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Genetic Evidence That Naive T Cells Can Contribute Significantly to the Human Immunodeficiency Virus Intact Reservoir: Time to Re-evaluate Their Role

To the Editor—We read with interest the article by Zerbato et al [1] on the human immunodeficiency virus (HIV) reservoir harbored in naive $CD4^+$ T cells (TN cells). We would like to share our genetic evidence that TN cells harbor a substantial fraction of the intact reservoir to reinforce the message that TN cells may play a central role in shaping the reservoir in comparison with central memory $CD4^+$ T cells (TCM cells).

We sorted TN cells (defined as $CD45RA^+$, $CCR7^+$, $CD27^+$) and TCM cells (defined as $CD45RA^-$, $CCR7^+$, $CD27^+$) from $CD3^+CD8^-$ peripheral blood mononuclear cells of 2 chronically HIV-1–infected donors who were virally suppressed on antiretroviral therapy (ART) for 2 years. We measured total HIV DNA and amplified HIV proviruses at limiting dilution in each subset. We sequenced a total of 365 and 227 near-full-length proviruses in subjects 1 and 2, respectively, as described [2]. All proviruses with an intact envelope (n = 159) in subject 1 were CCR5-tropic while the majority of evaluable proviruses (n = 106) in subject 2 were CXCR4-tropic.

Consistent with Zerbato et al [1] and previous reports [3–5], total HIV DNA was higher in TCM cells (4583 and 3717 HIV/million cells for subjects 1 and 2) than in TN cells (654 and 1236, respectively). After proviral sequencing of sorted subsets, we calculated the contribution of intact proviruses/million $CD4^+$ cells by these 2 subsets. Even though the HIV DNA levels were higher in TCM cells for both subjects, the contribution of intact proviruses by TN cells was far greater than by TCM cells, representing 38.5% and 58.7% of the intact reservoir (Figure 1A). Similarly, the percentages of intact proviruses were also higher in TN cells than in TCM cells (Figure 1B).

These findings could explain why the authors found equal or even higher levels of HIV RNA in TN than in TCM cells after latency reversal, even if the level of

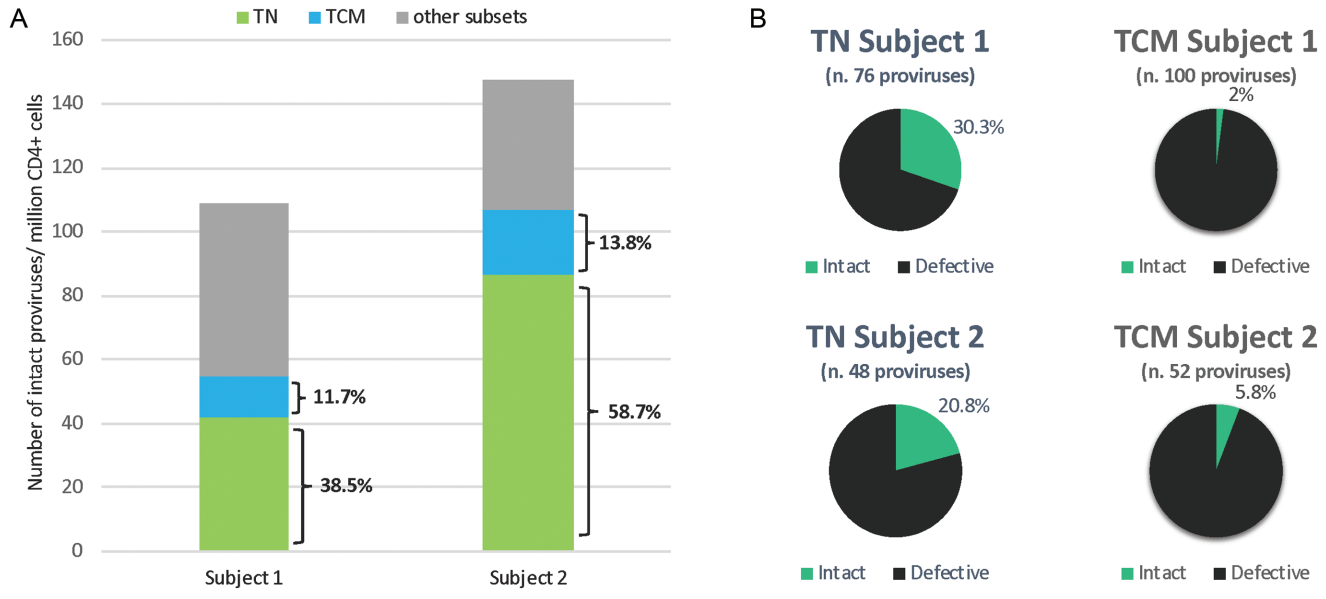

Figure 1. Genetic evidence that naive T cells contribute significantly to the replication-competent reservoir. *A*, The number of intact proviruses per million $CD4^+$ T cells for both subjects was determined after 2 years on suppressive ART. By sorting PBMCs into TN cells (green), TCM cells (blue), and other subsets (gray), the numbers of intact proviruses in all of the subsets were determined. TN cells contributed more intact proviruses than TCM cells. *B*, The percentages of intact (teal) and defective (black) proviruses within the 2 subsets are shown. Abbreviations: ART, antiretroviral therapy; PBMC, peripheral blood mononuclear cell; TCM, central memory $CD4^+$ T cells; TN, naive $CD4^+$ T cells.

total HIV DNA was significantly higher in TCM cells.

Notably, these 2 subjects represent 2 classes of chronic infection based on viral tropism and clinical evolution, yet both showed a prominent contribution of TN cells to the reservoir, consistent with Zerbato et al [1]. Specifically, subject 1 had a protracted $CD4^+$ cells decline with only CCR5-tropic proviruses and a nadir of 295 $CD4^+$ cells/μLafter 21 years of infection, while subject 2 had more rapid progression with a majority of CXCR4-tropic proviruses and a nadir of 0 $CD4^+$ cells/μL after 6 years of infection.

We do agree with the authors that the role of TN cells has been underestimated so far because of the low integration level of HIV DNA in this subset.

TN and TCM cells likely experience different selection pressures, which lead to differences in proviral decay and preservation of intact proviruses within TN cells. The potential for TCM cells to differentiate into effector memory cells may explain their less prominent contribution to the intact reservoir. A limitation of our study is that we cannot prove what fraction of TN cells are truly naive. Due to the longer life span of TN cells and their potential to convert into more differentiated T cells, TN cells may play a crucial role in maintaining and replenishing the HIV reservoir in patients on ART.

Notes

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