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Divergent CD4+ T Memory Stem Cell Dynamics in Pathogenic and Nonpathogenic Simian Immunodeficiency Virus Infections

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Recent studies have identified a subset of memory T cells with stem cell-like properties (T_{SCM}) that include increased longevity and proliferative potential. In this study, we examined the dynamics of CD4+ T_{SCM} during pathogenic SIV infection of rhesus macaques (RM) and nonpathogenic SIV infection of sooty mangabeys (SM). Whereas SIV-infected RM show selective numeric preservation of CD4+ T_{SCM}, SIV infection induced a complex perturbation of these cells defined by depletion of CD4+CCR5+ T_{SCM}, increased rates of CD4+ T_{SCM} proliferation, and high levels of direct virus infection. The increased rates of CD4+ T_{SCM} proliferation in SIV-infected RM correlated inversely with the levels of central memory CD4+ T cells. In contrast, nonpathogenic SIV infection of SM evidenced preservation of both CD4+ T_{SCM} and CD4+ central memory T cells, with normal levels of CD4+ T_{SCM} proliferation, and lack of selective depletion of CD4+CCR5+ T_{SCM}. Importantly, SIV DNA was below the detectable limit in CD4+ T_{SCM} from 8 of 10 SIV-infected SM. We propose that increased proliferation and infection of CD4+ T_{SCM} may contribute to the pathogenesis of SIV infection in RM. The Journal of Immunology, 2014, 192: 4666–4673.

Pathogenetic HIV infection of humans and SIV infection of rhesus macaques (RM) are characterized by progressive depletion of CD4+ T cells and development of a lethal state of immunodeficiency termed AIDS (1). In contrast, SIV infection of African nonhuman primate species that are natural hosts for the virus, such as the sooty mangabeys (SM) and the African green monkeys, is typically nonpathogenic despite high virus replication (2). Although the mechanism responsible for the development of AIDS remains incompletely understood, a series of recent studies have emphasized the role played by chronic immune activation and the direct infection of CD4+ central memory T cells (T_{CM}), with these two phenomena being significantly reduced in SIV-infected SM as compared with RM (3). During pathogenic HIV and SIV infections, high levels of direct virus infection of CD4+ T_{CM} are associated with the depletion of CD4+ T cells in blood, lymph nodes, and mucosal tissues, thus suggesting a direct link between virus-mediated CD4+ T_{CM} killing, CD4+ T_{CM} depletion, and onset of a clinically relevant immunodeficiency (4–8). According to this model, CD4+ T_{CM} are essential to maintain the overall CD4+ T cell homeostasis due to their in vivo longevity and high proliferative potential, resulting in the ability to maintain their pool as well as the more differentiated pool of CD4+ effector memory T cells (T_{EM}) (9,10). Interestingly, infection of CD4+ T_{CM} seems to play a significant role in the persistence of reservoirs of latently infected cells in HIV-infected individuals treated with antiretroviral therapy (11), and low levels of latent CD4+ T_{CM} infection are present in HIV-infected individuals who control virus replication either spontaneously (7) or after antiretroviral therapy (ART) (8).

A series of recent studies has identified a phenotypically and functionally novel subset of memory T cells with stem cell-like properties that were termed T_{SCM} memory stem cells (T_{SCM}) (12–15). These cells represent the stem cells of the memory T cell compartment, as they are uniquely able to self-renew as well as differentiate into all other memory T cell subsets (i.e., T_{EM}, T_{CM}, and transitional memory) (13). Additional properties of T_{SCM} include increased longevity and higher proliferative potential when compared with other T cell memory subsets (13). Phenotypically, T_{SCM} are defined in humans as CD45RA+CD45RO−CD62L+CCR7+CD27+CD28+CD95−CD122+, and in RM and pig-tailed macaques as CD45RA−CD45RO−CD62L−CCR7−CD27−CD28−CD127−CD95−CD122+, and in RM and pig-tailed macaques as CD45RA−CD45RO−CD62L−CCR7−CD27−CD28−CD127−CD95−. In both humans and RM, T_{SCM} express levels of CXCR3, Bcl-2, and LFA-1 intermediate between naïve and T_{CM} (13,15). T_{SCM} are found predominantly in peripheral blood and secondary lymphoid tissues, but are rare in mucosal tissues (15). In the context of SIV infection, CD8+ T_{SCM} are involved in the long-term maintenance of virus-specific CD8+ T cell–mediated responses (15). At this time, however, the contribution of CD4+ T_{SCM} to HIV or SIV pathogenesis remains unknown.
To our knowledge, this is the first study to examine CD4+ TSCM in both healthy and SIV-infected RM and SM. We found that the absolute number of CD4+ TSCM is preserved during both pathogenic and nonpathogenic SIV infections, but SIV-infected RM showed a selective depletion of CD4+CCR5+ TSCM. We also found that, in SIV-infected RM, but not in SIV-infected SM, CD4+ TSCM display significantly higher levels of proliferation that correlate inversely with both percentage and absolute number of CD4+ TCM. Importantly, substantial levels of direct virus infection of CD4+ TSCM were seen only in SIV-infected RM, with the majority of SIV-infected SM lacking SIV DNA within CD4+ TSCM. Based on these data, we propose that increased proliferation and infection rates of CD4+ TSCM may play a role in the pathogenesis of SIV infection in RM. Based on their longevity and high levels of direct virus infection in pathogenic SIV infection, we postulate that CD4+ TSCM may be an important site for the HIV/SIV reservoir as well as for maintaining memory T cell homeostasis.

**Materials and Methods**

**Animals**

Twenty-seven SIV-uninfected RM and 13 SIV-uninfected SM, plus 39 chronically SIV-infected RM and 19 chronically SIV-infected sooty SM, were included in this study. All SIV-infected RM had been previously infected i.v. with SIVmac239 or SIVmac251. To obtain frequency of infection data from SM, blood was collected from 6 experimentally infected and 4 naturally infected SM. Four experimentally infected SM were infected i.v. with 0.5 ml plasma (titrated to 10^7 SIV RNA copies/ml) from a naturally infected SM. Twenty-seven SIV-uninfected RM and 13 SIV-uninfected SM, plus 39 SIV-infected RM and 19 SIV-infected sooty SM, were included in this study. All SIV-infected RM had been previously infected i.v. with SIVmac239 or SIVmac251. To obtain frequency of infection data from SM, blood was collected from 6 experimentally infected and 4 naturally infected SM. Four experimentally infected SM were infected i.v. with 0.5 ml plasma (titrated to 10^7 SIV RNA copies/ml) from a naturally infected SM. Two experimentally infected SM were inoculated i.v. with 25 ng p27 equivalent of SIVsmE041 (primary isolate derived from a naturally infected SM) (16). Of the SIV-infected SM, 8 were heterozygous for the wild-type CCR5 allele and the previously described Δ 2 or Δ 24 alleles that are not associated with reduced susceptibility to infection in the heterozygous state (17). In RM, acute infection was defined as day 7–14 postinfection, early stage chronic infection was defined as day 42–84 postinfection, and late chronic infection was defined as day 128–365 postinfection. We obtained complete blood counts for 16 SIV-uninfected, 18 acutely infected, 11 early chronic, and 10 late chronic RM; thus, absolute numbers could only be calculated from these animals. All animals were anesthetized prior to the performance of any procedure, and proper steps were taken to ensure the welfare and to minimize the suffering of all animals in these studies. The animals were housed at the Yerkes National Primate Research Center of Emory University and maintained in accordance with National Institutes of Health guidelines under Institutional Animal Care and Use Committee–approved protocols. Anesthesia was used for all blood collections.

**Tissue processing**

PBMCs were freshly isolated from whole blood by density centrifugation or sodium citrate CPT (BD Biosciences) tubes. Frozen PBMCs were thawed in 37°C water bath and used immediately.

**Immunophenotyping**

Immunophenotyping was performed according to standard procedures, and mAbs cross-reactive in both SM and RM were used. The following Abs were used for immunophenotyping of CD4+TSCM in SM and RM PBMCs: Live/Dead Fixable Aqua from Invitrogen; CD14-V500 (M5E2), CD16-V500 (3G8), CD3-allophycocyanin-Cy7 (SP34-2), CD45RA-allophycocyanin (SH9), CCR7-PE-Cy7 (3D12), Ki67-Alexa 700 (B56), CCR5-PE (3A9), CD95-PE-Cy5 (DX2), CXCXR3-PerCP-Cy5.5 (IC6/CXCXR3), CD11a-FTTC (H111), CD122-biotin (Mik-b31), and streptavidin-PE, all from BD Biosciences; CD4-BV650 (OKT4), CD8-BV711 (RPA-T8), CD27-BV570 or -BV605 (O323), and streptavidin-605 all from BioLegend; and CD28-EC (CD28.2) from Beckman Coulter. Flow cytometric acquisition was carried out on a LSRII flow cytometer driven by the FACS DiVa software package (BD Biosciences). Analysis of the acquired data was carried out using FlowJo (Tree Star) and PRISM (GraphPad) software.

**Cell sorting**

Sorting of CD4+ naïve T cells (T_N), TSCM, TCM, and TEM from SIV-infected RM and SM was performed on a FACS Aria II flow cytometer (BD Biosciences). Cells were initially gated on the basis of light scatter, followed by positive staining for CD3 and CD4 and negative staining for Live/Dead Aqua. CD4+ T_N (CD28−/CD95±/CCR7−), TCM (CD45RA−/CD28−/CD95−/CCR7−), and TEM (CD45RCR7−) subsets were gated on characteristic patterns of CD28, CD95, CCR7, and CD45RA. CD4+ TSCM were sorted as CD45RA−/CD95−/CCR7−/CD122+. This population was determined to be uniformly positive for CD27, CD28 (Fig. 1A), and CD127 (data not shown).
Plasma viral load

Plasma viral RNA quantification was determined, as previously described (18).

Quantitative PCR for SIV gag DNA

Quantification of SIV<sub>mac</sub> gag or SIV<sub>smm</sub> utr DNA was performed, as described previously (19, 20). For cell number quantification, quantitative PCR was performed simultaneously for monkey albumin gene copy number. Albumin primers and probe along with quantitative PCR conditions were previously described (5). The sensitivity of the assay is five SIV DNA copies per 10<sup>5</sup> cells. Samples with undetectable SIV DNA were assigned a level of half of the lower limit of detection for graphical purposes and statistical analysis.

Statistical analyses

Comparisons between frequencies in RM and SM (Fig. 1B–E, Supplemental Fig. 1) and SIV-uninfected and SIV-infected SM (Fig. 5) were carried out using a nonparametric Mann–Whitney U test. Comparisons between frequencies of CD4<sup>+</sup> T cell subsets over time during pathogenic infection of RM (Figs. 2–4) were carried out using a Kruskal–Wallis test. Comparisons between frequency of infection in RM and SM (Fig. 6) were carried out using nonparametric Mann–Whitney U test. Correlations were determined using the non-Gaussian Spearman correlation. Significance was attributed at \( p < 0.05 \). All analyses were conducted using GraphPad Prism 5.0.

Results

Identification of CD4<sup>+</sup> T<sub>SCM</sub> in healthy RM and SM

CD4<sup>+</sup> and CD8<sup>+</sup> T<sub>SCM</sub> have been phenotypically identified in humans as CD45RA<sup>+</sup>CD45RO<sup>2</sup>CD62L<sup>+</sup>CCR7<sup>+</sup>CD27<sup>+</sup>CD28<sup>+</sup>CD127<sup>+</sup>CD95<sup>+</sup>CD122<sup>+</sup>, and in RM and pigtailed macaques are defined as CD45RA<sup>+</sup>CCR7<sup>+</sup>CD27<sup>+</sup>CD28<sup>+</sup>CD127<sup>+</sup>CD95<sup>+</sup> (13, 15).

We have first confirmed this immunophenotypic definition in healthy RM (see Fig. 1A for gating strategy) and SM (data not shown). As expected, CD4<sup>+</sup> T<sub>SCM</sub> isolated from both RM and SM expressed intermediate levels of CXCR3 and LFA-1 that were between those of naive and CD4<sup>+</sup> T<sub>CM</sub> (Supplemental Fig. 1). We also confirmed in healthy RM that CD4<sup>+</sup> T<sub>SCM</sub> can be readily identified in the blood, lymph nodes, bone marrow, and spleen but are present at a lower frequency in the intestinal mucosa (data not shown). We next compared the levels of circulating CD4<sup>+</sup> T<sub>SCM</sub> in healthy SIV-uninfected RM and SM as percentage of total CD4<sup>+</sup> T cells. As shown in Fig. 1B, the percentage of CD4<sup>+</sup> T<sub>SCM</sub> from SIV-uninfected SM ranged between 1 and 8% in RM and 0.5 and 3% in SM, with the levels observed in RM being significantly higher (\( p = 0.0004 \)). Interestingly, CD4<sup>+</sup> T<sub>SCM</sub> from SIV-uninfected SM show higher levels of proliferation (measured as expression of Ki67) as compared with RM (\( p = 0.0034 \), Fig. 1C), perhaps suggesting that a relatively smaller pool of CD4<sup>+</sup> T<sub>SCM</sub> maintains CD4<sup>+</sup> memory T cell homeostasis through higher baseline rates of proliferation in SM. We also found that CD4<sup>+</sup> T<sub>SCM</sub> from RM also express slightly higher levels of the inhibitory marker PD-1 as compared with CD4<sup>+</sup> T<sub>SCM</sub> from SM, although this trend was not statistically significant (Fig. 1D).

Several previous studies have shown that CD4<sup>+</sup> T cells of both SIV-infected and uninfected SM express lower levels of the SIV coreceptor CCR5 than CD4<sup>+</sup> T cells of humans and RM, and that this difference is particularly evident for CD4<sup>+</sup> T<sub>CM</sub> (3, 21). We next examined the levels of CCR5 expression on CD4<sup>+</sup> T<sub>SCM</sub> from healthy RM and SM, and, consistent with previous findings, we found significantly higher percentages of CCR5<sup>+</sup> CD4<sup>+</sup> T<sub>SCM</sub> from RM compared with SM (\( p = 0.0009 \), Fig. 1E). Three representative examples of CCR5 staining on CD4<sup>+</sup> T<sub>SCM</sub> are shown in Fig. 1F, which emphasize the almost complete absence of CCR5 on CD4<sup>+</sup> T<sub>SCM</sub> of SM.

![FIGURE 2.](http://www.jimmunol.org/)

**CD4<sup>+</sup> T<sub>SCM</sub> DYNAMICS IN SIV INFECTION**

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**A.** Total CD4<sup>+</sup> Frequency

**B.** Absolute Number of CD4<sup>+</sup> T cells

**C.** CD4<sup>+</sup> Subsets

**D.** Absolute Number of CD4<sup>+</sup> subsets

Data in (A) and (C) represent the following RM: 27 SIV uninfected, 29 acutely infected (days 7–14), 22 early chronic infection (days 65–84), and 19 late chronic infection (days 128–365). Data in (B) and (D) represent the following RM: 16 SIV uninfected, 18 acutely infected (days 7–14), 11 early chronic (day 65), and 10 late chronic (days 128–365). Bars are drawn at the median. *\( p < 0.05 \), **\( p < 0.01 \), ***\( p < 0.001 \) (Kruskal–Wallis test, compared with SIV uninfected).
Numeric preservation of CD4+ T_{SCM} during pathogenic SIV infection of RM

Pathogenic SIV infection of RM is characterized by a progressive depletion of CD4+ T cells from blood and mucosal tissues, which is typically associated with the loss of CD4+ T_{CM} homeostasis (4). To examine the dynamics of CD4+ T_{SCM} during pathogenic SIV infection of RM, we examined a total of 51 RM, including healthy SIV-uninfected animals and SIV-infected animals at different stages of infection. Consistent with many previous studies (22–25), the animals studied in this work exhibited the well-characterized progressive depletion of circulating CD4+ T cells associated with SIV infection, measured as the fraction of CD3+ T lymphocytes (Fig. 2A) or absolute number of cells per microliter of blood (Fig. 2B). We next examined, in the same animals, the relative distribution of four key CD4+ T cell subsets, that is, naive (CD28+CD95-); CD4+ T_{SCM} (CD45RA+CCR7+CD27+CD28+CD95-CD122+); CD4+ T_{CM} (CD45RA-CD28+CD95+CCR7+); and CD4+ T_{EM} (CD95+CCR7-). As expected, levels of both CD4+ T_{CM} and T_{EM} (primary targets for SIV infection) were altered by SIV infection, with a significant decline of the fraction and absolute number of CD4+ T_{CM} in both early and late chronic SIV infection ($p < 0.01$ and $p < 0.001$, respectively, Fig. 2C, 2D) and a significant decline of the absolute number of CD4+ T_{EM} in late chronic SIV infection ($p < 0.001$, Fig. 2D). Interestingly, neither the fraction nor the absolute number of CD4+ T_{SCM} was decreased in either acute or chronic SIV infection of RM (Fig. 2C, 2D). As such, these data indicate that pathogenic SIV infection of RM is not associated with a significant numerical decline of circulating CD4+ T_{SCM}.

Pathogenic SIV infection of RM is associated with a significant depletion of CCR5+CD4+ T_{SCM}

CCR5 is the main coreceptor for both HIV and SIV, and depletion of CD4+CCR5+ T cells, particularly in mucosal tissues, is a well-known hallmark of pathogenic HIV and SIV infections (23, 26, 27). In this study, we first confirmed the depletion of circulating CCR5+ T cells that begins in acute infection and continues during early and late chronic SIV infection of RM (Fig. 3A). We next measured, in our four groups of animals, the levels of CCR5 expression on the four studied subsets of CD4+ T cells (T_N, T_{SCM}, T_{CM}, and T_{EM}). As shown in Fig. 3B, we found that the fraction of CCR5+ cells is significantly decreased during late chronic SIV infection of RM in both CD4+ T_{CM} and T_{EM} ($p < 0.05$ and $p < 0.01$, respectively). Interestingly, we observed that the fraction of CCR5+CD4+ T_{SCM} was significantly decreased in SIV-infected RM examined during early chronic SIV infection compared with

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**FIGURE 3.** Pathogenic SIV infection of RM is associated with significant depletion of CCR5+CD4+ T_{SCM}. (A) Frequency of total CCR5+CD4+ T cells as a frequency of CD3+ lymphocytes during pathogenic SIV infection of RM. (B) Frequency of CCR5+ cells found in each of the four subsets (T_N, T_{SCM}, T_{CM}, and T_{EM}). Data in (A) and (B) represent the following RM: 26 SIV uninfected, 26 acutely infected (days 7–14), 18 early chronic infection (days 65–84), and 19 late chronic infection (days 128–365). (C) Absolute number of CCR5+CD4+ T_{SCM} during pathogenic SIV infection of RM/ml peripheral blood. Data in (C) represent the following RM: 15 SIV uninfected, 15 acutely infected, 6 early chronic SIV infection, and 10 late chronic SIV infection. Bars are drawn at the median. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (Kruskal–Wallis test, compared with SIV uninfected).
uninfected animals ($p < 0.05$). Although the median level of CCR5$^+$CD4$^+$ T$_{SCM}$ was also decreased in late chronic SIV infection, this difference was not statistically significant when compared with SIV-uninfected animals, most likely due to a wide range of values. Importantly, however, we found a significant depletion of the absolute number of CCR5$^+$CD4$^+$ T$_{SCM}$ (Fig. 3C) during both early and late chronic SIV infection. Together with the data shown in Fig. 2, these results indicate that pathogenic SIV infection of RM is associated with a depletion of CCR5$^+$CD4$^+$ T$_{SCM}$ that occurs in the context of an overall preservation of the CD4$^+$ T$_{CM}$ pool.

Pathogenic SIV infection of RM is associated with increased proliferation of CD4$^+$ T$_{SCM}$ that correlates inversely with circulating level of CD4$^+$ T$_{CM}$

To further investigate whether and to what extent pathogenic SIV infection of RM perturbs the homeostasis of CD4$^+$ T$_{SCM}$, we next measured, in our four groups of animals, the expression of the proliferation marker Ki67 in the studied subsets of CD4$^+$ T cells (T$_N$, T$_{SCM}$, T$_{CM}$, and T$_{EM}$). Consistent with previous studies (4), we observed that early and late chronic SIV infection is associated with increased proliferation of both T$_{CM}$ and T$_{EM}$ (Fig. 4A). Interestingly, the percentage of Ki67$^+$CD4$^+$ T$_{SCM}$ was also significantly increased in SIV-infected RM examined during both early ($p < 0.05$) and late ($p < 0.001$) stages of infection as compared with healthy uninfected animals (Fig. 4A). The observed increase in the fraction of cycling CD4$^+$ T$_{SCM}$ could be the result of homeostatic proliferation in response to the overall depletion of memory CD4$^+$ T cells, due to chronic immune activation, or both. In an attempt to assess whether homeostatic proliferation may be responsible for the increased proliferation of CD4$^+$ T$_{SCM}$ in SIV-infected RM, we investigated the relationship between the fraction of Ki67$^+$CD4$^+$ T$_{SCM}$ and the levels of the four studied CD4$^+$ T cell subsets. As shown in Fig. 4B–D, we found a significant inverse correlation between the percentage of Ki67$^+$CD4$^+$ T$_{SCM}$ and the percentage of circulating CD4$^+$ T$_{CM}$ ($p = 0.02$), but not with the level of any of the other memory CD4$^+$ T cell subsets. These data suggest that the increased proliferation of CD4$^+$ T$_{SCM}$ observed in SIV-infected RM represents, at least in part, a compensatory response to the CD4$^+$ T$_{CM}$ depletion induced by SIV infection.

**Dynamics of CD4$^+$ T$_{SCM}$ during nonpathogenic SIV infection of SM**

Previous studies have shown that nonpathogenic SIV infection of SM is typically associated with preserved CD4$^+$ T cell counts and low levels of immune activation (18, 28–30). To our knowledge, this is the first study to examine how SIV infection impacts the levels of CD4$^+$ T$_{SCM}$ in SM. As shown in Fig. 5A, we found no difference in the levels of any of the four studied subsets of CD4$^+$ T cells (T$_N$, T$_{SCM}$, T$_{CM}$, and T$_{EM}$) in SIV-infected SM as compared with uninfected animals, in contrast to the depletion of T$_{CM}$ and T$_{EM}$ seen in chronic SIV infection of RM (Fig. 2C). Similarly, no differences were found between SIV-infected and uninfected SM with respect to the absolute number of CD4$^+$ T$_{SCM}$ per microliter of blood (Fig. 5B). We next investigated whether SIV infection of SM is associated with a selective depletion of CD4$^+$ CCR5$^+$ T$_{SCM}$ and, unlike the loss of these cells in SIV-infected RM (Fig. 3B), we found similar levels of CD4$^+$CCR5$^+$ T$_{SCM}$ in SIV-infected and uninfected SM (Fig. 5C). To determine whether SIV infection of SM is associated with increased proliferation of CD4$^+$ T$_{SCM}$, we measured the fraction of these cells expressing Ki67. As shown in Fig. 5D, we found no difference in the fraction of CD4$^+$ T$_{SCM}$ expressing Ki67 among SIV-infected and uninfected SM. Taken together, these data indicate that the nonpathogenic SIV infection of SM is characterized by overall preservation of the CD4$^+$ T$_{SCM}$ compartment, involving both CD4$^+$CCR5$^+$ and CD4$^+$CCR5$^-$ cells, and does not result in increased turnover of this memory cell subset, consistent with the previously described maintenance of peripheral CD4$^+$ T cell homeostasis in SIV-infected SM (3).

**FIGURE 4.** Pathogenic SIV infection of RM is associated with increased proliferation of CD4$^+$ T$_{SCM}$ that inversely correlates with the level of CD4$^+$ T$_{CM}$. (A) Frequency of Ki67$^+$ T$_N$, T$_{SCM}$, T$_{CM}$, and T$_{EM}$ in PBMC during pathogenic SIV infection. Data represent the following RM: 22 SIV uninfected, 29 acutely infected (days 7–14), 22 early chronic infection (days 65–84), and 18 late chronic infection (days 128–365). Bars are drawn at the median. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (Kruskal–Wallis test, compared with SIV uninfected). (B–D) Correlations of the fraction of Ki67$^+$CD4$^+$ T$_{SCM}$ and fraction of circulating CD4$^+$ T$_{SCM}$, CD4$^+$ T$_{CM}$, and CD4$^+$ T$_{EM}$ during late chronic infection. $R$ and $p$ values were determined by Spearman correlation.
Robust levels of CD4+ TSCM infection in vivo are observed in SIV-infected RM but not in SIV-infected SM

In two prior studies, we have shown that pathogenic SIV infection of RM is associated with higher levels of SIV DNA in both circulating CD4+ TCM as well as lymph node–based CD4+ T cells as compared with SIV-infected SM (3, 5). These results led us to hypothesize that preservation of the CD4+ TCM compartment is a key determinant of the nonpathogenic nature of SIV infection of SM. To expand upon these observations, in this study we measured the levels of SIV DNA in flow-cytometrically sorted samples of the four studied subsets of CD4+ T cells (TN, TSCM, TCM, and TEM) in 9 SIVmac251-infected RM and 10 SIVsmm-infected SM. Please note that for sorting we have phenotypically defined TCM as CD45RA−CD28−CD95+CCR7+ and TEM as CD95+ CCR7−, which differs slightly from the markers used previously in Paiardini et al. and Brenchley et al. (3, 5). The reason for this choice was to incorporate the definition of TSCM in nonhuman primates established by Lugli et al. (15). As shown in Fig. 6A, we observed a robust frequency of infection (i.e., >1/1000 cells) in CD4+ TSCM isolated from 9 of 9 SIV-infected RM. In contrast, SIV DNA levels in CD4+ TSCM were undetectable in 8 of 10 SIV-infected SM. As previously reported (3, 5), the level of SIV DNA was higher in CD4+ TN and CD4+ TCM of RM as compared with SM. Shown in Fig. 6B, plasma viral loads of SIV-infected RM tended to be higher than those of SM, although this trend was not statistically significant. Overall, these results indicate that in vivo infection of CD4+ TSCM is frequent during pathogenic SIV infection of RM, but is absent or rare during nonpathogenic SIV infection of SM.

Discussion

In the past several years, the mechanisms responsible for AIDS pathogenesis have been extensively investigated in the pathogenic model of SIV infection of RM and the nonpathogenic model of SIV infection of SM (2, 31). These comparative studies led to the definition of a model in which chronic immune activation and disrupted homeostasis of CD4+ TCM are the key mechanisms re-

![FIGURE 5.](image-url) CD4+ TSCM are unperturbed during nonpathogenic SIV infection of SM. Comparison of frequency (A) and absolute number (B) of CD4+ TN, TSCM, TCM, and TEM in SIV-uninfected and chronically SIV-infected SM. (C) Frequency of CCR5+ T cells in each CD4+ T cell subset in SIV-uninfected and chronically SIV-infected SM. (D) Frequency of proliferating CD4+ T cell subsets, as measured by Ki67 expression in both uninfected and chronically SIV-infected SM. Bars are drawn at the median. **p < 0.01 (Mann–Whitney U test).

![FIGURE 6.](image-url) Robust levels of CD4+ TSCM infection in vivo are observed in SIV-infected RM but not in SIV-infected SM. (A) Fraction of SIV-infected CD4+ TN, TSCM, TCM, and TEM cells, as determined by quantitative PCR for the number of SIV gag (RM) or SIV utr (SM) DNA copies/cell in nine SIVmac251-infected RM, six experimentally SIVsmm-infected SM, and four naturally SIVsmm-infected SM. Cell number was determined using simultaneous PCR for albumin gene copy number. (B) Plasma viral load of RM and SM shown in (A) as determined by RT-PCR. Bars are drawn at the median. *p < 0.05, **p < 0.01, ***p < 0.001 (Mann–Whitney U test).
sponsible for the lentivirus-associated immunodeficiency (3, 4, 6, 32). More recently, a novel subset of memory CD4+ and CD8+ T cells has been identified, and named T_{SCM} based on their unique ability to generate all other memory T cell subsets de novo (13, 15). In this study, we sought to determine how CD4+ T_{SCM} are affected by pathogenic and nonpathogenic SIV infections of RM and SM, respectively. To the best of our knowledge, this study represents the first systematic investigation of the dynamics of CD4+ T_{SCM} during SIV infection.

The main results of this study are as follows: 1) CD4+ T_{SCM} are numerically preserved during both pathogenic and nonpathogenic SIV infections, with SIV-infected RM showing a selective depletion of CD4+CCR5+ T_{SCM}; 2) CD4+ T_{SCM} show significantly higher levels of proliferation that correlate inversely with the percentage of CD4+ T_{CM} in SIV-infected RM, but not SM; and 3) robust levels of direct virus infection of CD4+ T_{SCM} are found only in SIV-infected RM, with the majority of SIV-infected SM showing no evidence of CD4+ T_{SCM} infection. The observation that CD4+ T_{SCM} of healthy, SIV-uninfected RM express higher levels of CCR5 as compared with CD4+ T_{SCM} of healthy SM is consistent with previous findings in T_{CM} and a potential inherent resistance to direct infection at the virus entry level in SM (3).

Taken together, these data allow us to delineate a model for the role of CD4+ T_{SCM} in SIV pathogenesis. In SIV-infected RM, we observe significant perturbation of the homeostasis of CD4+ T_{SCM} in three ways, as these cells can be directly infected by the virus, are depleted in the percentage of cells expressing CCR5, and manifest increased proliferation. In contrast, none of these perturbations in the T_{SCM} pool are present in SIV-infected SM. The significant inverse correlation between CD4+ T_{SCM} proliferation and CD4+ T_{CM} depletion that we observed in SIV-infected RM suggests that CD4+ T_{SCM} proliferate at least in part to compensate for the progressive loss of CD4+ T_{CM}. Whereas the overall numeric homeostasis of CD4+ T_{SCM} is not altered in SIV-infected RM, it is possible that this cellular compartment loses, in time, the ability to effectively support the maintenance of CD4+ T_{CM}. Whether and to what extent the deficit of CD4+ T_{CM} that is associated with progression to AIDS is related to a functional exhaustion of CD4+ T_{SCM} as opposed to the direct depleting effects of virus infection and/or bystander apoptosis remains to be determined.

A striking difference between SIV-infected RM and SIV-infected SM is the level of virus infection of these cells as measured by fraction of SIV DNA-positive cells. Whereas all SIV-infected RM showed a calculated percentage of CD4+ T_{SCM} infection between 0.3 and 10%, 8 of 10 SIV-infected SM show undetectable levels of CD4+ T_{SCM} infection (i.e., <0.005%). Given the lower levels of CCR5 expression on CD4+ T_{SCM} of SM as compared with RM, one possibility is that these cells are resistant to virus infection at the entry level, analogous to what has been observed for CD4+ T_{CM} (3). It should be noted, however, that other coreceptors, in addition to CCR5 (17, 33), as well as postentry factors, may be involved in determining the different levels of SIV infection in CD4+ T_{SCM} of RM and SM. Unfortunately, due to the relatively small number of CD4+ T_{SCM} that can be isolated from SM, we were not able to directly confirm in vitro that these cells are intrinsically more resistant to in vitro SIV infection than CD4+ T_{CM} of RM.

A recent compelling study indicates that CD4+ T_{SCM} represent an increasingly important component of the persistent reservoir of latently infected cells in HIV-infected individuals treated with ART (34). The observation that CD4+ T_{SCM} are infected at high levels during pathogenic SIV infection of RM is consistent with the possibility that, once virus replication is suppressed by ART, a subset of these cells remains latently infected and may seed the previously described persistent reservoir in T_{CM} (11). Under these circumstances, the contribution of CD4+ T_{SCM} to the persistent reservoir may increase over time simply as a consequence of their enhanced proliferative ability. An intriguing corollary of this hypothesis is that, in SIV-infected SM, the absence of virus infection in CD4+ T_{SCM} may result in an inability to maintain a persistent reservoir of latently infected CD4+ T cells when virus replication is suppressed by ART. An experiment in which SIV-infected SM are treated with ART for increasing periods of time is currently ongoing in our laboratory. We hope that the results of these studies will further elucidate the role of direct CD4+ T_{SCM} infection as an obstacle to a functional cure for HIV infection.

In conclusion, this study provides evidence that pathogenic SIV infection of RM, but not nonpathogenic SIV infection of SM, is associated with significant infection and homeostatic perturbation of CD4+ T_{SCM}. We therefore propose that CD4+ T_{SCM} play an important role both in the pathogenesis of disease progression as well as the persistence of infection under ART.

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Disclosures
The authors have no financial conflicts of interest.

References


Supplemental Materials

A.

Supplementary Figure 1. Expression of CXCR3, LFA-1, and Bcl-2 in SIV-uninfected RM and SM. (A) Frequency of circulating CXCR3+ T_N, T_{SCM}, T_{CM}, and T_{EM} in 22 SIV-uninfected RM and 11 SIV-uninfected SM. (B) Expression of LFA-1 on circulating T_N, T_{SCM}, T_{CM}, and T_{EM} expressed as a ratio of MFI on each subset compared to T_N from 6 SIV-uninfected RM and 6 SIV-uninfected SM. (C) Frequency of circulating Bcl-2+ T_N, T_{SCM}, T_{CM}, and T_{EM} in 6 SIV-uninfected RM and 6 SIV-uninfected SM. ** p<0.01 (Mann-Whitney test). Bars are drawn at the median.