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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

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