Age but not disease progression defines CD4+ and CD8+ T stem cell memory levels in human retroviral infections: contrasting effects of HTLV-1 and HIV-1

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Abstract:

Background: Human $CD4^+$ and $CD8^+$ stem cell memory T cells (T_{SCM}) represent a minor fraction of circulating lymphocytes characterized by stemness and long-term *in vivo* persistence. $CD4^+$ T_{SCM} are preferentially infected and constitute a reservoir for HIV-1, whereas $CD8^+$ T_{SCM} appear to play a protective role. However, little is known about $CD4^+$ and $CD8^+$ T_{SCM} in the only other human pathogenic retroviral infection, human T-cell leukemia virus type 1 (HTLV-1). HTLV-1 is the etiological agent of both Adult T-cell Leukemia (ATL) and HTLV-1 associated myelopathy/tropical spastic paraperesis (HAM/TSP), a neuroinflammatory disorder. In ATL, $CD4^+$ T_{SCM} cells were identified as the hierarchical leukemic stem cell, but data in HAM/TSP are lacking. Age is a major risk factor for both ATL and HAM/TSP, as both diseases generally manifest several decades after infection. Therefore, we explored a possible link between T_{SCM}, age and disease status in human retroviral infections in a cross-sectional study, using multiparametric flow cytometry.

Results: We found that $CD4^+$ or $CD8^+$ T_{SCM} levels (quantified as $CD3^+CD45RA^+CD45RO^-CD27^+CCR7^+Fas^{hi}$) do not differ between healthy controls and untreated HTLV-1 infected individuals with and without neuroinflammatory disorder. However, we found both T_{SCM} as well as $CD8^+$ T_{SCM} significantly accumulated with age, resulting in a >400% increase in elderly HTLV-1 infected individuals (>60 years). A significant correlation between age and T_{SCM} signature genes was validated at the transcriptome level in an independent cohort. $CD8^+$ but not $CD4^+$ T_{SCM} were significantly decreased in untreated HIV-1 infection. Unexpectedly, $CD8^+$ T_{SCM} recovery upon successful antiretroviral treatment was essentially complete (92.2±11.0%) in younger (<45 years) individuals, but significantly lower (37.3±6.1%) in older (>45 years) individuals (p=0.0003).

Conclusion: In HTLV-1 infection, an age-dependent accumulation of $CD4^+$ and $CD8^+$ T_{SCM} points towards a possible protective role of CD8 T_{SCM} in the elderly against leukemic but not neuroinflammatory disease. HIV-1-infected individuals lose their ability to restore $CD8^+$ T_{SCM} levels upon successful antiretroviral therapy at later age (>45 years), which might eventually lead to immunological failure and decreased vaccine efficacy.

BRIEF REPORT

Introduction

Human $CD4^+$ and $CD8^+$ stem cell memory T cells (T_{SCM}) represent a small fraction (2-3%) of circulating lymphocytes characterized by intrinsic apoptosis resistance, proliferation and long-term *in vivo* persistence (Gattinoni et al., 2011). $CD4^+$ T_{SCM} are preferentially infected and constitute a long-lived cellular reservoir for human immunodeficiency virus-1 (HIV-1) (Buzon et al., 2014), whereas $CD8^+$ T_{SCM} appear to play a protective role (Ribeiro et al., 2014; Vigano et al., 2015). However, little is known about $CD4^+$ and $CD8^+$ T_{SCM} in the only other human pathogenic retroviral infection, namely human T-cell leukemia virus type 1(HTLV-1). HTLV-1 is the etiological agent of both Adult T-cell Leukemia (ATL) and HTLV-1 associated myelopathy/tropical spastic paraperesis (HAM/TSP), a neuroinflammatory disorder. Age is a major risk factor for both ATL and HAM/TSP, as both diseases generally manifest several decades after infection. In ATL, $CD4^+$ T_{SCM} cells were identified as the hierarchical leukemic stem cell (Nagai et al., 2015). In HAM/TSP, we recently documented a predominant Fas^{hi} phenotype linked to lymphoproliferation and inflammation (Menezes et al., 2017), but its possible link to $CD4^+$ and $CD8^+$ T_{SCM} subsets is unknown. Interestingly, *FAS* polymorphisms determine both ATL susceptibility (Farre et al., 2008) and $CD4^+$ and $CD8^+$ Tscm levels in a large twin study (Roederer et al., 2015). Pulko et al. recently identified a novel human memory T cell subset with a naïve phenotype, accumulating with age (Pulko et al., 2016). While Pulko et al. elaborately explored various $CD8^+$ naïve and memory cellular subsets and elegantly described a $CD45RA^+IFN\text{-}\gamma^+CXCR3^+Fas^{lo}$ subset of memory T cells with a naïve phenotype (T_{MNP}) and responsiveness to chronic but not acute viral infections, their study did not include T memory stem cells (T_{SCM}) (Pulko et al., 2016). Therefore, we explored a possible link between T_{SCM}, age and disease status in human retroviral infections in a cross-sectional study.

Results and Discussion

We found that $CD4^+$ or $CD8^+$ T_{SCM} levels (quantified as $CD3^+CD45RA^+CD45RO^-CD27^+CCR7^+Fas^{hi}$) do not differ between healthy controls (HC, seronegative for HIV-1 and

HTLV-1) and untreated HTLV-1 infected individuals with and without neuroinflammatory disorder (AC and HAM/TSP patients, Fig. 1A). Whereas the hallmark of HIV-1 infection is $CD4^+$ depletion, HTLV-1 infection propels $CD4^+$ cells into the cell cycle while protecting $CD8^+$ cells from apoptosis (Sibon et al., 2006). However, $CD4^+$ T_{SCM} abundance was not significantly different between HC, untreated HTLV-1 (HIV-1 negative), untreated HIV-1 and treated HIV-1 infection (successful antiretroviral therapy with undetectable viral load), as shown in Fig. 1B. Nevertheless, $CD8^+$ T_{SCM} was significantly decreased in untreated HIV-1 infection compared to all other groups (Fig. 1C). In parallel to HTLV-1 infection, $CD4^+$ and $CD8^+$ T_{SCM} levels (%) did not differ in untreated HIV-1-infected individuals with different degrees of disease progression, i.e. controllers, non-controllers and immunological progressors (Ribeiro et al., 2014).

In a large cohort of healthy controls (n=460), $CD4^+$ T_{SCM} were found to modestly increase by 25% over a 30-year period in HC (p=0.06, Roederer et al. unpublished), whereas $CD8^+$ T_{SCM} levels decrease 25% over the same period (p=0.004, Roederer et al., unpublished). Unexpectedly, in untreated HTLV-1-infected individuals, both $CD4^+$ T_{SCM} as well as $CD8^+$ T_{SCM} significantly accumulated with age (r=0.60, p=0.016 and r=0.73, p=0.0019, respectively), resulting in a >400% increase in the elderly (>60 years Fig. 2A). This effect was not due to differences in viral factors, as $CD4^+$ and $CD8^+$ T_{SCM} levels did not correlate to proviral load nor viral mRNAs (Tax and HBZ). Furthermore, age-dependent accumulation was observed in the combined HTLV-1-infected group, as well as in the asymptomatic and HAM/TSP subgroups, implying that age, but not disease status, determines $CD4^+$ T_{SCM} and $CD8^+$ T_{SCM} levels in HTLV-1 infection.

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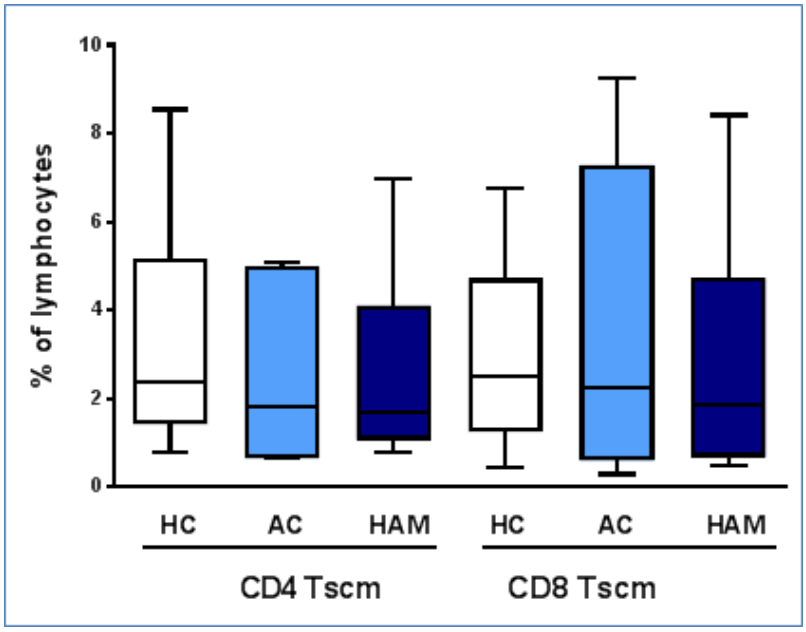

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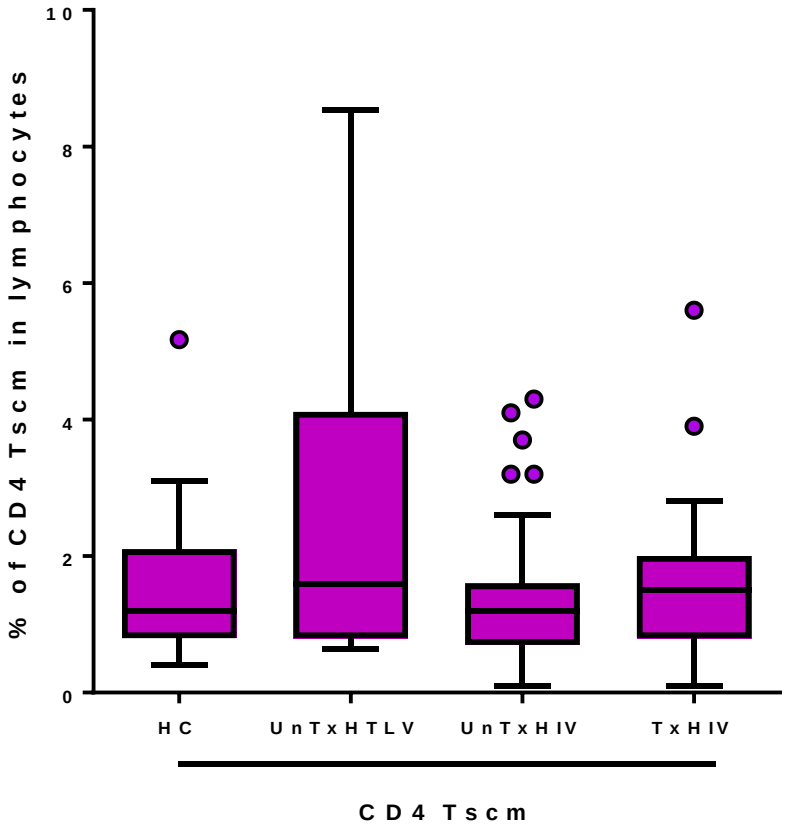

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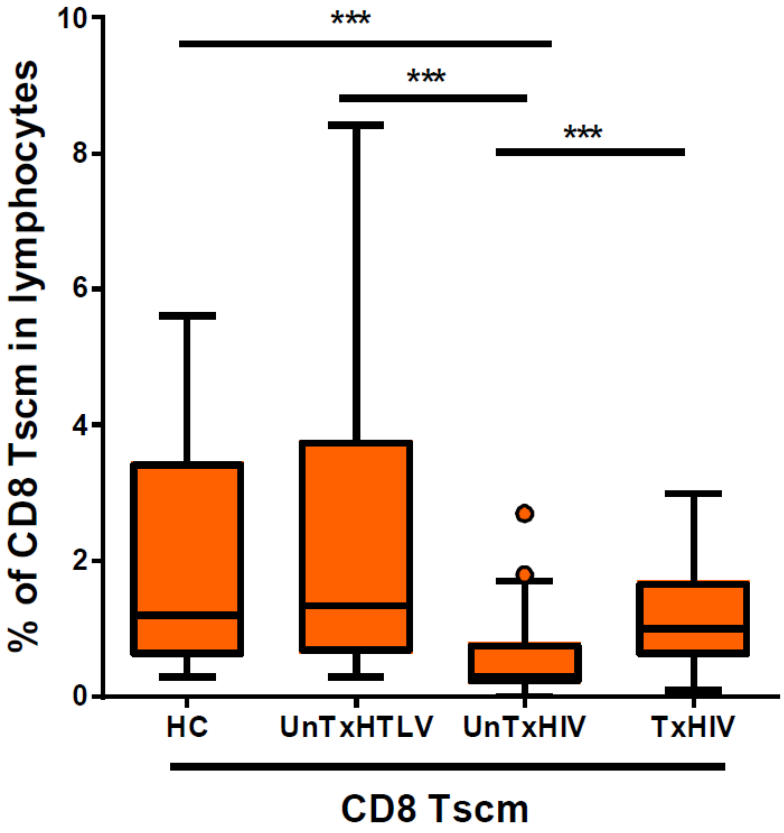

Figure 1. CD4⁺ and CD8⁺ T_{SCM} levels do not differ between matched healthy controls (HC), HTLV-1 asymptomatic carriers (AC) and HAM/TSP patients, while CD8⁺ T_{SCM} cells are depleted in untreated HIV-1-infected individuals. *(A) No significant difference in the levels of CD4⁺ and CD8⁺ T_{SCM} cells ($CD3^+CD45RA^+CD45RO^-CD27^+CCR7^+Fas^{hi}$) quantified by flow cytometry in HC (n=7 CD4⁺ T_{SCM}, n=9 CD8⁺ T_{SCM}), AC (n=6) and HAM/TSP patients (n=9). (B) No significant difference in the levels of CD4⁺ T_{SCM} between HC (n=19), untreated HTLV-1- (n=13), untreated HIV-1- (n=77) and treated (n=27) HIV-1-infected individuals. (C) CD8⁺ T_{SCM} levels are decreased in untreated HIV-1-infected individuals when compared to HC, untreated HTLV-1 and treated HIV-1 infected individuals. (***p < 0.001; Kruskal-Wallis test with Dunn's post-test).*

We validated these findings at the transcriptome level using an independent, previously published cohort (Tattermusch et al., 2012). Transcript levels of T_{SCM} signature markers (Gattinoni et al., 2011) *FAS* (p=0.0005) and *CD58* (p=0.026) were positively correlated to age but not disease in HTLV-1-infected individuals (n=30), forming a distinct cluster with other T_{SCM} signature genes (*FAM129A* and *PLAUR*, upper cluster Fig. 2B). On the other hand, transcript levels of T_{MNP} signature genes *ITGA4*, *CXCR3* and *IFNG* were not correlated to age or disease status but significantly correlated to each other ($p<0.001$, in agreement with Pulko et al.), forming a second cluster (middle in Fig. 2B), which included memory (*PRDM1/HNRNPLL/KLRG1*) and proliferative (*PCNA*) markers. A third distinct cluster, negatively correlated to age, was comprised of "classical" naïve T cell marker genes (*IL7R, FOXP1, IL6ST, LEF1, TAF4B, CCR7*) and telomerase (*TERT*). Supporting HTLV-1 specificity, no naïve or memory subset marker genes were correlated to age in transcriptomes from HC (data not shown). This age-dependent increase is quite surprising given the role of $CD4^+$ T_{SCM} in ATL pathogenesis (Nagai et al., 2015). Since the oldest HTLV-1-infected individuals (both AC and HAM/TSP) did not develop ATL, even at a mean age of 66±4.5 years, the parallel increase of $CD8^+$ T_{SCM} cells might keep the pre-leukemic $CD4^+$ T_{SCM} in check, in agreement with a proposed protective role of $CD8^+$ cytotoxic effector cells in ATL (Rowan et al., 2016), which can be derived from the $CD8^+$ T_{SCM} pool. A wealth of recent studies has indeed demonstrated the superior anti-tumoral properties and clinical potential of $CD8^+$ T_{SCM} cells for adaptive immunotherapy, as compared to other $CD8^+$ naïve or memory subsets.

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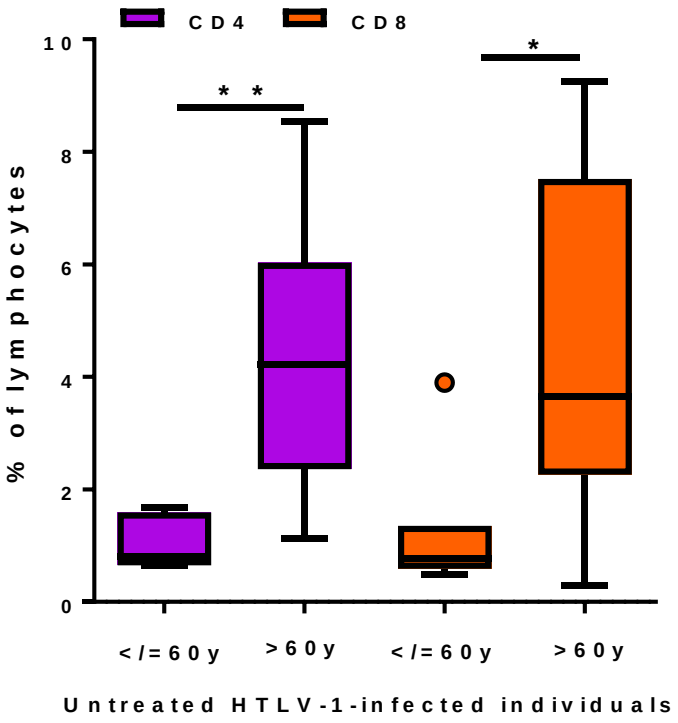

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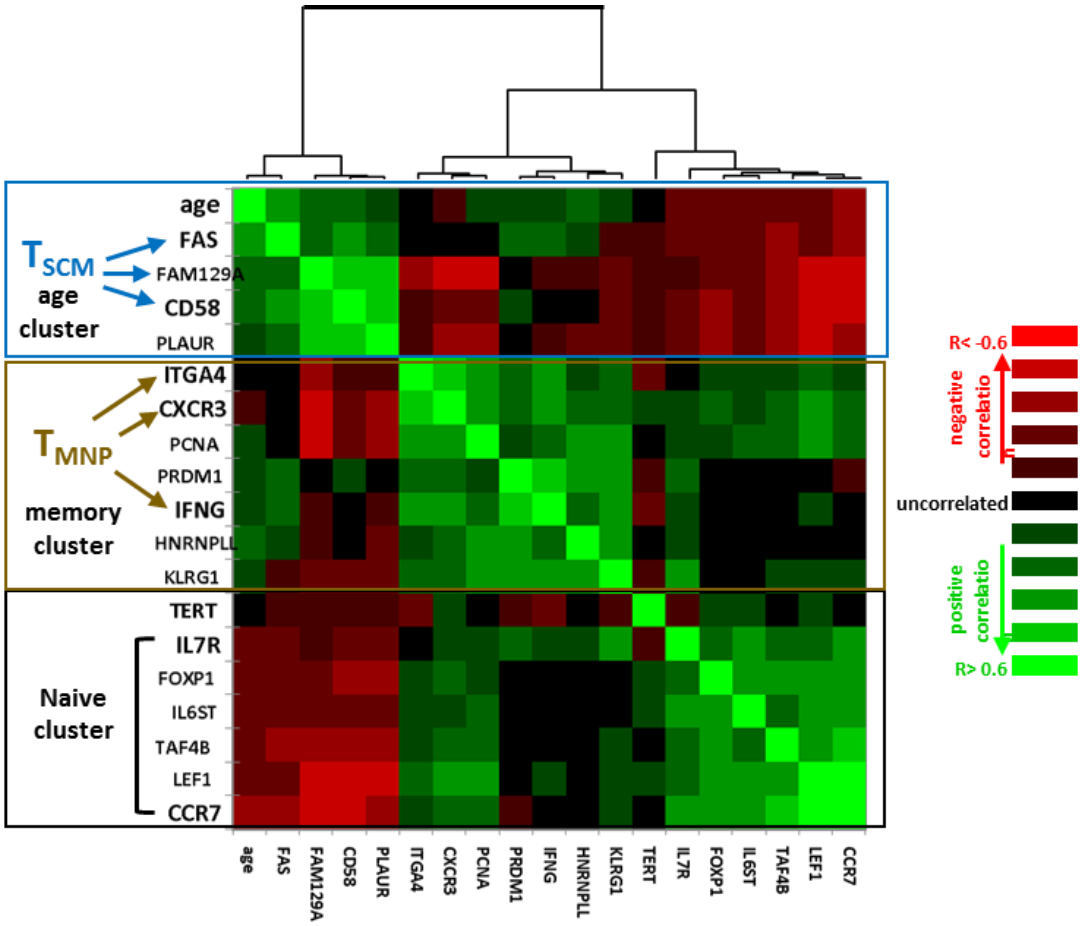

Figure 2: T_{SCM} cells accumulate with age and T_{SCM} but not T_{MNP} signature genes correlate to age in untreated HTLV-1 infection. *(A) $CD4^+$ (**p = 0.0024, unpaired t test) as well as $CD8^+$ (*p = 0.016) T_{SCM} levels increase in older HTLV-1 infected individuals (> 60 years, n=9 vs. <60 years, n=7).(B) Transcriptomic analysis of T_{SCM}, T_{MNP} and Naïve/Memory markers, clustered according to Spearman's correlation (heatmap) reveals three distinct clusters: T_{SCM} -Age, T_{MNP}-Memory and Naïve, in HTLV-1 infected individuals from an independent published UK cohort (n=30).*

In contrast to HTLV-1-infected individuals, we observed a significant age-dependent decline of $CD8^+$ T_{SCM} (p=0.0017, r=-0.57) but not $CD4^+$ T_{SCM} levels in treated HIV-1 patients (Fig. 3A). There was no correlation of $CD4^+$ T_{SCM} or $CD8^+$ T_{SCM} with age in untreated HIV-1 patients (Fig. 3B), again independent of disease status (data not shown). When calculating the percentage of $CD8^+$ T_{SCM} depletion (relative to HC), $CD8^+$ T_{SCM} depletion was strikingly similar between younger (<45 years) and older (>45 years) untreated HIV-1-infected individuals (Figure 3C). However, the percentage of $CD8^+$ T_{SCM} recovery upon successful treatment was essentially complete (92.2±11.0%) in younger (<45 years) individuals, but significantly lower (37.3±6.1%) in older (>45 years) individuals (p=0.0003, Figure 3D), which parallels HIV-associated $CD8^+$ senescence in this age group (Cobos Jimenez et al., 2016). Thus, contrary to HTLV-1 infection, disruption of $CD8^+$ T_{SCM} homeostasis occurs at an early age in HIV-1 infection, but is relatively stable over the age groups (defective CD8 T_{SCM} recovery is apparent at different cut-offs, i.e. in age groups >40, >50 and >60 years, data not shown).

Hence, our findings have obvious clinical implications in the light of current life-long requirement of antiviral therapy for HIV-1-infected individuals. Although successful as measured by suppressed viral load and increased $CD4^+$ levels, antiretroviral therapy was unable to restore protective $CD8^+$ T_{SCM} levels at middle age. This eventually might lead to immunological failure and disease progression, highlighting the need for long-term in-depth immunological follow-up of large cohorts, beyond the current routine $CD4^+$ and viral load monitoring. In addition, this novel age-T_{SCM} link should be considered in ongoing therapeutic vaccination trials aiming at a functional cure for HIV. On a larger scale, given the extensively documented role of $CD8^+$ T_{SCM} in life-long immunological memory and an increasingly ageing human population, natural as well as vaccine-induced herd immunity against other known as well as “novel” pathogens (e.g. pandemic flu, arboviruses) might wane or disappear over time, considering the 25% decline observed in a large cohort of healthy controls. In conclusion, our findings indicate vigilance is warranted in the light of age-dependent disruption of T_{SCM} homeostasis.

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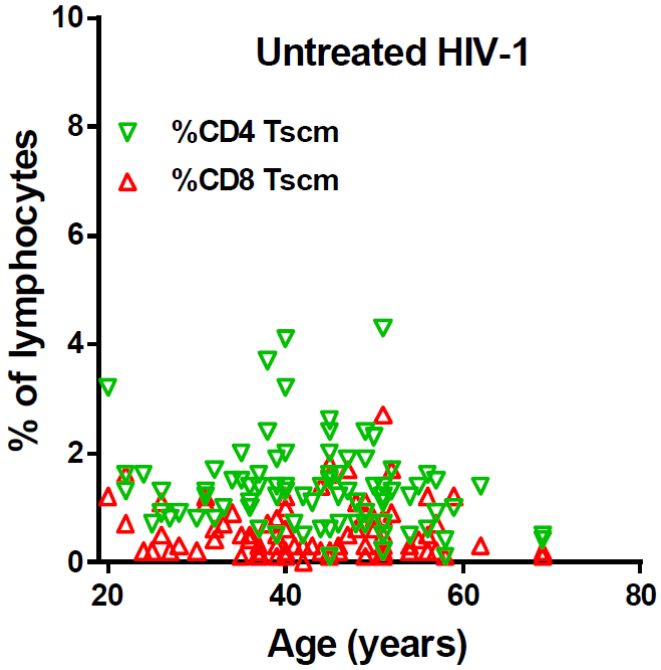

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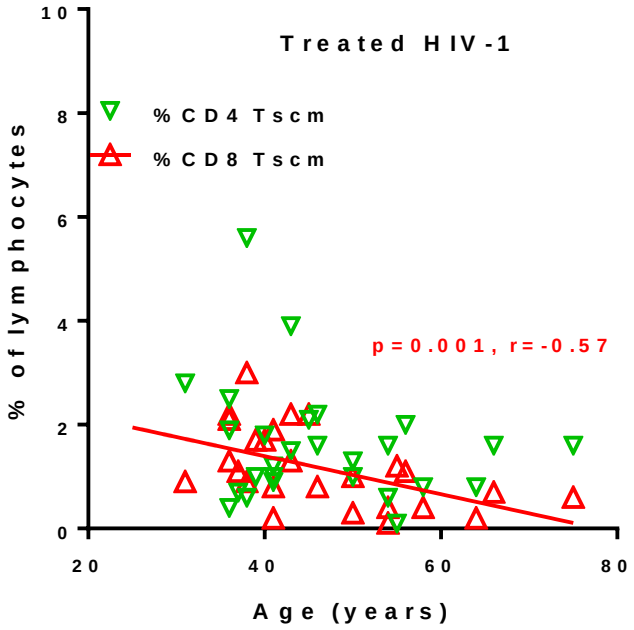

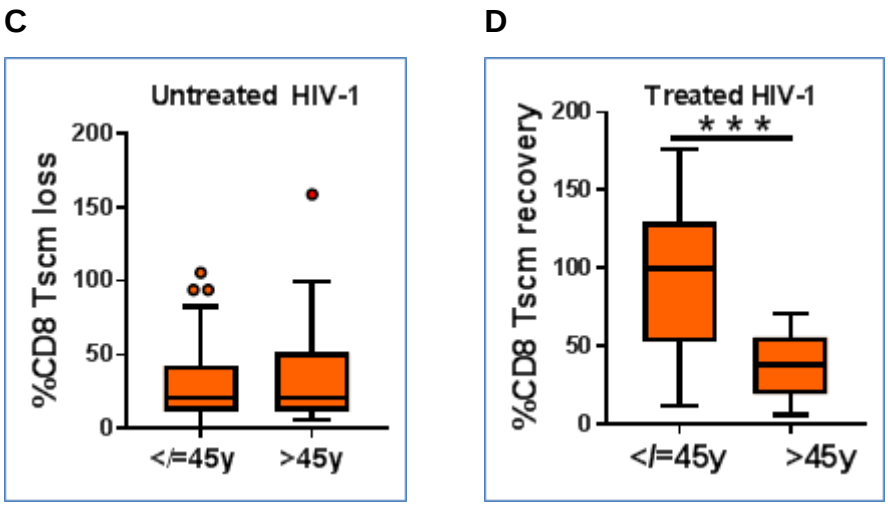

Figure 3: HIV-1 infected individuals over 45 years lose their ability to restore CD8⁺ T_{SCM} levels following successful ART. *(A) In treated HIV-1-infected individuals, CD8⁺ but not CD4⁺ T_{SCM} levels negatively correlate with age. (**p=0.001, Spearman's r=0.57, n=27). (B) Neither CD4⁺ nor CD8⁺ T_{SCM} levels were correlation of age to in untreated HIV-1-infected individuals (n=77). (C) No age effect in CD8⁺ T_{SCM} depletion (relative to matched HC) in untreated HIV-1 infected individuals (> and < 45 years). (D) HIV-1 infected individuals on successful ART treatment >45 years recover less than half of CD8⁺ T_{SCM} levels (relative to matched HC) compared to those <45 years. (***p<0.001, Unpaired t test, with Welch correction)*

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