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Research Letter In-Press Preview **AIDS/HIV** **Immunology** **Virology**

Characterization of elite controllers with undetectable intact HIV DNA reservoir

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Elite controllers (EC) are people with HIV (PWH) who can naturally control HIV infection without antiretroviral therapy (ART). A better understanding of the mechanisms for this control could inform novel therapeutic strategies for achieving a cure for HIV. Multiple factors, including HIV DNA reservoir size, the landscape of viral integration, viral fitness, protective HLA alleles, and potent T-cell responses, may contribute to elite control (1, 2). Previous studies have demonstrated significantly smaller HIV DNA reservoirs, including intact proviral DNA (IPD), in ECs compared to chronically ART-treated PWH (3, 4). In this study, we examined 34 ECs to identify and characterize those without detectable IPD, and to elucidate the mechanisms underlying their exceptional virologic control.

We first examined virological parameters, including intact, defective, total HIV DNA, and cell-associated HIV RNA. Surprisingly, 13 ECs had undetectable IPD (EC-IPD0), whereas 21 ECs had detectable IPD (EC-IPD+) (Figure 1A). When the ECs were stratified by IPD levels, there were no significant differences in age, sex, duration of infection after diagnosis, or $CD4^+$ and $CD8^+$ T-cell counts between the groups (Supplemental Table 1). However, the total ($P<0.0001$) and 3' defective ($P=0.0319$) HIV DNA levels were significantly lower in the EC-IPD0 compared to the EC-IPD+ group, while levels of 5' defective HIV DNA ($P=0.0834$) and cell-associated HIV RNA ($P=0.0770$) did not differ between the groups (Figure 1A). Levels of cells harboring replication-competent HIV were significantly lower in the EC-IPD0 compared to the EC-IPD+ participants ($P=0.0173$; Figure 1B), indicating that EC-IPD0 participants maintain extraordinarily low levels of productively infected cells. Notably, the HLA-B*57 haplotype was significantly more prevalent in the EC-IPD0 (61.5%) versus the EC-IPD+ group (33.3%) ($P=0.0324$; Figure 1C and Supplemental Table 1). Of note, confirmatory HIV antibody tests

yielded indeterminate results in three ECs from the EC-IPD0, whereas none were observed in the EC-IPD+ group (Supplemental Table 2).

To gain further insights into the low infectious HIV burden of the EC-IPD0 participants, we conducted high-dimensional spectral flow cytometry to examine T-cell immunophenotypes. Based on a panel of 28 cell-surface markers, we identified significantly reduced frequencies of effector memory (T_{EM}; $P=0.0397$) and effector memory RA (T_{EMRA}; $P=0.0040$) CD4$^+$ T cells, as well as significantly elevated frequencies of central memory CD8$^+$ T cells (T_{CM}; $P=0.0154$) in the EC-IPD0 compared to the EC-IPD+ group (Figure 1D). Additional analysis using the FlowSOM algorithm identified two of the 30 meta-clusters that were significantly reduced in the EC-IPD0 group (Figure 1E). These included T_{EM}/terminally differentiated (T_{TD}) CD8$^+$ T cells expressing senescent (CD57) and exhaustion (2B4) markers (cluster 12, $P=0.0406$) and transitional (T_{TM})/T_{EM} cytotoxic (Tc1) CD8$^+$ T cells expressing high levels of activation markers CD38 and HLA-DR (cluster 25, $P=0.0242$). Antigen-specific T-cell analysis showed comparable levels of polyfunctional HIV Gag-specific CD4$^+$ ($P=0.5937$) and CD8$^+$ T cells ($P=0.2800$) between the groups (Figure 1F). Finally, we measured the levels of biomarkers PD-L1, CXCL10, IL-2Ra, IL-6, granzyme B, perforin, and RANTES in the plasma of the study participants. Among them, only IL-6 levels were significantly elevated in EC-IPD0 vs. EC-IPD+ participants ($P=0.0154$; Supplemental Figure 1). Of note, analyses of innate immune cells, including natural killer and plasmacytoid dendritic cells, revealed no differences between the groups (Supplemental Figure 2).

The identification of a sizable number of ECs with undetectable IPD could potentially advance our understanding of the mechanisms of HIV control without ART. They exhibit extraordinarily low levels of intact and infectious HIV, suggesting the near-complete elimination

of infected cells. Our findings indicate several distinguishing immunologic features in EC-IPD0 participants, including enrichment of the HLA-B*57 haplotype, reduced frequencies of effector $CD4^+$ and exhausted/activated $CD8^+$ T cells. In particular, the predominance of the HLA-B*57 haplotype in EC-IPD0 aligns with previous findings demonstrating its association with superior HIV control, driven by a strong $CD8^+$ T-cell response and subsequent clearance of infected cells (2, 5). However, the functionality of $CD8^+$ T cells (i.e., proliferative and cytotoxic responses to additional HIV antigens) between the two groups requires further investigation. Our data further suggest that this genetic trait within the EC cohort may facilitate enhanced elimination of the persistent HIV reservoir, in addition to effective control of plasma viremia in the absence of ART. Additionally, the reduced frequency of terminally differentiated $CD8^+$ T cells expressing exhaustion markers suggests preservation of functional immune responses, potentially enabling the elimination of detectable HIV reservoirs; however, this could be due to lower antigen exposure in the EC-IPD0 versus the EC-IPD+ group. Although the elevated IL-6 level in EC-IPD0 is intriguing (6), its role in the clearance of infected cells warrants further investigation.

The limitations of our work include the small sample size, as well as the lack of longitudinal timepoints and lymphoid tissue analyses. Nonetheless, we demonstrated that some ECs achieved near-complete elimination of infectious HIV reservoirs and identified correlates of this remarkable virologic control. Our findings provide important insights into the mechanisms underlying the elimination of persistent HIV reservoirs in the EC population that can apply to the development of therapeutic strategies aimed at achieving HIV cure.

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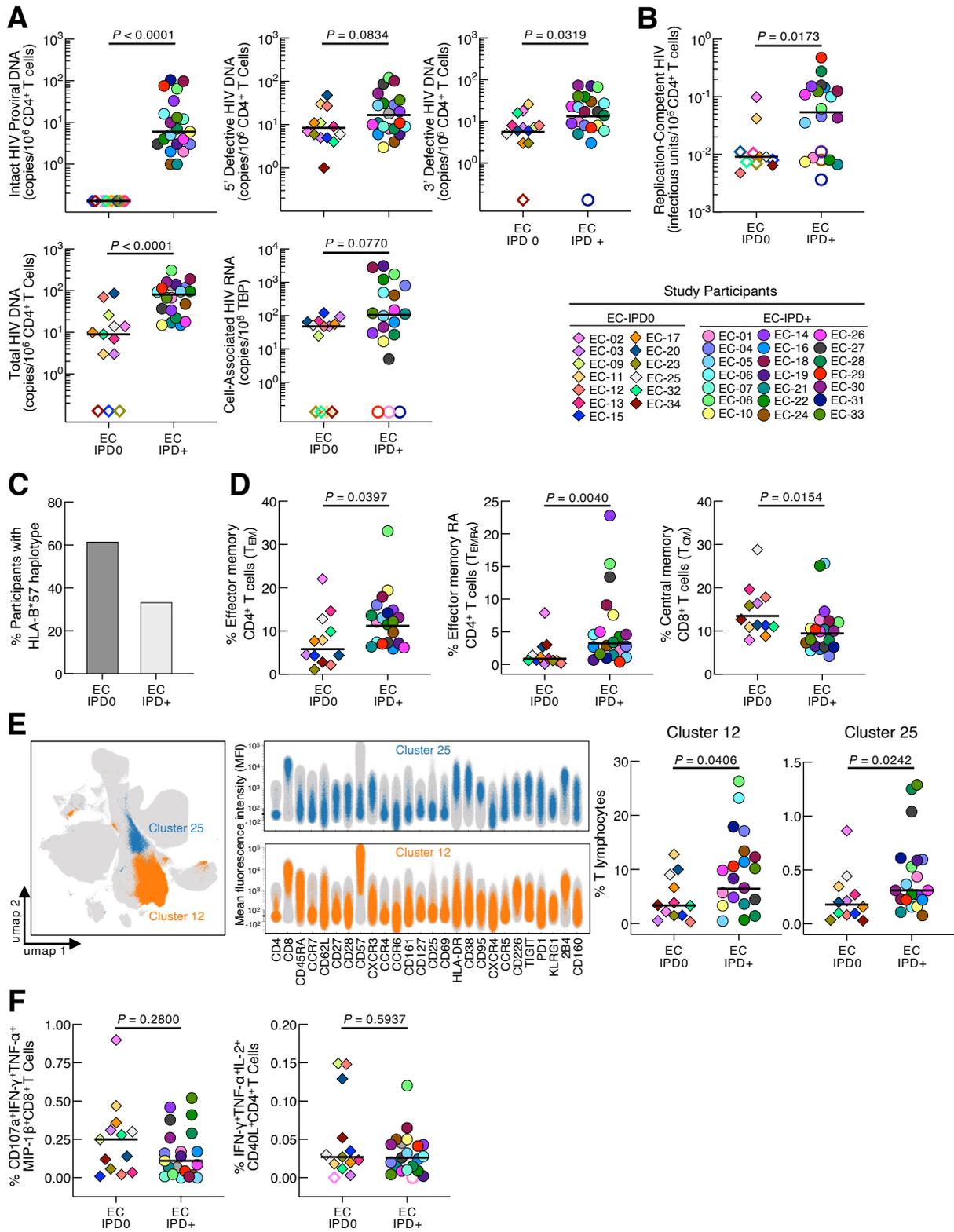
Figure 1. HIV reservoirs and immune parameters in elite controllers (EC) with undetectable (EC-IPD0) versus detectable (EC-IPD+) intact HIV DNA. Comparisons of (**A**) intact, defective, and total HIV DNA, cell-associated HIV RNA, and (**B**) replication-competent virus. (**C**) Proportion of participants with HLA-B*57 phenotype. (**D**) Frequencies of effector memory (T_{EM}) and effector memory RA (T_{EMRA}) CD4$^+$ and central memory (T_{CM}) CD8$^+$ T cells. (**E**) Uniform Manifold Approximation and Projection (UMAP) map of T-cell clusters identified by FlowSOM clustering, mean fluorescent intensity (MFI) plots showing the level of expression of individual markers in two significantly different clusters, Cluster 12 and 25, and comparison of the frequencies of those clusters in T lymphocytes. (**F**) Comparisons of frequencies of HIV Gag-specific polyfunctional CD4$^+$ and CD8$^+$ T cells. Open symbols indicate values under limits of detection. Black bars represent the median values. *P* values were determined using the Mann-Whitney test.