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 paraparesis (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 paraparesis (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-γ⁺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.

A

B

C

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/HNRNP11/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.

A

B

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 ($92.2\pm11.0\%$) in younger (<45 years) individuals, but significantly lower ($37.3\pm6.1\%$) 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.

A

B

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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