Permanent control of HIV-1 pathogenesis in exceptional elite controllers

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INTRODUCTION

Elite controllers (EC) represent a small subset of HIV-1 infected people able to spontaneously control viral replication. However, natural virological supression and absence of immune dysfunction are not always long-term sustained. Exceptional EC (EEC) are HIV-1 subjects who mantain the EC characteristics without disease progression for more than 10 years.

METHODS

- Subjects: 3 HIV-infected ART naïve from "Centro Sanitario Sandoval"
- Quantitative viral outgrowth assay: 28 to 63 milion bulk CD4$^+$ T cells were used to measure the replicative competent provirus
- Total HIV-DNA and cell-associated HIV-RNA: measured in CD4$^+$ T cells by droplet digital PCR amplifying Gag and/or LTR regions. The *RPP30* and *TBP* cellular genes were quantified in parallel to normalize sample input for total HIV-DNA and cell-associated HIV-RNA respectively
- Ultrasensitive viral load: 9 ml of plasma were ultracentrifuged and HIV-RNA quantified using Abbot VL platform
- Genetic variability analysis: we estimated the average diversity sequence pairs for each time point in each individual and calculated the average number of base differences per site (p-distance) with 500 bootstrap replicates using MEGA6. All positions containing gaps and missing data were eliminated. Differences in the average p-distance per individual between groups were tested for significance using pairwise Wilcoxon Rank-Sum Test, applying the Benjamini-Hochberg procedure for multiple comparisons correction (cut-off for significance False Discovery rate of 0.05)
- Immunophenotyping and intracellular cytokine staining: PBMCs were in vitro stimulated with overlapped HIV Gag-specific peptide pool, and stained with surface and intracellular marker antibodies. For dendritic cell immunostaining, myeloid dendritic cells (mDCs) were defined as Lin2$^-$HLA-DR$^+$CD11c$^+$CD123$^-$
- Susceptibility and viral inhibition assay: activated CD4$^+$ T cells alone or in combination with CD8$^+$ T cells from each individual were infected by spinoculation with 200ng/ml of p24 from laboratory viral strains CXCR4 and CCR5 tropic. Supernatants were collected at day 7 and quantified with HIV-1 p24^{GAG} ELISA
- Soluble biomarkers: levels of high sensitivity C-Reactive Protein (hsCRP) and β-microglobulin, D-dimer, IL-6 and sCD163 were performed as previously described in *de Pablo-Bernal. J Gerontol A Biol Sci Med Aci.2016*
- HIV-1 antibody determination: plasma titers of HIV-1 antibodies were measured in an enzyme immunoassay (GenescreenTM HIV-1/2 Version 2)

RESULTS

3.1 Clinical characteristics

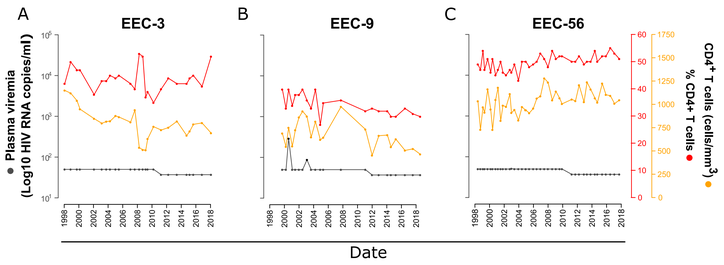


Figure 1. Clinical evolution of CD4$^+$ and CD8$^+$ T cells, and viral load of the EEC individuals since the moment of HIV-1 infection.

Table 1. Clinical, epidemiological and host-genetic factors

	EEC-3	EEC-9	EEC-56
Year of birth	1956	1957	1957
Sex	Male	Female	Female
Estimated year of infection[a]	1983±2	1986±2	1984±2
Year of HIV-1 diagnosis	1988	1992	1989
Transmission route[b]	IVDU	IVDU	IVDU
Remarks	–	HIV-1 negative child	HIV-1 negative child
HCV coinfection	1996	1992	1993
Treatment	RBV/IFNα (2008)	Spontaneous clearance	DAA (2017)
Genetic markers			
CCR5 Δ32 rs333[c]	11	11	11
CCR2 V64I rs1799864[c]	11	11	11
HLA C rs9264942[c]	22	22	22
HLA A	02:01, 02:05	02:01, 31:01	01:01, 02:01
HLA B	27:05, 58:01	39:01, 57:01	14:02, 57:01
Genetic Score	4	3	4

[a] Viral dating performed in the last sample and estimated according to *Bello G.J Gen Virol.2004*; [b] No further expositions after HIV-1 diagnosis; [c] "1" indicates the most frequent allele and "2" the mutant; IVDU= intravenous drug user, RBV= ribavirin, IFNα= interferon α, DAA= direct acting antivirals.

3.2 Viral persistence

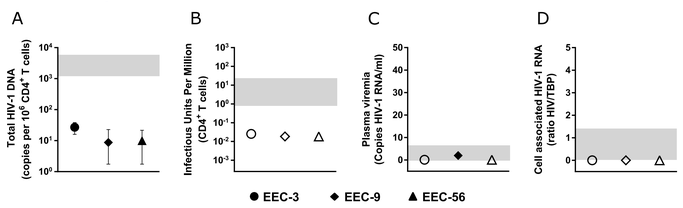


Figure 2. Viral measurements of (A) total HIV-1 DNA, (B) Infectious Units per million cells (IUPM), (C) ultrasensitive plasma viral load and (D) cell associated HIV-1 RNA. Open sybmbols indicate undetectable values. Light grey bands are the interquartile range from standard HIV-1 infected individuals on ART (*Moron-López. J Infect Dis.2016*).

3.3 Immune responses

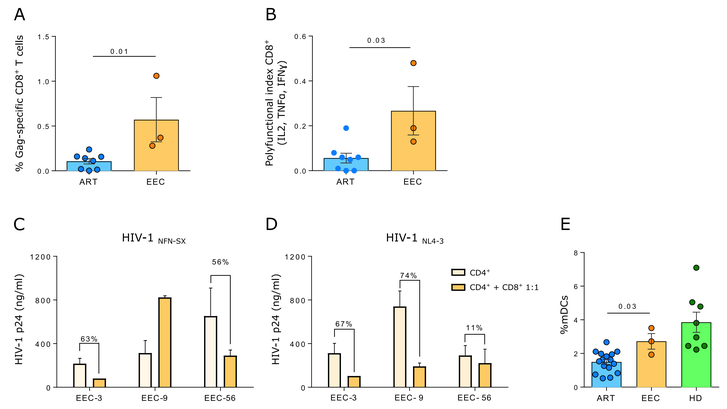


Figure 3. Cellular immune responses. (A) Total CD8$^+$ T cell Gag-specific response from EEC and HIV-1 infected individuals on supressive ART. (B) Index of polyfunctionality of Gag-specific total CD8$^+$ T cells from EEC and HIV-1 infected individuals on ART based on the proportions of cells producing intracellular combinations of IL-2,TNFα, IFNγ. (C-D) Assay of the ex vivo ability of CD8$^+$ T cells to inhibit superinfected autologous CD4$^+$ T cells of the three individuals. The figure shows day 7 of an infection with a laboratory viral strain (C) HIV-1$_{NFN-SX}$ (CCR5 tropic) and (D) HIV-1$_{NL4-3}$ (CXCR4 tropic). Percentage of inhibition of CD4$^+$ vs CD4$^+$CD8$^+$ T cells is indicated in each individual. (E) Total myeloid dendritic cell quantification, comparing EEC individuals with HIV-1 infected individuals on ART and healthy donors (HD). (A-B and E) Differences between groups were determined by Mann-Whitney U test.

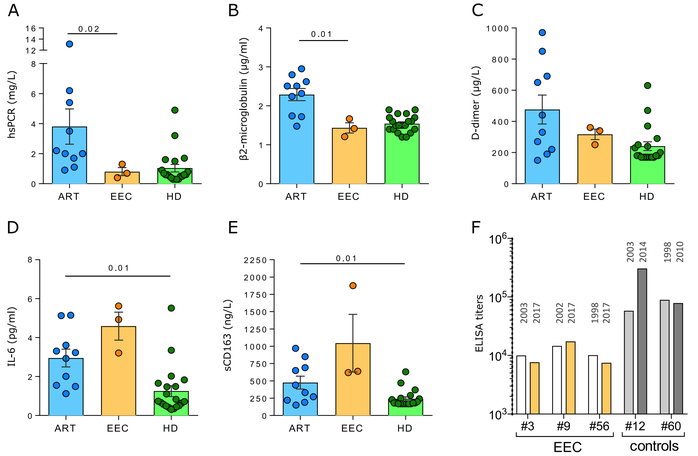


Figure 4. Inflammation biomarkers and antibody titers. (A-E) Different inflammatory markers were analyzed including (A) hsPCR, (B) β2-microglobulin, (C) D-dimer, (D) IL-6 and (E) sCD163. EEC individuals were compared with HIV-1 infected individuls on ART and healthy donors (HD). (F) Plasma antibody titers in two samples 14-19 years apart.

3.4 Genetic variability

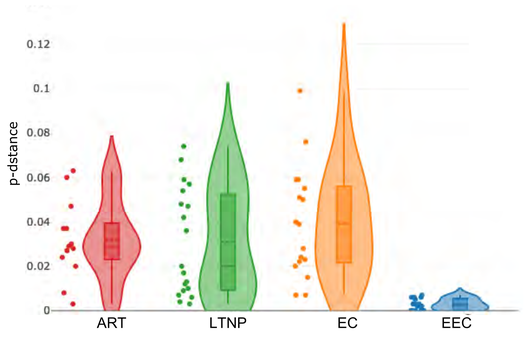


Figure 5. Genetic variability analysis of samples from different groups of HIV-1 individuals with controlled infection. ART=Antiretroviral therapy; LTNP=Long-Term Non Controllers; EC=Elite Controllers; EEC=Exceptional Elite Controllers.

CONCLUSIONS

- We postulate that these EEC should be considered unique cases of spontaneous functional HIV-1 cure.
- Low genetic diversity and lack of viral evolution distinguish these individuals from other EC.
- The combined non-functional HIV-1 reservoir, extremely low viral diversity and an HIV-1 specific immune response seems to be key to mimic these cases of spontaneous functional cure in future eradication strategies.

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