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The CD8$^+$ Memory Stem T Cell (T_{SCM}) Subset Is Associated with Improved Prognosis in Chronic HIV-1 Infection

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ABSTRACT

Memory stem T cells (T_{SCM}) constitute a long-lived, self-renewing lymphocyte population essential for the maintenance of functional immunity. The hallmarks of HIV-1 pathogenesis are CD4$^+$ T cell depletion and abnormal cellular activation. We investigated the impact of HIV-1 infection on the T_{SCM} compartment, as well as any protective role these cells may have in disease progression, by characterizing this subset in a cohort of 113 subjects with various degrees of viral control on and off highly active antiretroviral therapy (HAART). We observed that the frequency of CD8$^+$ T_{SCM} was decreased in all individuals with chronic, untreated HIV-1 infection and that HAART had a restorative effect on this subset. In contrast, natural controllers of HIV-1 had the highest absolute number of CD4$^+$ T_{SCM} cells among all of the infected groups. The frequency of CD4$^+$ T_{SCM} predicted higher CD8$^+$ T_{SCM} frequencies, consistent with a role for the CD4$^+$ subset in helping to maintain CD8$^+$ memory T cells. In addition, T_{SCM} appeared to be progenitors for effector T cells (T_{EM}), as these two compartments were inversely correlated. Increased frequencies of CD8$^+$ T_{SCM} predicted lower viral loads, higher CD4$^+$ counts, and less CD8$^+$ T cell activation. Finally, we found that T_{SCM} express the mucosal homing integrin α4β7 and can be identified in gut-associated lymphoid tissue (GALT). The frequency of mucosal CD4$^+$ T_{SCM} was inversely correlated with that in the blood, potentially reflecting the ability of these self-renewing cells to migrate to a crucial site of ongoing viral replication and CD4$^+$ T cell depletion.

IMPORTANCE

HIV-1 infection leads to profound impairment of the immune system. T_{SCM} constitute a recently identified lymphocyte subset with stem cell-like qualities, including the ability to generate other memory T cell subtypes, and are therefore likely to play an important role in controlling viral infection. We investigated the relationship between the size of the CD8$^+$ T_{SCM} compartment and HIV-1 disease progression in a cohort of chronically infected individuals. Our results suggest that HAART restores a normal frequency of CD8$^+$ T_{SCM} and that the natural preservation of this subset in the setting of untreated HIV-1 infection is associated with improved viral control and immunity. Therefore, the CD8$^+$ T_{SCM} population may represent a correlate of protection in chronic HIV-1 infection that is directly relevant to the design of T cell-based vaccines, adoptive immunotherapy approaches, or the pharmacologic induction of T_{SCM}.

Human immunodeficiency virus type 1 (HIV-1) causes a slow and progressive disease course characterized by a gradual decline in CD4$^+$ T helper cell numbers. In addition to the loss of CD4$^+$ cells, there is widespread degeneration of the immune system resulting from immune activation caused in part by gut barrier disruption (1, 2). After infection, cellular activation and continued viral replication drive the immune system to exhaustion, reflected by an increase in cellular markers of aging and senescence. Nonhuman primate studies suggest that the dynamics of memory T cell subsets also play a role in the pathogenesis of the disease (3). During untreated infection, HIV-1 preferentially infects activated effector memory T cells (T_{EM}) (4). Initiation of highly active antiretroviral therapy (HAART) is accompanied by a reduced frequency of T cells harboring active HIV-1 replication (5), although viral DNA is still detected at low levels in resting memory T cells. T cell survival and homeostatic proliferation are two major mechanisms implicated in the maintenance of the memory T cell pool.

Conventionally, memory T cells have been divided into subsets of central memory T cells (T_{CM}) and effector memory T cells (T_{EM}), and these cells home to secondary lymphoid and peripheral tissues, respectively (6). More recently, a new subset of memory T cells with stem cell-like properties (T_{SCM}) has been identified (7, 8). These cells are the least differentiated of all distinct memory populations, expressing multiple naive markers as well as the memory antigen CD95. Functionally, T_{SCM} cells can generate multiple memory T cell populations, and they possess an enhanced capacity for self-renewal (7). In addition, they are en-

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TABLE 1 Clinical data for HIV-1-infected subjects

Subject category	Median (IQR[a]) values		
	CD4$^+$ count (cells/mm^3)	HIV-1 load (copies/ml)	Age (yrs)
HAART suppressed	674 (534–981)	50 (0–75)	45 (38–54)
Controllers	813 (585–1,260)	96 (11–489)	47.5 (39–54)
Noncontrollers	576 (446–730)	35,650 (24,586–52,675)	43 (34–49)
Immunologic progressors	228 (175–296)	71,585 (29,925–191,067)	41.5 (36–49)

[a] IQR, interquartile range.

dowed with superior immune reconstitution potential in immunodeficient hosts and can mediate antitumor immunity in a humanized mouse model (7). Given the crucial immune properties of T_{SCM}, we evaluated the presence of these cells in HIV-1-infected individuals on and off HAART and their relationship with viral load, CD4$^+$ count, and activation of the immune system. We observed that HAART had a restorative effect on both the frequency and absolute number of cells in the CD8$^+$ T_{SCM} compartment. This could represent an important benefit of treatment, as these cells are thought to be the progenitors of long-term T cell memory in the setting of viral infection (9). We found that the T_{SCM} population was also present in gut-associated lymphoid tissue (GALT) but at a lower frequency than in the peripheral blood. Furthermore, the frequency of circulating CD8$^+$ T_{SCM} cells in untreated individuals was inversely correlated with T cell activation and viral load, suggesting that they may contribute to protection from disease progression in chronic HIV-1 infection.

MATERIALS AND METHODS

Study subjects. Samples of cryopreserved peripheral blood mononuclear cells (PBMCs) were selected from participants in the San Francisco-based HIV-1-infected SCOPE cohort. Samples from HIV-1-seronegative controls were obtained from 20 donors to the Stanford blood bank. The study was approved by the local Institutional Review Board (University of California San Francisco Committee on Human Research), and individuals gave written informed consent. PBMC samples were obtained from the following numbers and categories of HIV-1-infected individuals: 29 untreated virologic "controllers" (viral load, <2,000 HIV-1 copies/ml), 27 HAART-suppressed patients (viral load, <50 to 75 HIV-1 copies/ml), and 28 untreated "virologic noncontrollers" (viral load, >10,000 copies/ml). All had CD4$^+$ T cell counts of >250 cells/mm^3. A fourth group of untreated HIV-1-infected patients was also tested. These 29 individuals were defined as "immunologic progressors," with an HIV-1 load of >10,000 copies/ml and CD4$^+$ T cell counts of <250 cells/mm^3. All patients had been diagnosed with HIV-1 at least 1 year prior to inclusion in this study. See Table 1 for baseline subject characteristics.

GALT samples. Gut-associated lymphoid tissue (GALT) from rectosigmoid biopsy specimens and paired fresh PBMC samples were obtained from two HIV-1-infected viremic subjects, five HIV-1-infected controllers, and three HIV-1-seronegative subjects. Rectal mononuclear cells (RMCs) were isolated from biopsy specimens by collagenase type II digestion at 37°C for 1 h, followed by blunt dissection, passage through a 70-μm-pore-size cell strainer, and washing with R10 media and fluorescence-activated cell sorter (FACS) buffer (phosphate-buffered saline [PBS] with 0.5% bovine serum albumin and 2 mM EDTA) prior to staining with antibodies for flow cytometry analysis.

Antibodies and flow cytometry. Cryopreserved PBMCs were thawed in RPMI 1640 with 10% fetal bovine serum (FBS) and washed in FACS buffer. When available, 1 million fresh lymphocytes from disrupted GALT biopsy specimens and paired PBMC samples were stained. Phenotypic staining was performed on 10^6 cells by incubation with a viability marker (AmCyan live-dead kit from Invitrogen) and with antibodies conjugated to CD3, CD4, CD8, CD45RA, CCR7, CD27, CD95, and α4β7 (BD Biosciences, San Diego, CA) for 30 min on ice. Subsequently, cells were washed, fixed with 4% paraformaldehyde for 5 min, washed, and acquired with an LSR-II flow cytometer (Becton Dickinson).

Statistical analysis. Statistical analysis was performed using GraphPad Prism statistical software, version 6b (GraphPad Software, San Diego, CA). Nonparametric Kruskal-Wallis and Mann-Whitney U tests were used for group comparisons. Dunn's *post hoc* test (which incorporates the Bonferroni adjustment to correct for multiple comparisons) was used for between-group analyses. The Spearman rank test with linear regression was used for correlation analyses. *P* values of less than 0.05 were considered significant. The Benjamini-Hochberg test was applied to correct for multiple comparisons, with a q value (false-discovery rate) threshold of less than 0.05.

RESULTS

Comparison of T_{SCM} distributions in HIV-1-infected subjects. In this cross-sectional study of chronically HIV-1-infected subjects, we studied the following groups: (i) controllers who naturally suppress HIV-1 with a viral load of <2,000 copies/ml in the absence of treatment; (ii) noncontrollers who are untreated and have a viral load of >10,000 copies/ml but maintain CD4$^+$ counts of >250 cells/mm^3; (iii) immunological progressors who are also untreated with viral loads of >10,000 copies/ml and have progressed to immunodeficiency with CD4$^+$ counts of <250 cells/mm^3; and (iv) HAART-suppressed individuals with viral loads of <75 copies/ml on treatment (see Table 1). A cohort of age-matched healthy control subjects was also included. There were no significant differences in the median ages of the five groups (including the HIV-1-infected and uninfected subjects) or in the durations of diagnosis among the HIV-1-infected groups (P = >0.05 for all pairwise comparisons).

We evaluated the relative frequencies of the T_{SCM} populations within the CD4$^+$ and CD8$^+$ T cell compartments in each of the subject groups. The gating strategy to define this subset is shown in Fig. 1. Briefly, singlet cells were defined, followed by gating on lymphocytes and live cells. Among the live cells, CD3$^+$ T lymphocytes were identified, followed by the definition of CD4$^+$ and CD8$^+$ subpopulations. Subsequently, the expression of CD45RA and CCR7 was analyzed in the CD4$^+$ and CD8$^+$ T lymphocytes and further gated based on CD27 and CD95 expression. Central memory T cells (T_{CM}) are CD45RA$^-$ CCR7$^+$ CD27$^+$, effector memory T cells (T_{EM}) are CD45RA$^-$ CCR7$^-$ CD27$^-$, naive T cells are CD45RA$^+$ CCR7$^+$ CD27$^+$ CD95$^-$, and T_{SCM} are CD45RA$^+$ CCR7$^+$ CD27$^+$ CD95$^+$. Consistent with previous reports (10), comparisons of conventional memory T cell subsets among the healthy controls and HIV-1-infected subjects revealed a skewing toward a terminally differentiated T_{EM} phenotype in the infected groups, particularly in the individuals with the most advanced disease (immunologic progressors) (Fig. 2A and B). Confirming the findings of Gattinoni et al. (7), we observed that the T_{SCM} represent a relatively small percentage of all circulating CD4$^+$ and CD8$^+$ T lymphocytes. In our cohort, healthy uninfected controls had a median of 1.2% CD4$^+$ cells and 1.5% CD8$^+$ T_{SCM}. Comparing the frequencies of this subset among all HIV-1-infected groups, we did not observe any difference in the CD4$^+$ T cell compartment (Fig. 2A). However, in the CD8$^+$ T cell compartment, there was a decrease in the frequency of this subpopulation

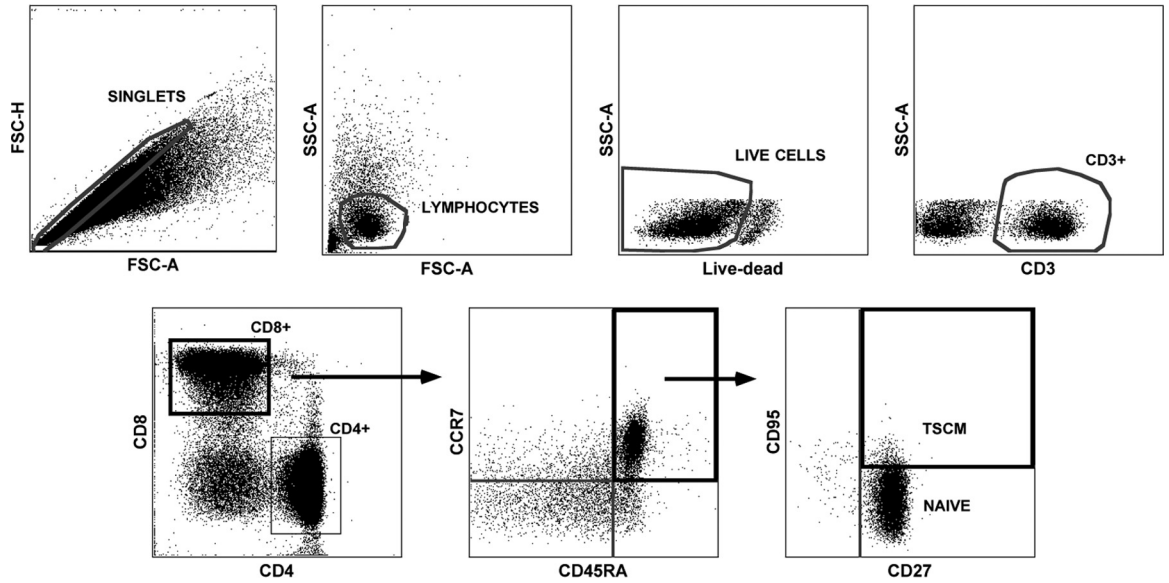

FIG 1 Gating strategy for T_{SCM}. Lymphocytes were stained and acquired as described in Materials and Methods. Cells were gated as follows: (top left to top right) forward scatter A (FSC-A) × FSC-H for singlets (i), FSC-A × side scatter A (SSC-A) for lymphocytes (ii), live-dead (AmCyan negative) for live cells (iii), and $CD3^+$ for T lymphocytes (iv); (bottom left to bottom right) $CD4^+$ or $CD8^+$ (v), CD45RA and CCR7 (vi) for conventional memory subsets and CD27 and CD95 for T_{SCM} versus naive T cells (vii). Central memory T cells (T_{CM}), $CD45RA^-CCR7^+$ $CD27^+$; effector memory T cells (T_{EM}), $CD45RA^-CCR7^-CD27^-$; naive T cells, $CD45RA^+$ $CCR7^+$ $CD27^+$ $CD95^-$; T_{SCM}, $CD45RA^+$ $CCR7^+$ $CD27^+$ $CD95^+$.

in the untreated groups compared to the HAART-suppressed group ($P = <0.001$, $P = <0.0001$, and $P = <0.001$ for HAART-suppressed subjects versus controllers, immunologic progressors, and noncontrollers, respectively; Fig. 2B) and the healthy control group ($P = <0.001$, $P = <0.0001$, and $P = <0.001$ for healthy controls versus controllers, immunologic progressors, and noncontrollers, respectively; Fig. 2B). Controllers had the greatest absolute numbers of $CD4^+$ T_{SCM} among the untreated groups ($P = <0.0001$ and $P = <0.05$ for controllers versus immunologic progressors and noncontrollers, respectively; Fig. 2C) and showed a trend toward greater $CD4^+$ T_{SCM} numbers than the HAART-suppressed group. The HAART-suppressed subjects had higher numbers of $CD8^+$ T_{SCM} than the untreated subjects ($P = <0.01$, $P = <0.0001$, and $P = <0.05$ for HAART-suppressed subjects versus controllers, immunologic progressors, and noncontrollers, respectively; Fig. 2D). Of note, the duration of antiretroviral treatment did not affect T_{SCM} frequencies or absolute numbers (not shown). Among all subjects, the levels of $CD4^+$ and $CD8^+$ T_{SCM}, measured by either frequency ($r = 0.51$, $P = <0.0001$) or absolute number ($r = 0.56$, $P = <0.0001$), were positively correlated, indicating that within an individual, the maintenance of these two subsets may be linked (Fig. 2E and F, respectively). The correlation within each group as well as across all subjects is shown within Fig. 2.

T_{SCM} and memory T cell maintenance. T_{SCM} have been reported to be important in the homeostasis of the immune system due to their ability to rapidly generate other memory subtypes. In all infected untreated subjects, we observed that the frequency of $CD4^+$ T_{SCM} was positively correlated with the frequency of $CD4^+$ central memory cells (T_{CM}) ($r = 0.20$, $P = 0.03$; Fig. 3A) and inversely correlated with the frequency of $CD4^+$ effector memory cells (T_{EM}) ($r = -0.63$, $P = <0.0001$; Fig. 3B), potentially indicating that the T_{SCM} subset supports the T_{CM} population, which may then subsequently differentiate into the peripheral tissue-homing T_{EM}. The same pattern was observed in the $CD8^+$ T_{SCM} compartment ($r = 0.55$, $P = <0.0001$ for $CD8^+$ T_{SCM} versus T_{CM} [Fig. 3C]; $r = -0.47$, $P = <0.0001$ for $CD8^+$ T_{SCM} versus T_{EM} [Fig. 3D]). The relationships between the T_{SCM} subset and the conventional memory T cell subset were consistent within each of the subject groups, except for the $CD4^+$ T_{SCM} versus $CD4^+$ T_{CM} correlation, which was driven primarily by the noncontrollers.

T_{SCM}, clinical outcome, and immune activation. Next we investigated whether the T_{SCM} subset was correlated with markers of disease progression in our untreated cohort. The HAART group was excluded given the known effect of antiretroviral treatment on viral replication and immune activation. Across all three untreated groups (controllers, noncontrollers, and immunologic progressors), we found that the frequency of $CD8^+$ T_{SCM} was inversely correlated with HIV-1 RNA levels ($r = -0.25$, $P = 0.02$; Fig. 4F) and positively correlated with $CD4^+$ T cell count ($r = 0.24$, $P = 0.03$; Fig. 4N). The positive correlation with $CD4^+$ count was largely driven by the controller group ($r = 0.54$, $P = 0.003$), while the inverse relationship between T_{SCM} and viral load was found within all three groups. This relationship was also observed for the $CD8^+$ naive (Fig. 4E and M) and T_{CM} (Fig. 4G and O) subsets, whereas the frequency of T_{EM} was correlated with higher HIV-1 loads (Fig. 4D and H) and lower $CD4^+$ T cell counts (Fig. 4L and P). $CD4^+$ T_{SCM} were not correlated with HIV-1 RNA levels ($r = -0.10$, $P = 0.37$; Fig. 4B) or $CD4^+$ T cell counts ($r = 0.05$, $P = 0.64$; Fig. 4J).

We then sought to determine the relationship between T_{SCM} and the level of $CD4^+$ and $CD8^+$ T cell activation based on the expression of CD38 and HLA-DR. As expected, the immunologic progressors had higher frequencies of activated $CD4^+$ and $CD8^+$ T cells than the HAART-suppressed and controller groups and levels similar to those seen with the noncontroller group (data not

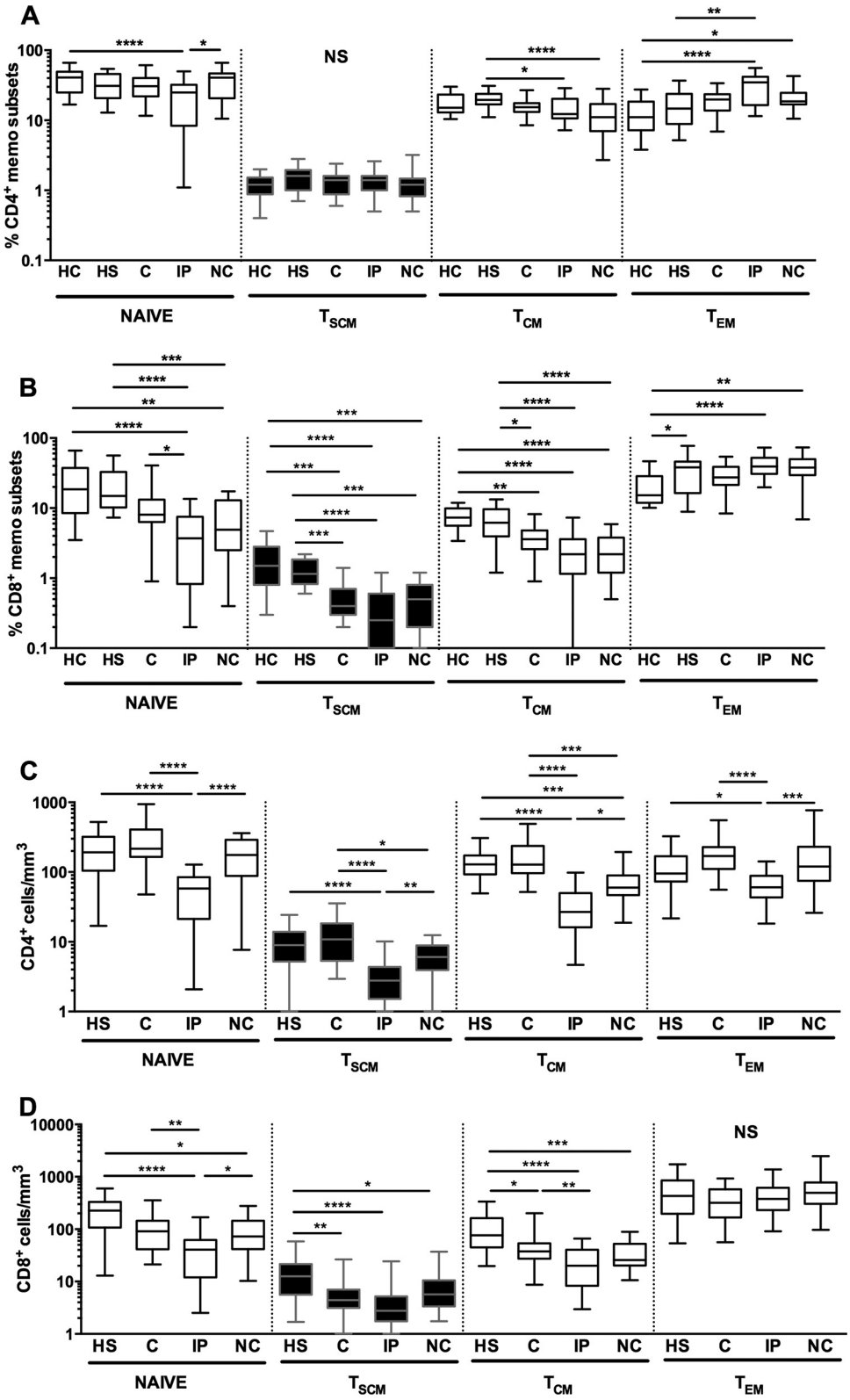

FIG 2 T_{SCM} prevalence in HIV-1-infected subjects. (A and B) Percentages of CD4$^+$ (A) and CD8$^+$ (B) naive and memory (memo) T cell subsets across uninfected and infected-subject groups. (C and D) Absolute numbers (cells/mm^3) of CD4$^+$ (C) and CD8$^+$ (D) memory T cells across infected-subject groups. Nonparametric Kruskal-Wallis and Dunn's *post hoc* tests for multiple comparisons were used. (E and F) Results of a correlation Spearman rank test comparing the percentages (E) as well the absolute numbers (F) of CD4$^+$ and CD8$^+$ T_{SCM} are depicted. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$. HC, healthy controls; HS, HAART-suppressed patients; C, controllers; IP, immunologic progressors; NC, noncontrollers. Individual *r* and *P* values are shown for each subject group within the legends in panels E and F.

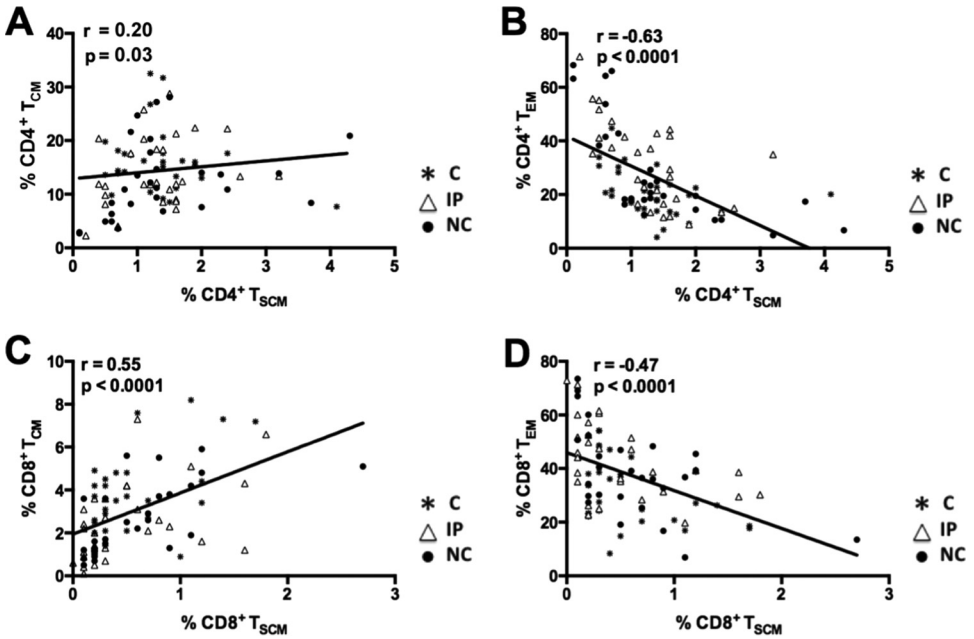

FIG 3 T_{SCM} are the progenitors for T_{EM} cells and help the maintenance of T_{CM}. Results of correlation Spearman rank tests comparing frequencies of $CD4^+$ T_{SCM} and $CD4^+$ central memory cells (T_{CM}) (A) and $CD4^+$ effector memory cells (T_{EM}) (B) and comparing frequencies of $CD8^+$ T_{SCM} and $CD8^+$ central memory cells (T_{CM}) (C) and $CD8^+$ effector memory cells (T_{EM}) (D) are shown for untreated subjects. C, controllers; IP, immunologic progressors; NC, noncontrollers.

shown). The frequency of the $CD8^+$ T_{SCM} subpopulation was inversely correlated with the frequency of $CD8^+$ $CD38^+$ T cells across all subjects ($r = -0.25$, $P = <0.05$; Fig. 5F) as well as within each group. The same inverse correlation was observed when considering $CD8^+$ T_{naive} and T_{CM} ($P = <0.01$ and $P = <0.0001$, respectively; Fig. 5E and G). In contrast, the presence of T_{EM} was positively correlated with the percentage of $CD8^+$ $CD38^+$ T cells (Fig. 5D and H), which is similar to the findings in another recent study (11). The only significant relationship between populations coexpressing CD38 and HLA-DR and memory subsets was that between the frequency of $CD8^+$ T_{CM} and the percentage of $CD4^+$ $CD38^+$ $HLA\text{-}DR^+$ ($r = -0.30$, $P = 0.006$; not shown).

T_{SCM} in gut-associated lymphoid tissue (GALT). In a smaller cohort of HIV-infected and uninfected individuals, we interrogated the T_{SCM} subset in the GALT, as this is a primary site of HIV-1 replication and $CD4^+$ depletion. To our knowledge, this cell population has not previously been studied in the mucosal compartment of humans. Consistent with their naive T cell-like phenotype, we found that both $CD4^+$ T_{SCM} and $CD8^+$ T_{SCM} are more prevalent in peripheral blood than in GALT (Fig. 6A). However, the frequency of $CD4^+$ T_{SCM} in GALT was inversely associated with that of peripheral $CD4^+$ T_{SCM} ($r = -0.93$, $P = <0.01$), suggesting the potential migration of this population to the gut mucosa (Fig. 6B). Interestingly, in the total cohort of all healthy and infected subjects, about 34% of $CD4^+$ and 30% of peripheral $CD8^+$ T_{SCM} expressed the gut-homing marker $\alpha4\beta7$, close to twice the level seen with the circulating T_{EM}, which more frequently localize to peripheral tissues (not shown).

DISCUSSION

We report here the distribution of the multipotent memory stem T cell (T_{SCM}) subset in chronically HIV-1-infected subjects with various degrees of disease control. Furthermore, we describe for the first time the relationship of T_{SCM} with key parameters linked to HIV-1 pathogenesis. In summary, we observed that untreated HIV-1-infected subjects had lower frequencies of circulating $CD8^+$ T_{SCM} cells than individuals on antiretroviral treatment. This subset was directly correlated with $CD4^+$ T cell count and inversely correlated with viral load in the untreated groups. Furthermore, higher levels of the $CD8^+$ T_{SCM} subpopulations were associated with lower levels of $CD8^+$ T cell activation, suggesting an association with a more functional immune system. T_{SCM} were also present in gastrointestinal lymphoid tissue but to a lesser extent than in the peripheral circulation, and the frequencies of $CD4^+$ T_{SCM} in these two sites were inversely correlated. Although our study design precludes the demonstration of causality, our data are broadly consistent with a model in which preservation of the $CD8^+$ T_{SCM} subset contributes to improved control of HIV-1, which in turn contributes to maintenance of immune function and lower levels of T cell activation. Our data also suggest that antiretroviral treatment is associated with a frequency of these cells closer to that of healthy individuals, which may explain in part the beneficial effect therapy has on immune recovery and function.

It has been reported that one mechanism for maintenance of long-term T cell memory derives from the unique homeostatic properties of T_{SCM} cells (9). The superior persistence of T_{SCM} cells following antigen loss suggests that they are the main precursors of T cell memory in the post-antigen phase. In this context, we observed that the numbers of $CD4^+$ and $CD8^+$ T_{SCM} were positively correlated with the T_{CM} compartment and inversely correlated with the T_{EM} compartment, suggesting that the differentiation of T_{SCM} leads to a shift in the proportions of these cell populations as a possible mechanism to reestablish the homeostasis of the immune system.

The role of $CD8^+$ T cells in virus control and consequent main-

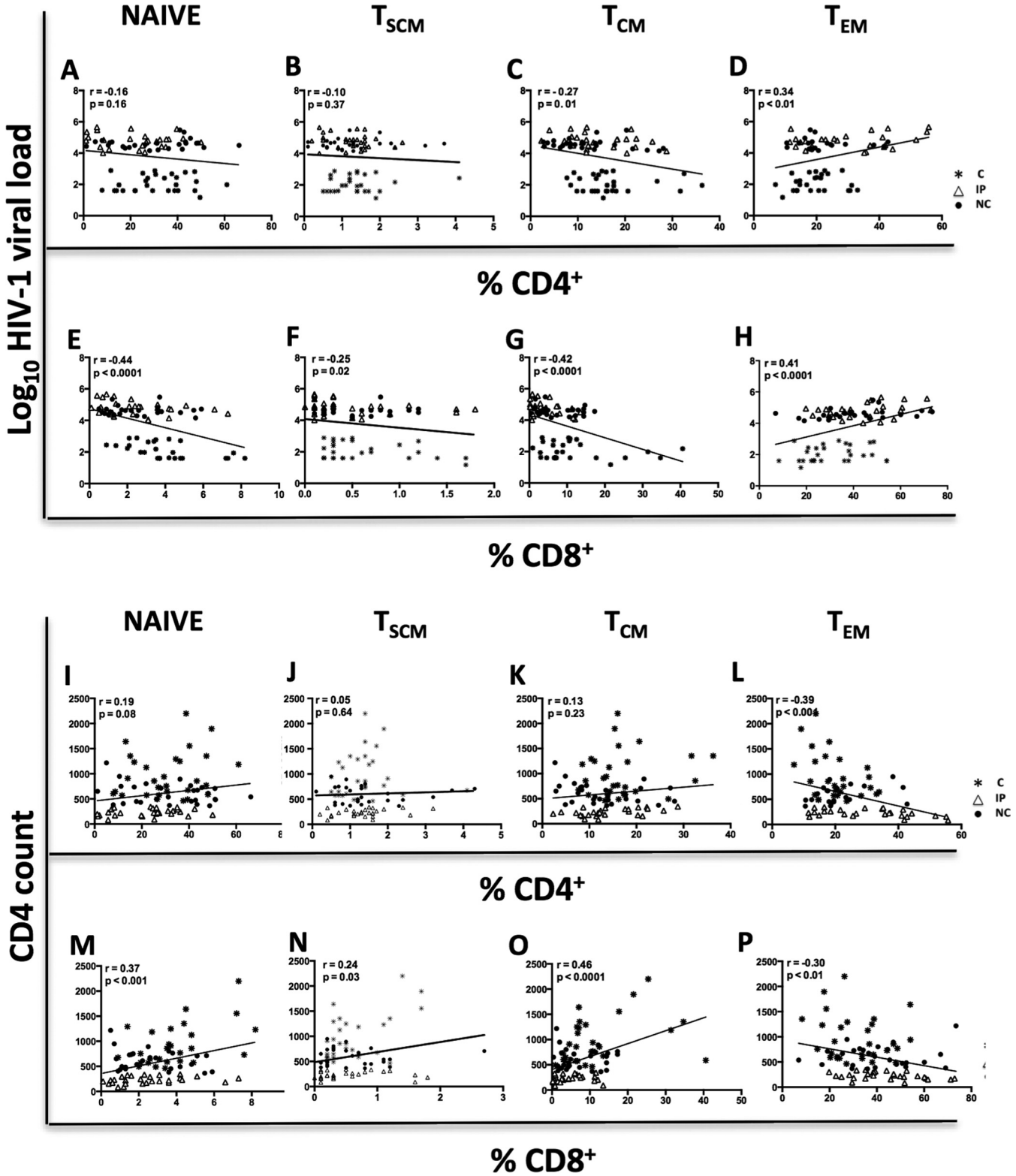

FIG 4 CD8$^+$ T_{SCM} cells are correlated with low HIV-1 load (VL) and high CD4$^+$ T cell count. Results of correlation Spearman rank tests comparing the percentages of CD4$^+$ (A to D) and CD8$^+$ (E to H) memory T cell subsets and HIV-1 (log_{10}) load and comparing CD4$^+$ (I to L) and CD8$^+$ (M to P) memory T cell subsets and CD4$^+$ count are shown. Results are depicted for untreated subjects. C, controllers; IP, immunologic progressors; NC, noncontrollers.

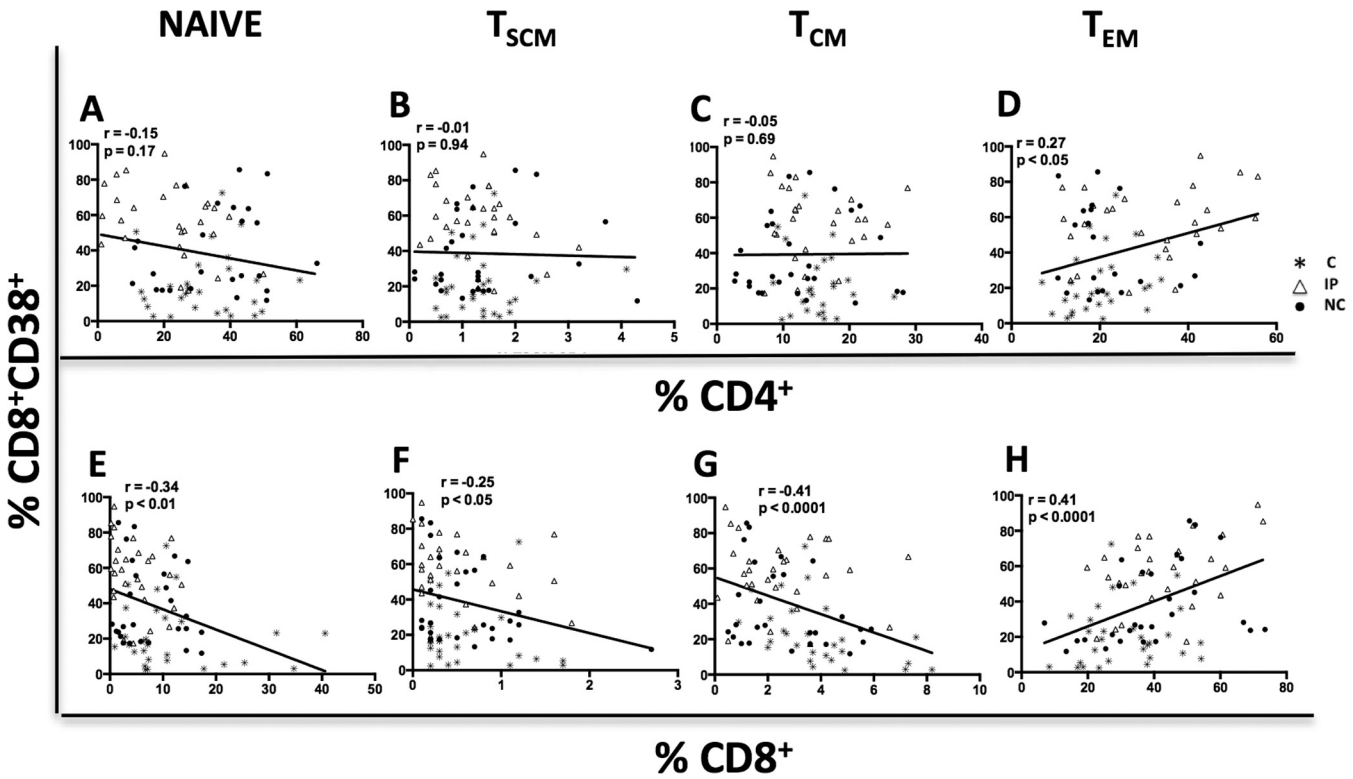

FIG 5 $CD8^+$ T_{SCM} cell numbers are inversely correlated with T cell activation in untreated subjects. Results of correlation Spearman rank tests comparing the frequencies of $CD4^+$ (A to D) and $CD8^+$ (E to H) memory T cell subsets and % $CD8^+$ $CD38^+$ are shown. Results are depicted for untreated subjects. C, controllers; IP, immunologic progressors; NC, noncontrollers.

tenance of $CD4^+$ T cell counts is well described (12, 13). Here, we observed that numbers of $CD8^+$ T_{SCM} were inversely correlated with viral loads and positively correlated with $CD4^+$ counts, indicating that a high percentage of this subset is associated with a good prognosis. Of note, the naive and T_{CM} subpopulations were also associated with less-advanced disease in this cohort. As previous studies have shown that T_{SCM} can generate T_{CM} and are preferentially maintained compared to T_{CM} following escape from cognate antigen in a model of chronic simian immunodeficiency virus (SIV) infection (9), HIV-1-specific $CD8^+$ T_{SCM} may be an optimal target for therapeutic vaccine induction. In contrast, the $CD4^+$ T_{SCM} population was not correlated with any measured disease parameters. Interestingly, the $CD4^+$ T_{SCM} subset was recently described as being permissive to HIV-1 infection *in vitro* (14) and was also described as contributing disproportionately to a long-lasting latent reservoir (15). However, we found that virologic controllers had preserved levels of $CD4^+$ T_{SCM}. A recent study (16) reported that another rare subset of HIV-infected individuals known as viremic nonprogressors (VNPs) also maintain $CD4^+$ T_{SCM} counts, which is associated with decreased HIV infection of these cells. The mechanism of T_{SCM} resistance to infection in VNPs and potentially the classic controllers in our study remains to be determined.

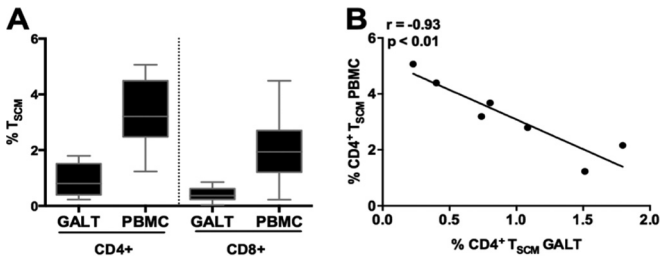

FIG 6 Prevalence of T_{SCM} cells in peripheral blood and GALT. (A) Percentages of $CD4^+$ T_{SCM} and $CD8^+$ T_{SCM} in peripheral blood (PBMC) and GALT. (B) Results of correlation Spearman rank test comparing percentages of $CD4^+$ T_{SCM} cells in GALT and PBMC. Data from untreated HIV-1-infected and healthy subjects are shown.

It is well known that HIV-1 infection is associated with systemic immune activation, in part through bacterial translocation after injury of the epithelial barrier of the gut, as well as through other mechanisms (17). This activation leads to immune exhaustion, increased non-AIDS-related comorbidities such as cardiovascular disease, and, eventually, progression to AIDS (18). Here we found that in untreated subjects, the $CD8^+$ T_{SCM} subset was correlated with lower frequencies of activated $CD8^+$ T cells. The mechanism for a possible protective effect on immune activation by $CD8^+$ T_{SCM} is not known but may be linked to the maintenance of gut barrier function or through the provision of a stable precursor pool of antigen-specific memory T cells.

In a recent study of healthy nonhuman primates, Lugli et al. (9) showed that T_{SCM} have a tropism for secondary lymphoid tissues, with a distribution most similar to that of naive T (T_N) cells, while central and effector memory T cells predominate in mucosal surfaces. In the present work, we observed medians of 0.8% and 0.4% of $CD4^+$ T_{SCM} and $CD8^+$ T_{SCM}, respectively, in GALT samples obtained from infected and healthy subjects. This corroborates the findings of Lugli et al. (9), as we found a median of 3.2% of

CD4$^+$ and 2% of CD8$^+$ T_{SCM} in the peripheral blood. We also observed an inverse correlation between the CD4$^+$ T_{SCM} subsets in PBMC and GALT, indicating a possible migration of these cells to the gut, the main site of HIV-1 infection and replication. In support of this, we found that there are high frequencies of CD4$^+$ T_{SCM} expressing the gut-homing marker $\alpha4\beta7$ in the circulation. Further longitudinal and cross-sectional studies of T_{SCM} in human and animal models within the context of viral infection will be useful in defining the function of this cell type distributed across various tissues.

Our study had a number of limitations. With a cross-sectional design, we are unable to demonstrate causality. While we suggest here that preservation of CD8$^+$ T_{SCM} cells is causally associated with improved immune function and virus control and that the mechanism may be related to homeostatic maintenance of other memory T cell subsets, it is also possible that lack of disease progression (defined by stable CD4$^+$ T cell counts and/or low viral load) contributes to higher levels of T_{SCM}. Longitudinal studies in humans should help untangle these associations, although the only definitive method to demonstrate the causal role of T_{SCM} cells in disease control would be to therapeutically increase their number in a controlled manner. It is also possible that the function rather than the number of T_{SCM} might prove to be the most important prognostic characteristic. Although we lacked sufficient cells to characterize the HIV-1-specific T_{SCM} population, experiments in a nonhuman primate model of SIV infection have demonstrated that SIV-specific T_{SCM} preferentially survive after antigen elimination compared to other memory subsets and are fully functional even in chronic infection (9). Therefore, HIV-1-specific CD8$^+$ T_{SCM} can presumably directly contribute to HIV-1 control and should be investigated in future studies.

Conclusions. In summary, this report describes the distribution of stem cell-like memory T cells in HIV-1-infected humans and identifies CD8$^+$ T_{SCM} as a correlate of protection from disease progression. Our findings suggest an important role for T_{SCM} in supporting durable immunity *in vivo* and are therefore directly relevant to the design of T cell-based vaccines, adoptive immunotherapy approaches, or the pharmacologic induction of T_{SCM}.

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