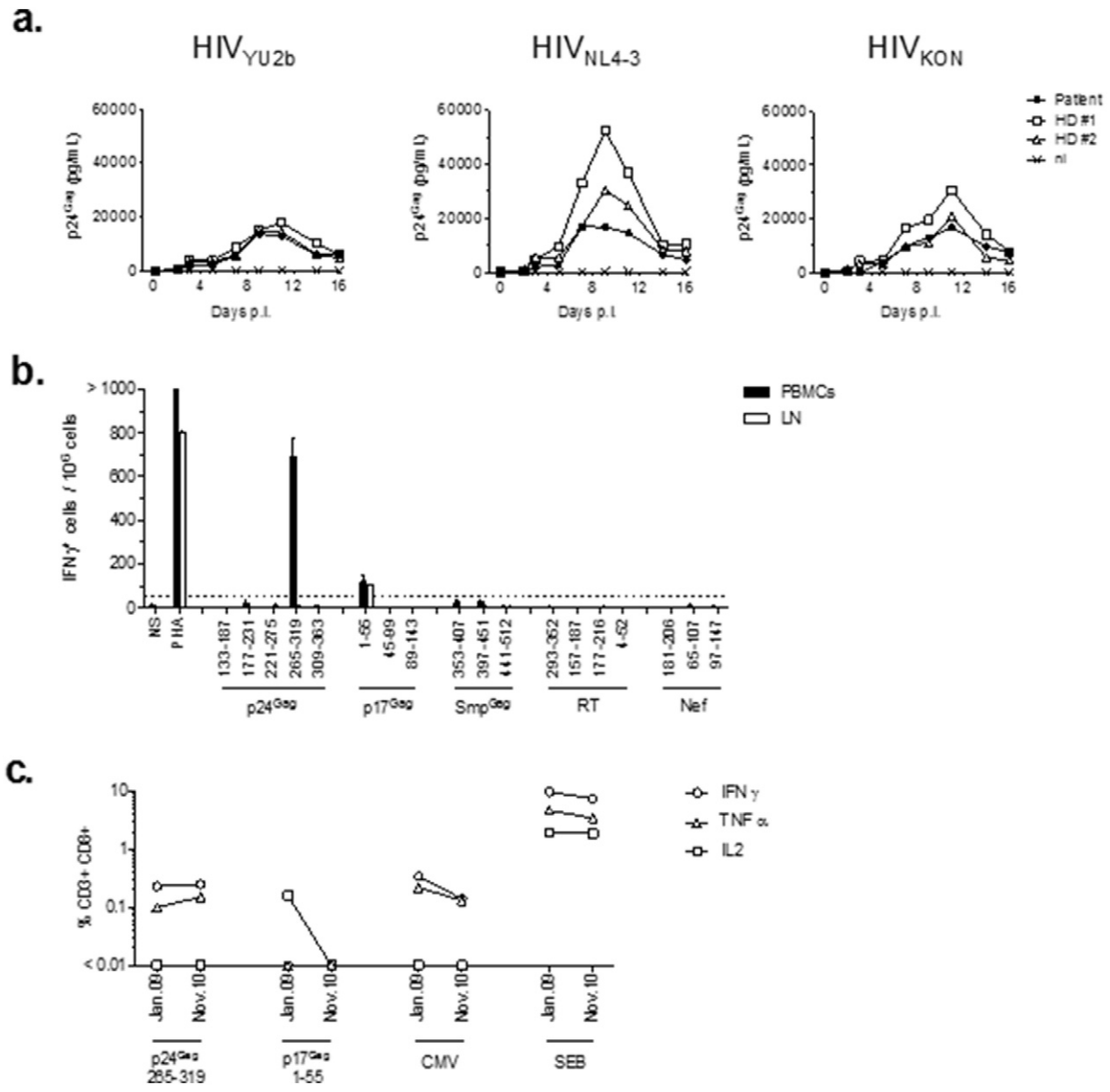


Fig. 2. HIV infection and T-cell responses of PBMC from the case patient. (a) *In vitro* susceptibility to HIV infection in 2009.[9] CD4 + T-cells of the case patient or healthy donors (HD) were PHA-activated and infected *in vitro* with 100 ng $p24^{Gag}$ of HIV_{YU2b} (R5-tropic), HIV_{NL4-3} (×4-tropic), and HIV_{KON} a primary isolate of the same clade than CRF02_AG isolate. Viral replication was monitored by $p24^{Gag}$ ELISA during 16 days post-infection (p.i.). (ni = non infected). (b) *Ex vivo* IFN-γ-ELISpot assay was performed in November 2009 using PBMC and lymph node (LN) cells loaded with 18 HIV-1 pools of 15-mers peptides overlapping by 11 amino acids. Eleven pools covered the three HIV type 1 Gag proteins: three pools for p17 Gag (1–55, 45–99, and 89–143), five pools for p24 Gag (133–187, 177–231, 221–275, 265–319, and 309–363), three pools for the small Gag proteins (Smp Gag) (p2/p7/p1/p6) (353–407, 397–451, and 441–512), four pools corresponding to poly-epitopic RT regions (293–352, 157–187, 177–216, and 4–52), and three pools corresponding to poly-epitopic Nef regions (181–206, 65–107, and 97–147). All results are expressed as specific IFN-γ-producing cells after subtracting the number of SFCs observed with cells alone, without stimulation. The IFN-γ producing HIV-specific CD8 + T cells were first investigated in an ELISpot assay detecting in 2009 significant responses to p17Gag (1–55) in PBMC and lymph node cells (110 SFC/10^6 cells) and to p24Gag (265–319) in PBMC alone (680 SFC/10^6 cells. (c) PBMC obtained in November 2009 were stimulated with the p17Gag 1 to 55 pool or with the p24 Gag 265 to 319 pool that elicited an IFN-γ-ELISpot production. The percentages of CD3 + CD8 + T cells producing IFN-γ, TNF-α or IL-2 were analyzed by intracellular cytokine staining and flow cytometry. The percentages of activated cells not subjected to peptide stimulation were subtracted. The immune-dominant CMV-derived HLA-B*07-restricted epitope (pp65 $_{417}$TPRVTGGGAM$_{426}$), and staphylococcal enterotoxin B (SEB) were used as positive controls. Cells alone served as negative control.

From 2009 to 2015, no major significant difference between our case report and healthy individuals were observed. In contrast, the partner showed some functional markers which are significantly increased in HIV-1 progressors (Gumá Cabrera et al., 2006). The partner had a massive loss of capacity to producing ADCC in August 2011 (Fig. 4). Polyfunctional analysis revealed that case report and the partner are only (but strongly) different in their ability to product ADCC (Raji + anti-CD20), but not direct lysis (K562) (Fig. 4).

HIV-1 WB reactivity has previously been reported in cohorts of elite controllers (Simek et al., 2009; Canouï et al., 2017; Boufassa et al., 2011). Canouï et al. investigated an extreme HIC subset with undetectable viral load (uHIC) and no viral blips using semi-quantitative HIV-1 WB to detect IgG antibodies against HIV proteins. They found that 10 of the 47 uHICs (21%) lacked at least 1 HIV-specific antibody and were described as having weak IgG responses. Seven of these 10 uHICs lacked either anti-p68 or anti-p34 antibodies. This data further supporting the lack of recent antigenic stimulation in these patients (Canouï et al., 2017). In the study from Pereyra et al. (2009), reactivity was detectable to multiple proteins (range 2 to 10), and the majority of individuals (77%) had a complete pattern against all the proteins tested. The most commonly detected antibodies were anti-gp120 and anti-gp160, which were detected in all individuals, followed by anti-p24, -p40 and -gp41, in

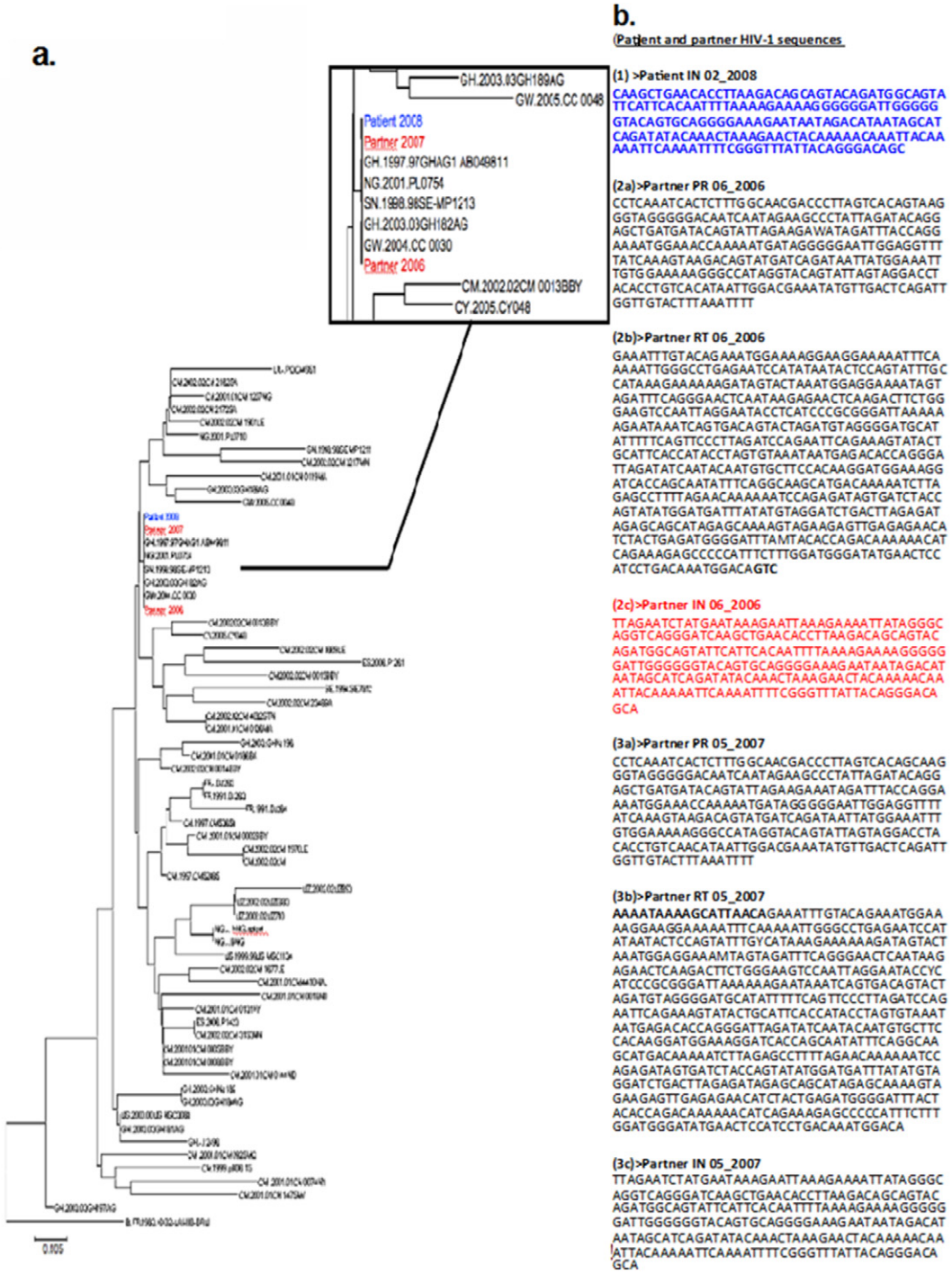
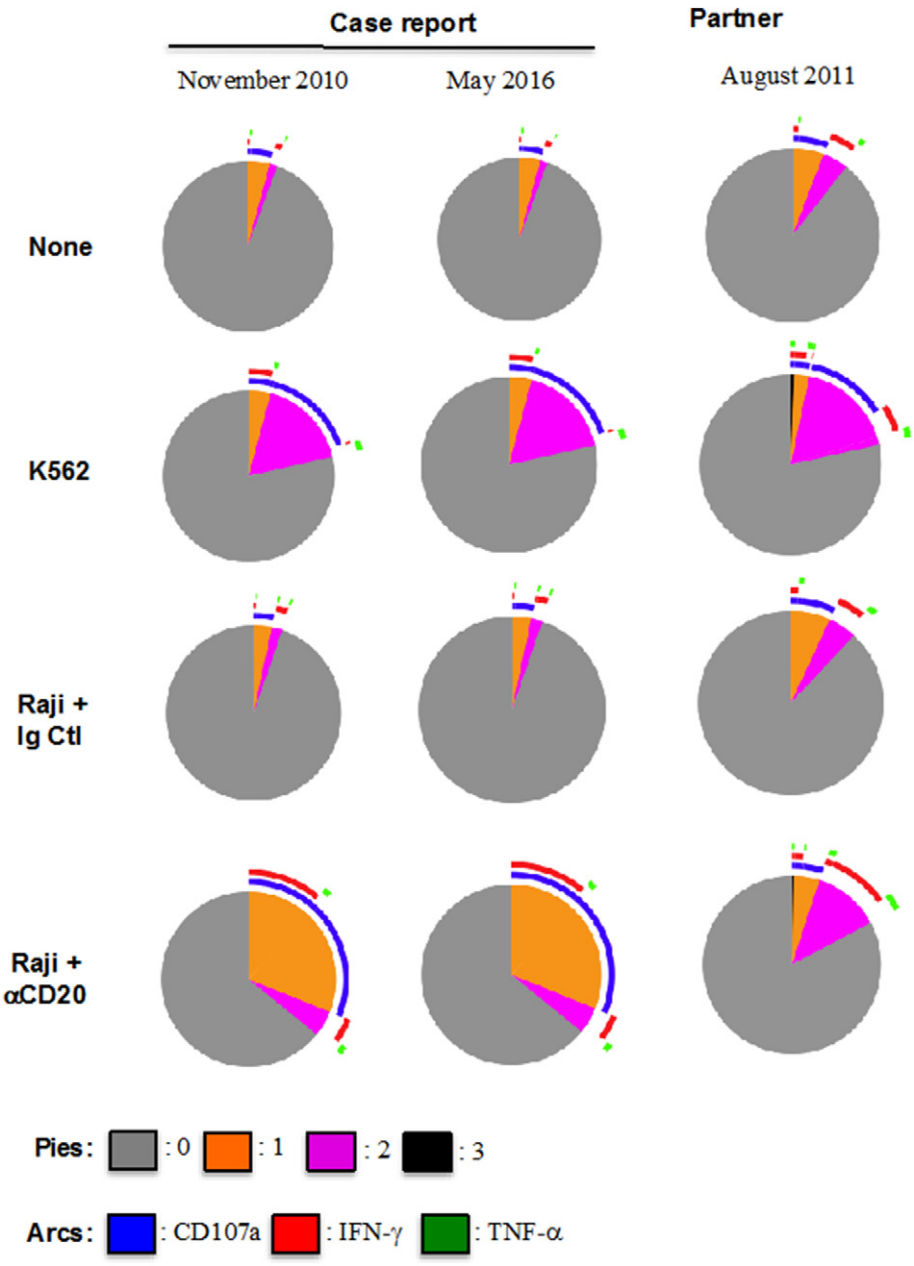


Fig. 3. Phylogenetic analysis of IN-sequences from the case report and of Protease, Reverse Transcriptase and IN-sequences from the partner. (3a) Phylogenetic analysis of viral sequences obtained from the case patient's PBMC DNA collected in 2008, and from plasma samples collected from his only sexual partner in June 2006 and May 2007 (HIV-1 RNA). The phylogenetic tree was constructed using 61 sequences of CRF02_AG variants available in the Los Alamos HIV sequence database (http://hiv-web.lanl.gov). The 241-bp IN coding sequences were analyzed comparatively using the MEGA analysis program (http://www.megasoftware.net) with the neighbor-joining method. Distances were calculated using the Kimura two-parameter matrix (ratio T/t = 2.0). Bootstrap analysis (500 replications) was used to test the reliability of the branching order. The tree was rooted using the sequence of the reference subtype B strain HXB2-LAI-IIIB-BRU. The scale bar at the bottom of the figure indicates the number of base substitutions per site. (3b) HIV-1 IN-sequences from the case patient's PBMC DNA collected in February 2008 (Antoni et al., 2013), and the Protease, Reverse Transcriptase and IN-sequences from partner's plasma samples collected in June 2006 (2a; 2b; 2c respectively) and May 2007 (3a; 3b; 3c respectively), before partner's ARV initiation.

respectively 98%, 96% and 93% of individuals. The least commonly detected antibodies were those against p18, p31 and p65, which were found in respectively 80%, 84% and 86% of individuals (Pereyra et al., 2009). In the previous study, by using an ultrasensitive assay, the results showed that the number of antibodies detected correlated directly with the plasma HIV-1 RNA level, and that full WB reactivity was present in all patients with plasma viral load above 13 copies/mL. In our case report, only one on the eight samples had a VL detectable at 2 copies of HIV-1-RNA/mL of plasma (detection limit <1 copy/mL) by using an ultrasensitive PCR (Fig. 1b). His sequential patterns of the antibodies to anti-gp160, anti-gp110, anti-gp41, anti-p40, anti-p24, and anti-p18 were in accordance with the previous study, WB remaining incomplete until May 2017 (Fig. 1a). This report provided extensive longitudinal data on an EC with incomplete WB over a 10-years period.



Figs. 4. Poly-functional analysis of NK cells. (4) Poly-functional analysis of NK cells was performed to simultaneously detect degranulation (anti-CD107a mAb, BD Biosciences) and cytokine production (intracellular expression IFN-γ (BD Biosciences) and TNF-α (*E*-Biosciences) controls· Cell-surface expression of CD107a and intracellular expression of TNF-α and IFN-γ were assessed on PBMC from the case report (in November 2010 and May 2016) or his partner (in August 2011) in the absence of target (Alone), and in the presence of MHC class-I negative K562 cells or CD20 + RAJI cells treated by 1 μg/mL of anti-CD20 mAb (αCD20; Rituximab, Roche), or an Isotype control (Ig Ctl). Effector and target are used at a 1/1 ratio. The values were analyzed with a Boolean gate algorithm (FlowJo; Tree Star). Data are presented as pie charts created with Pestle and Spice software. Colored arcs represent the frequencies of cells producing CD107a, TNF-α and IFN-γ. Pie fractions represent cells performing 0, 1, 2, or 3 functions simultaneously.

The extremely rare uHICs differ from HICs with blips by significantly lower ultrasensitive plasma HIV-RNA loads and HIV-DNA levels in PBMCs, higher $CD4^+$ T-cell count at enrollment and during follow-up and with lower T-cell activation levels. In our case, the only 2 copies of HIV-1-RNA/mL of plasma detected from 2009 to 2017 (Fig. 1b) and the constantly high CD4 + T cell count above 1000/mm^3 from 2007 until 2017, with, in 2017, CD4 + T-cell count higher than in 2007 (Fig. 1c), are consistent with the uHIC cohort (Boufassa et al., 2011). Our patient's blood HIV-1 DNA reservoir appeared smaller than usually observed in ECs and in LTNPs (Fig. 1b) (Boufassa et al., 2011). This small blood HIV-1 DNA reservoir was accompanied by a very small HIV-1 DNA reservoir in gut-associated lymphoid tissue, as previously reported in several LTNP groups, including untreated patients with primary infection (Avettand-Fenoel et al., 2008). Half of the uHICs have a protective HLA allele (B*57 or *58 or *B27), weak $CD8^+$ T-cell responses, and very small HIV-DNA reservoir (Canouï et al., 2017). The case may be one of these extreme controllers. Our case report had poly-functional HIV-1 specific CD8 + T cell responses, coproducing IFN-γ and TNFα were albeit modest and fluctuating. These responses contrasted with his partner's lack of CD8 T cell responses and were markedly higher than observed in HAART-treated non viremic patients but did not differ from those seen elsewhere in chronically viremic patients. Nevertheless they had no significant impact on HIV-1 replication *in vitro*.

Our case patient was HLA-A*03-A*31, B*07, with C*07 homozygous pejorative alleles (Coloccini et al., 2014) whereas his partner was HLA-B*07-B*52, C*07 heterozygous and C*12 heterozygous. Elite controllers have a variety of genetic backgrounds, although protective HLA as B*27/B*57 alleles are usually over-represented. Interestingly, the case

report did not bear any of the classical protective HLA-B*57 and B*27 reported among 45% and 15% of Caucasian HIV controllers respectively *versus* 6.2% and 6.9%, respectively in the French Caucasian general population; www.allelefrequencies.net. Neither the case report had other protective HLA alleles like HLA-B*13 (Antoni et al., 2013), B*14 (Antoni et al., 2013; Peterson et al., 2013), B*51 (Brumme et al., 2011), B*52 (Antoni et al., 2013). Our case patient and his partner shared at least one pejorative allele: the case report was homozygous for B*07, the most frequent human allele consistently linked to accelerated disease progression in B-clade, but not in C-clade infection (Kløverpris et al., 2014). The influence of HLA-B*07 on the control of CRF02_AG variant being unknown to date, this allele cannot be considered as pejorative in this clinical setting. Moreover, the case report was also homozygous for the HLA-C*07 allele (Coloccini et al., 2014).

Transmission of HIV-1 from a patient who developed AIDS to a patient who has maintained undetectable viral loads has already been documented (Bailey et al., 2008), but unlike our case, either both patients shared the same protective HLA-B*57 allele, or the patient who has maintained undetectable viral loads was HLA-B*57:03 (Buckheit et al., 2012). Similarly, the four patients described by Mendoza (Mendoza et al., 2012) (without any data about the partners) carried protective HLA class-I alleles, two carried an additional B*57 allele, and none carried pejorative alleles. Moroni et al. (2014) published a case of HIV-1 control in a heterosexual couple lasting >20 years, exploring the genetic background in depth. The woman possessed multiple genetic polymorphisms, including HLA alleles (B*14, B*57, C*06 and C*08.02) and HLA-C single nucleotide polymorphisms (SNPs, rs9264942 C/C and rs67384697 del/del), that have been previously individually associated with spontaneous control of plasma viremia, maintenance of high CD4(+) T cell counts and delayed disease progression.

HLA-B*07 allele does not represent a true pejorative HLA-class I allele such as B35:02, (Martin and Carrington, 2013), and has been linked to disease progression with rapid seroconversion (Peterson et al., 2013). Its homozygosity for HLA-B*07-C*07, combined with the absence of a well-known protective HLA-B allele (B27+/B57+/B58+) represents at least a neutral, or a pejorative genetic background (Carrington et al., 1999). Although some ECs do not possess any protective allele, this might be the first description of an EC homozygous for at least one pejorative allele (HLA-C07 *07) and no protective allele. This is to be compared to the ANRS Visconti study (Samri et al., 2016) in which three of the 14 post-treatment controllers (PTCs) carried one HLA-B*07 allele, two of them carried an additional HLA-B*27 or B*57 allele, and the third carried a non-protective allele. Our findings, together with the Visconti study, clearly show that HLA-B*07 does not rule out PTC or EC status.

Carrying (one or more) same HLA class-I alleles in a previously HIV-infected partner and a person in acute HIV-1 infection might negatively impact virus control following transmission, likely because of CTL escape mutations selected in the previously HIV-infected partner. These patients usually progress rapidly to AIDS and death (Mónaco et al., 2016; Yue et al., 2013). Nevertheless, despite the fact that our case report and his partner shared HLA-B*07-C*07-DQB1 06, that was obviously not the case here.

HLA-B*52 is known as conferring a strong protective effect during early time points on viral load without significant effect on the long-term control of HIV-1 (Antoni et al., 2013). When associated with heterozygous deletion for the CCR5ΔD32 haplotype, it could explain the partner's low HIV-1 RNAs observed in acute infection and the fact that our patient has been exposed to low HIV-1 RNAs, albeit a role has not been demonstrated, in the observed outcome.

Early studies reported an increased frequency of HLA-C*04 and HLA-C*07 in HIV-1 infected patients, compared to non-infected controls. HLA-A and HLA-B are downregulated by the HIV nef protein, HLA-C is not. A larger study established the association of HLA-C*04, C*07 and HLA-B*35 with rapid development of AIDS (Zipeto and Beretta, 2012). HLA-C genotyping revealed that our case report was homozygote for HLA-C*07 and his partner HLA-C*07C*12 (Supplementary Table 2), suggesting that HLA-C was not associated with the unique HIV profile observed in this patient; we cannot however rule out that HLA-C is differentially expressed at the cell-surface level.

Specific KIR/ligand combinations are associated with the outcome of several viral infectious diseases, and more particularly in HIV-1. The dominant ligands for KIRs have been identified are the C1 and C2 epitopes of HLA-C, that recognized KIR2DL2/2DL3 and KIR2DL1, respectively and HLA-Bw4 that recognized KIR3DL1. Genetic association studies all indicate a role for KIR-HLA interactions in HIV infection, and generally demonstrate an association between disease progression and KIR3DL1 with HLA-Bw4 or/and KIR2DL1 with HLA-C1.

To analyze this interesting point, HLA-class I and KIR genotyping have been performed and analyzed together. This data show that patient and his partner were both HLA-C1/C1 but differed for KIR3DL1 in a context of HLA-Bw4 epitope (Supplementary Table 2). The case patient's group was B KIR haplotype, and was positive for KIR3DL1 in a context of HLA-Bw4 epitope, a combination classically-associated with HIV disease progression (Supplementary Table 2) (Bashirova et al., 2011), whereas his partner was of group A, with fewer activating genes, and less diverse repertoire, suggesting that the case patient's NK/T cells could be more functional that those from his partner. Indeed the lack of NK cell abnormality in our patient, compared to his partner (Gumá Cabrera et al., 2006), together with the more robust ADCC capacity of the case patient's NK cells than of his partner, might suggest a protective efficacy of those cells, as shown in the prophylactic RV144 vaccine trial (Haynes et al., 2012), and in slow progressors (Wren et al., 2013).

No neutralizing activity was detected, even towards the highly neutralization-sensitive NL4.3 strain (November 2015). Our results suggest that the extraordinary control of HIV-1 replication was not the result of a particular neutralizing activity. It is not surprising since development of a neutralizing activity requires antigenic stimulation which is only provided by a persistent level of HIV replication and HIV evolution.

The limits of our study were first that, despite we used ultrasensitive PCR for HIV-1 RNA measurement, the threshold varied between 1.1 log and 1.6 log according to the volume available. We could therefore not rule out definitively the possibility of viral blips. Secondly, we cannot exclude the possibility that the variant transmitted to the case patient harbored mutations affecting its replicative properties, as, despite using large volumes of plasma and PBMC, we could not isolate the case patient's whole virus. When the case patient was referred in November 2007, the partner was already treated with ARV since September 2007; for ethical reasons we did not withdraw partner's treatment to try to sequence the virus and to study the replicative capacity. Thirdly, we assessed NK cell potential by using rituximab plus Raji, but we were not able to assess the potential of serum antibodies from patients to perform ADCC function, or at least provide antibody isotypes and HIV recognition data, as there was not any plasma or serum available from our patient, and the partner moved to the provinces and was lost-to-follow-up.

A fragment of the integrase coding region (pol-IN) was amplified by PCR, but all subsequent amplification attempts, with targets including highly conserved regions of the env gene (gp41), were all negative. Phylogenetic analysis was thus performed based only on the pol-IN fragment. We suggest that such failure is due to the low virus load in the patient.

In conclusion, we described an extremely prolonged and spontaneous almost complete control of HIV-1 replication in the blood and different tissues despite the lack of protective alleles. Our study may imply that the genetic background of the exposed person, the genetic background of the source, and the viral strain could interact with each other to obtain an elite controller. This case which might belong to the extremely rare subset of extreme HIV controllers with undetectable virus loads, might be taken as a model and as a creative step, in a positive direction, for future control of HIV disease (Autran et al., 2011, Autran, 2015).

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Contributors

M. Bendenoun, A. Samri, and P. Sellier conceived and wrote the study.
V. Avettand-Fènoël performed virological studies: HIV-1 RNA measurements by ultra-sensitive assay and HIV-1 DNA measurements from cells.
S. Cardinaud, B. Descours, A. Urrutia, A. Moris, G. Carcelain and V. Vieillard performed immunological studies.
M-C. Mazeron performed virological studies from plasma.
S. Brunet and F. Barin performed phylogenic analysis.
M. Pocard and X. Dray performed the lymph nodes and GALT biopsies in the patient.
J-F. Bergmann, C. Rouzioux, F. Simon, P. Andre, and B. Autran supervised the study.

Conflicts of Interest

No conflicts of interest.

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none.

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Declaration of Interests

M. Bendenoun: received personal fees from ABBVIE, outside the submitted work;
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S. Cardinaud: no COI.
Descours: no COI.
G. Carcelain: received personal fees from MSD France, personal fees from Gilead, personal fees from ViiV healthcare, outside the submitted work;
M-C. Mazeron: no COI.
J-F. Bergmann: no COI.
Urrutia: no COI.
Moris: no COI.
Rouzioux:
F. Simon: disclosed board membership for Abbott, Beckman, bioMérieux; consultancy for Abbott, Beckman, bioMérieux, GSK,bioRad; grants for Abbott, bioMérieux, Beckman; meeting expenses: Abbott, Beckman, bioMérieux,bioRad, all outside the submitted work;
P. Andre: no COI.
M. Pocard: no COI.
X. Dray: no COI.
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