Longitudinal Immune-Virologic Study of HIV Exceptional Elite Controllers With Distinct Outcomes

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BACKGROUND

HIV-1 exceptional elite controllers (EECs) represent a model of functional cure, able to sustain undetectable viremia and prevent disease progression for decades without therapy. After 35 years of follow-up, we describe two EECs who lost viral control (transient controllers, T-EECs) and compare them with two persistent controllers (P-EECs) who maintained long-term remission. These cases provide a unique opportunity to elucidate mechanisms underlying durable spontaneous control.

METHODS

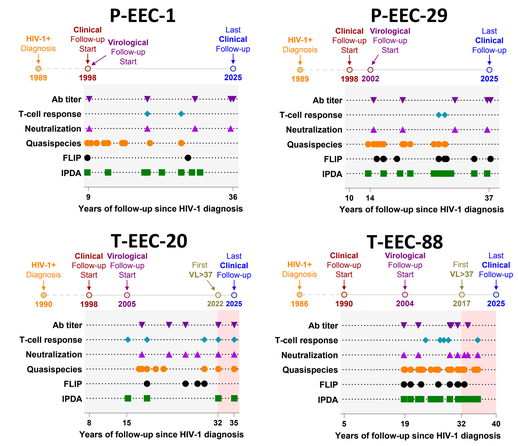


Figure 1: Longitudinal analysis.

We performed a longitudinal immuno-virological analysis on available samples from 2 P-EECs and 2 T-EECs (**Figure 1**). We measured:

- Reservoir integrity by intact proviral DNA assay (IPDA) and by near full-length individual proviral sequencing.
- Viral quasispecies evolution.
- Infectivity and fusogenicity of Env clones.
- HIV-antibodies titers
- Heterologous/autologous neutralization capacity
- HIV-1-specific T-cell responses
- T cell exhaustion

We generated three most common recent ancestor (**MRCA**) Env sequences (labeled 409, 440 and 535) from a phylogenetic tree regrouping a Spanish cohort of EECs Env sequences.

Table 1: Patient characteristics.

INDIVIDUAL IDENTIFIER	P-EEC 1	P-EEC 29	T-EEC 20	T-EEC 88
Years of HIV control	36	37	32	30
Sex	Female	Male	Male	Female
Age at diagnosis (years)	31	31	21	19
Age at loss of control (years)	-	-	53	49
Age at last sample	67	68	56	58
Risk Factor	IDU	IDU	MSM	IDU
Host Factors				
CCR5 Δ32 rs333 (1 = mutation)	0/0	0/0	0/0	0/1
CCR2 V64I rs1799864 (1 = mutation)	0/0	0/0	0/0	0/0
HLA-C rs9264942	1/1	1/1	0/1	0/1
HLA-B	14:02/57:01	27:05/58:01	14/51	39:01/44:02
Genetic Score	4	4	2	2

HIV evolution precedes loss of virological control in EECs.

RESULTS

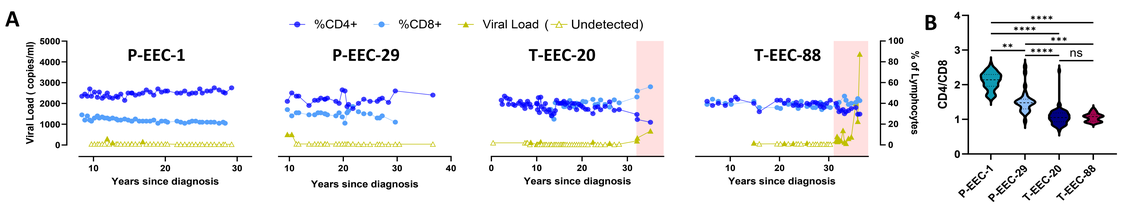


Figure 2: Clinical Follow-up: A. Longitudinal measures of CD4 and CD8 T-cell percentages of total lymphocytes and viral loads (HIV RNA copies/mL). For T-EECs, pink areas represents the loss of virological control period (detected VL for 3 consecutive measurements). **B.** Ratio of CD4 T cells over CD8 T cells in all samples acquired before loss of control (one-way Anova with multiple comparison) .

All four EECs (2 females and 2 males; **Table 1**) maintained undetectable viremia for at least 30 years (**Figure 2**). P-EECs maintained durable control for 35 and 37 years while T-EECs showed persistent viremia (>50 copies/mL) after 30 and 32 years. P-EECS carried protective alleles (B57:01/814:02 and B27:05/B58:01, respectively), whereas T-EECs lacked them (B14:B51 and B39:01/B44:02) (**Table 1**). P-EECS exhibited low proviral load predominantly composed of defective genomes (>90%), and highly homogeneous viral quasispecies with low divergent sequences to its MRCA (**Figure 3A and 3B**). P-EECs showed limited neutralization capacity and low antibody titers over time (**Figure 4A and 4B**), higher CD4/CD8 ratios (**Figure 2B**), and high polyfunctional Gag-specific T-cell responses which remained stable across of follow-up with no T-cell escape mutations. The results are consistent with the absence viral replication throughout follow-up. Prior to losing control, T-EECS displayed an increase in reservoir size and viral quasispecies diversity (**Figure 3A and 3B**). They developed autologous neutralizing responses; compensated by the emergence of new variants with higher functionality (**Figure 3C**) and ability to escape immune recognition (**Figure 4A**). At the same time, T-EECs also showed low magnitude and polyfunctionality of Gag-specific T-cell response (**Figure 4B**) alongside heightened T-cell activation and proliferation compared to P-EECs (**Figure 4C**).

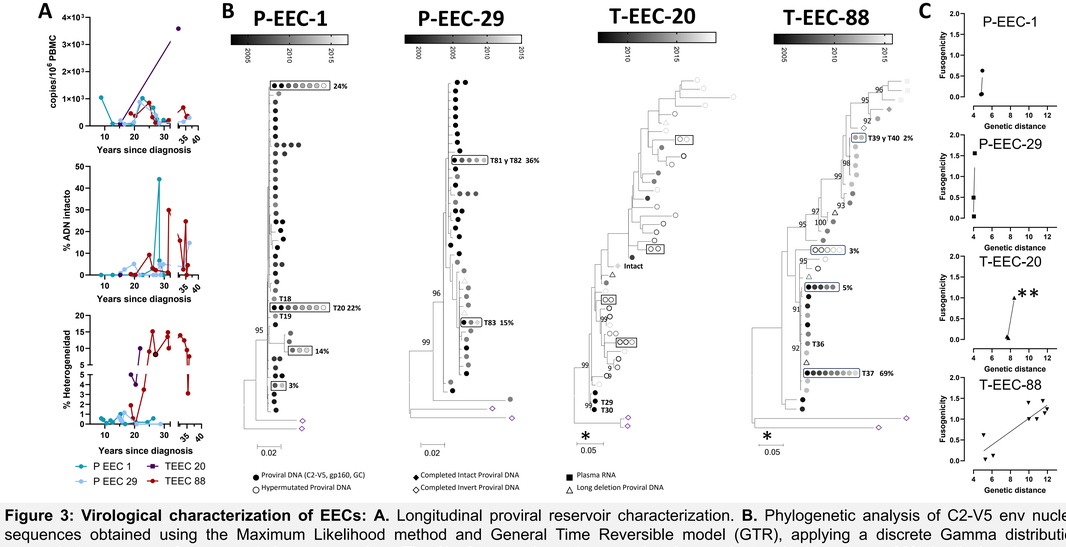


Figure 3: Virological characterization of EECs: A. Longitudinal proviral reservoir characterization. **B.** Phylogenetic analysis of C2-V5 env nucleotide sequences obtained using the Maximum Likelihood method and General Time Reversible model (GTR), applying a discrete Gamma distribution (5 categories) to account for rate variation among sites. The % of trees where the associated taxa clustered with values >90% is displayed next to the branches. *:Scale was reduced for T-EECs. **C.** Comparison of Env fusogenicity and genetic distance relative to the subtype B ancestral sequence (Los Alamos). **This specific Env was codon optimized, therefore, its fusogenic activity is probably exaggerated.

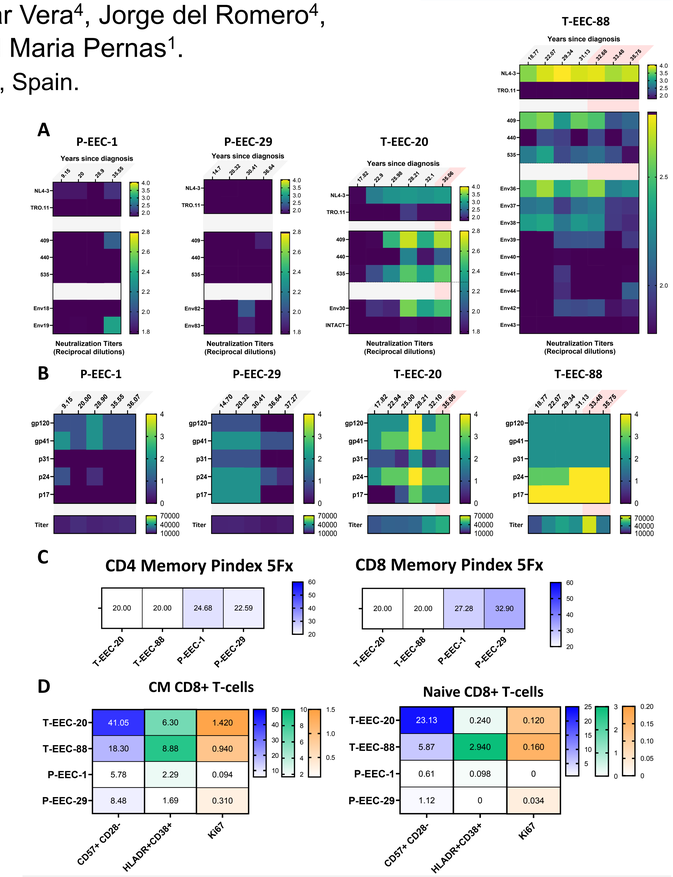


Figure 4: Anti-HIV responses in EECs. A. HIV-1 EEC plasma sample neutralization titers heatmap (reciprocal dilution) against reference strains (NL4-3 and TRO.11), MRCA sequences (409, 440 and 535) and autologous Env expressing pseudoviruses. **B.** Anti-HIV Humoral response heatmap measured by INNO-LIA (semiquantitative, Top panel) and titers (reciprocal dilutions) by LIAISON XL MUREX HIV Ab/Ag (Lower Panel). **C.** CD4 and CD8 T-cell effector function measured by intracellular staining and represented as a polyfunctional Index heatmap. **D.** Central Memory (CM) and Naive CD8 T cells phenotype heatmap including CD57/CD28 (senescence) HLADR/CD38 (activation) and Ki67 (proliferation) expression. In the case of T-EECs, T cell results are from the last sample before loss of control.

CONCLUSIONS

These findings suggest that host genetic factors, immune response and reservoir dynamics are critical determinants of long-term spontaneous HIV-1 control. Long-term follow-up revealed that EECs are a heterogeneous group. Distinguishing among both phenotypes is crucial for understanding durable remission and guiding functional cure strategies

PLAIN LANGUAGE SUMMARY

Rare individuals can control HIV to undetectable levels for several decades in absence of treatment. However, in some cases, loss of control is observed which could be predicted by changes in both the virus and the immune response.

ECRIS: AIDS Research Network HIV-controllers Cohort

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