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A Five-Year Longitudinal Analysis of Sooty Mangabeys Naturally Infected with Simian Immunodeficiency Virus Reveals a Slow but Progressive Decline in CD4$^+$ T-Cell Count Whose Magnitude Is Not Predicted by Viral Load or Immune Activation$^\nabla$

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Natural simian immunodeficiency virus (SIV) infection in sooty mangabeys (SMs) typically does not result in AIDS, despite high-level viremia and significant depletion of mucosal CD4$^+$ T cells. Here, we report the results of the first longitudinal study of a large cohort of SMs naturally infected with SIV ($n = 78$) housed at the Yerkes National Primate Research Center from which samples were obtained three times over a 5-year period. In this study, we observed (i) no signs of simian AIDS, (ii) stable SIV loads, (iii) a slow but progressive decline in CD4$^+$ T-cell counts (from a mean of 1,067.0 cells/mm^3 at time point 1 to 764.8 cells/mm^3 at time point 3) and increases in the numbers of animals with CD4$^+$ T-cell levels below 500 and 200 cells/mm^3 (from 8 to 28 of 78 and from 1 to 4 of 78, respectively), (iv) progressive declines in percentages of naïve CD4$^+$ and CD8$^+$ T cells (from 37.7 to 24.8% and from 21.0 to 13.0%, respectively), and (v) stably low levels of activated/proliferating T cells as well as CD4$^+$ CCR5$^+$ T cells. Since the level of total CD4$^+$ T cells and the fraction of naïve T cells in SIV-uninfected SMs also declined, it is possible that some of these observations are related to aging, as the SIV-infected animals were significantly older than the uninfected animals. In contrast to the decline in CD4$^+$ T cell counts in individuals infected with human immunodeficiency virus (HIV), the decline in CD4$^+$ T cell counts in SMs naturally infected with SIV over a 5-year period was not predicted by either plasma viremia or levels of T-cell activation. Taken together, these results confirm that natural SIV infection is nonprogressive from a clinical, virological, and immunological point of view and that stable levels of viremia associated with persistently low-level immune activation represent key differences from the natural course of HIV infection in humans.

Over the past few years, there has been renewed interest in understanding the immunology and virology of so-called natural simian immunodeficiency virus (SIV) infections in African nonhuman primate species (20, 41, 51). Natural SIV hosts include the chimpanzee (*Pan troglodytes*), the gorilla (*Gorilla gorilla*), the sooty mangabey (SM; *Cercocebus atys*), the mandrill (*Mandrillus sphinx*), African green monkeys (AGMs; *Chlorocebus* spp.), and numerous others (57). Of note, SIVs that infect two of these species, the chimpanzee-derived SIVcpz and the SM-derived SIVsmm, are the origins of the human immunodeficiency virus type 1 (HIV-1) and HIV-2 epidemics, respectively (7, 11). A striking feature of natural SIV hosts is that, in marked contrast to HIV-infected humans, they tend to have benign infections, with normal CD4$^+$ T-cell counts in the majority of observed animals and progression to simian AIDS in only a few cases (44). The only known exception to this rule is the recent observation that chimpanzees naturally infected with SIVcpz in the Gombe forest of Tanzania manifest an ~16-fold increase in mortality compared to uninfected apes (19). The mechanisms underlying the favorable outcome of SIV infection in monkey species such as SMs and AGMs have been the subject of intense studies but thus far have been incompletely elucidated. Since natural SIV infections are characterized by chronically high levels of virus replication occurring primarily in short-lived CD4$^+$ T cells, it does not appear that natural SIV hosts remain healthy due to stronger or more effective adaptive immune responses to the virus or that the virus is intrinsically less cytopathic in these animals (9, 41, 51). In recent studies, more emphasis has been placed on other factors, such as the absence of chronic immune activation, more vigorous CD4$^+$ T-cell regeneration, and lower levels of CCR5 expression on CD4$^+$ T cells (40, 43, 52, 56). Elucidating the mechanisms responsible for the AIDS resistance of natural SIV hosts may provide important insight into the mechanisms of AIDS pathogenesis in HIV-infected humans.

Among the numerous natural SIV hosts, SMs have been extensively studied due to the existence of a large colony of

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animals naturally infected and uninfected with SIV at the Yerkes National Primate Research Center of Emory University, Atlanta, GA. In this body of experimental work, we and others have described in substantial detail the interaction between SIV and the SM immune system (1, 2, 6, 9, 13, 14, 25, 32, 38, 39, 43, 50, 52, 56). One concept that has emerged from a recent comparative study of the impact of SIV infection on the transcriptional profiles of SMs and rhesus macaques is that the acute phase of infection is far from being immunologically silent in SMs, with initial strong upregulation of immune activation and upregulation of type I interferon response genes that resolve approximately 4 to 6 weeks later (4). In addition, it has become apparent that natural SIV infection in SMs, while by and large nonprogressive, does in fact have an impact on the homeostasis of the CD4+ T-cell compartment. First, SIV-infected SMs manifest rapid, severe, and persistent depletion of mucosal CD4+ T cells (14). Second, small subsets of SMs both naturally and experimentally infected with SIV experience severe systemic CD4+ T-cell depletion (32, 56). Third, in at least one animal, the development of classical AIDS has been observed (23). Collectively, these observations prompted us to reevaluate whether natural SIV infection in SMs is truly nonpathogenic, as we have often stated in the past, or whether it is simply less pathogenic or less progressive. To clarify this point, we here report the results of the first longitudinal assessment of the virologic and immunologic statuses of SMs naturally infected with SIV in a study of 78 infected individuals as well as a group of 22 SIV-uninfected animals housed at the Yerkes Center over a period of 5 years (2004 to 2009). We confirmed that natural SIV infection in SMs is ultimately nonprogressive, as it did not result in AIDS in any of these 78 animals. In addition, we observed that the natural history of SIV infection in SMs is associated with a triad of (i) stable viral loads, (ii) slowly decreasing CD4+ T-cell counts, and (iii) persistently low-level immune activation. The fact that neither the viral load nor the level of immune activation predicts the magnitude of decline in CD4+ T-cell counts in SMs naturally infected with SIV identifies a clear immunologic difference between the infections in these animals and the progressive HIV and SIV infections in humans and macaques.

MATERIALS AND METHODS

Animals. Seventy-eight SMs naturally infected with SIV and 22 uninfected SMs from the colony housed at the Yerkes National Primate Research Center of Emory University were included in this longitudinal study. Blood samples were collected three times, once between 2004 and 2005 (time point 1 [TP1]), once between 2006 and 2007 (TP2), and once between 2008 and 2009 (TP3). Eighty-two healthy, SIV-uninfected rhesus macaques were also included in this study. All animals were maintained in accordance with National Institutes of Health guidelines. For uninfected animals, negative results from PCR analyses of plasma samples for the presence of SIV and negative HIV-2 serology results confirmed the absence of SIV infection.

Viral load. SIVsmm loads in plasma samples were measured by real-time PCR as described previously (52, 56).

Flow cytometry and immunophenotyping analyses of peripheral blood lymphocytes. Immunophenotyping of whole-blood samples was performed according to standard procedures using multicolor flow cytometry and monoclonal antibodies (MAbs) that were originally designed for humans or macaques and have been found to be cross-reactive in SMs. CD4+ and CD8+ T cells were identified using anti-CD3 Alexa Fluor 700 (clone SP34-2; BD Pharmingen), anti-CD4 peridinin chlorophyll protein (PerCP)-Cy5.5 (clone L200; BD Pharmingen), and anti-CD8 Pacific Blue (clone RPA-T8; BD Pharmingen). Memory T-cell subsets were identified using anti-CD28 phycoerythrin (PE)-Cy7 (clone CD28.2; eBioscience) and anti-CD95 PE-Cy5 (clone DX2; BD Pharmingen). Anti-CCR5 allophycocyanin (clone 3A9; BD Pharmingen) and anti-Ki67 fluorescein isothiocyanate (clone B56; BD Pharmingen) were also used for T-cell immunophenotyping. Ki67 staining was performed intracellularly after the BD Pharmingen Cytofix/CytoPerm kit was used for fixation and permeabilization of samples first surface stained with appropriate antibodies. Flow cytometric acquisition and analysis of samples were performed with at least 10,000 events collected by an LSRII flow cytometer driven by FACSDiva software. High-resolution analysis of the acquired data was performed using Flow Jo software (Tree Star).

Statistical analysis. Spearman correlations were used to assess relationships between two different parameters for infected SMs at a given time point. In longitudinal comparisons in which the interest was in assessing differences only between TP1 and TP3, two-tailed paired *t* tests were conducted. Random-effect models were used to assess trends across the three time points, with Tukey's adjustment for multiple comparisons. These models are similar to analysis of variance (ANOVA) models but allow for incomplete/nonbalanced data, as well as the inherent within-animal correlation of longitudinal study results. Variables were transformed, as necessary, to meet distributional assumptions of the statistical tests. All analyses used an overall α value of 0.05. Statistics were calculated using GraphPad Prism 4.0c (GraphPad Software) for Macintosh and SAS 9.2 (SAS Inc.) for Windows.

RESULTS

Study design and animal population. The colony of SMs naturally infected with SIV and uninfected SMs housed at the Yerkes National Primate Research Center has been the subject of several virologic and immunologic studies (1, 2, 6, 9, 13, 14, 25, 32, 38, 39, 43, 50, 52, 56), including a large cross-sectional survey of 110 animals that revealed the existence of a small subset of SIV-infected SMs with low CD4+ T-cell counts but no signs of disease progression (56). However, none of the published studies have provided a longitudinal assessment of the immunologic features of natural SIV infection in SMs over a prolonged period of time. To address this specific issue, we have now conducted a longitudinal study involving a total of 78 SIV-infected and 22 uninfected SMs with analysis of data from three time points spanning a 5-year period (between 2004 and 2009). Of note, none of the 78 SIV-infected SMs that were included in this study developed any signs of simian AIDS between 2004 and 2009, thus confirming the generally benign nature of the infection.

SMs naturally infected with SIV show stable levels of viremia. In HIV-infected humans, the main markers of disease progression are viral load and CD4+ T-cell count, with a large number of studies showing that the natural history of infection is associated with increasing levels of viremia and declining CD4+ T-cell counts (29, 30, 55). To investigate whether and to what extent natural SIV infection in SMs may recapitulate these features of HIV infection, we first longitudinally measured plasma SIVsmm viremia in our cohort of animals naturally infected with SIV. As shown in Fig. 1A, the levels of viremia in SMs naturally infected with SIV were stable over time, with no significant differences between TP1 and TP3 observed (averages, 169,417 SIV RNA copies/ml of plasma and 120,207 copies/ml of plasma, respectively; *P* value, not significant). Figure 1B shows the longitudinal trend in viremia for each individual animal. The observation of a stable level of viremia during the natural history of SIV infection in SMs confirms a difference from pathogenic HIV-1 infection in humans (1).

Natural SIV infection in SMs is associated with a moderate but significant decline in CD4+ T-cell count. To then longitu-

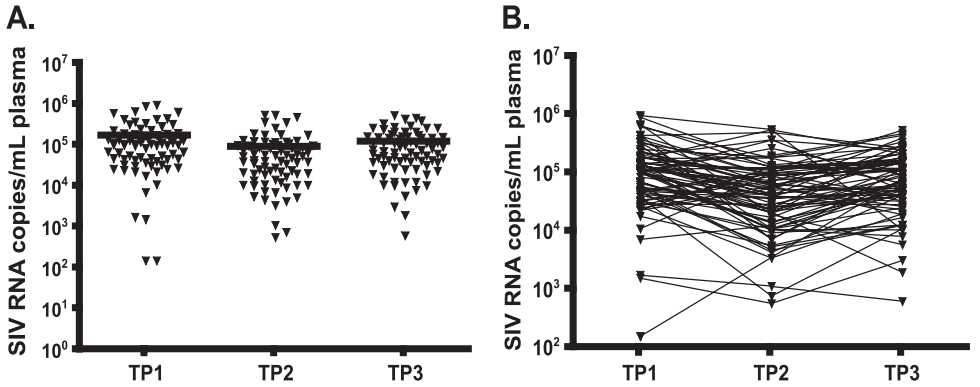

FIG. 1. SMs naturally infected with SIV show stable levels of viremia. (A) Viral loads in 78 SMs naturally infected with SIV were measured as numbers of SIV RNA copies per milliliter of plasma. Samples from animals were obtained at three time points, between 2004 and 2005 (TP1), 2006 and 2007 (TP2), and 2008 and 2009 (TP3). Triangles represent results for individual animals, and horizontal lines indicate means. (B) Viral load trends across three time points for each individual animal are shown by connecting lines.

dinally assess the effect of natural SIV infection on the counts of peripheral $CD4^+$ T cells in SMs, we measured these cells in our cohort of animals. Perhaps unexpectedly, our analysis revealed that SMs naturally infected with SIV manifested significant decline in $CD4^+$ T-cell counts over time, from an average ± standard deviation of 1,067 ± 57 cells/mm^3 at TP1 to 765 ± 64 cells/mm^3 at TP3 ($P < 0.001$) (Fig. 2A). Of note, we observed that the number of animals with $CD4^+$ T-cell counts below 500 cells/mm^3 increased from 8 (10.3%) at TP1 to 28 (35.9%) at TP3. The same degree of increase in the total number of SIV-infected SMs with $CD4^+$ T-cell counts below 200 cells/mm^3, from 1 (1.3%) at TP1 to 4 (5.1%) at TP3, was found. Interestingly, we also observed a similar decline in the $CD4^+$ T-cell counts in SIV-uninfected SMs, from an average of 1,076 ± 73 cells/mm^3 at TP1 to 818 ± 76 cells/mm^3 at TP3 ($P < 0.001$). This effect was most evident between TP2 and TP3 (Fig. 2A) and is consistent with a potential effect of aging in reducing the levels of circulating $CD4^+$ T cells. As the SIV-infected SMs were significantly older than their SIV-uninfected counterparts (17.2 ± 0.5 years of age versus 12.4 ± 0.7 years [$P < 0.0001$; unpaired t test]), the more rapid loss of $CD4^+$ T cells in the infected animals may be explained partially by the additive effect of aging. Of note, the effect of age on $CD4^+$ T cell count is not unique to SMs, as we found significant inverse correlation between $CD4^+$ T cell counts and age in a cross-sectional survey of 82 healthy, SIV-uninfected rhesus macaques ($P = 0.020$) (data not shown). Our analysis also indicated that SIV-infected SMs showed a significant decline ($P < 0.001$) in the average $CD8^+$ T-cell count, from 1,598 ± 108 cells/mm^3 at TP1 to 843 ± 79 cells/mm^3 at TP3 (Fig. 2C), and that this decline was significantly steeper than that in SIV-uninfected SMs ($P < 0.05$; linear mixed-effects models with Tukey's adjustment for multiple comparisons). This effect of SIV infection on the $CD8^+$ T-cell counts in SMs may be complex, as the levels we observed at TP3 are consistent with those found in uninfected animals at all time points, thus suggesting that the observed decline in SIV-infected SMs may represent normalization of a $CD8^+$ T-cell expansion previously induced by the infection.

Taken together, these results reveal for the first time that natural SIV infection in SMs is associated with a moderate but significant decline in $CD4^+$ T-cell counts over time. However, since a similar decline in $CD4^+$ T-cell counts in SIV-uninfected animals over the same time period was observed, it

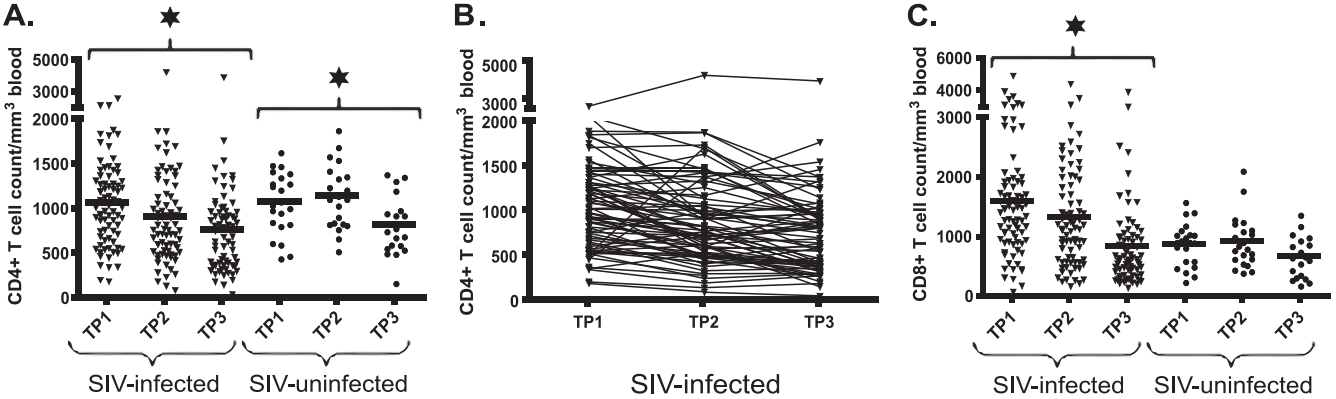

FIG. 2. Natural SIV infection in SMs is associated with moderate but significant decline in $CD4^+$ T-cell counts. (A and B) $CD4^+$ T-cell counts (A) and their longitudinal trends (B) for individual animals. (C) $CD8^+$ T-cell counts in 78 SMs naturally infected with SIV (▼) and 22 SIV-uninfected SMs (●) were determined at three time points. Asterisks indicate P values of <0.001, as determined by linear mixed-effects models with Tukey's adjustments for multiple comparisons.

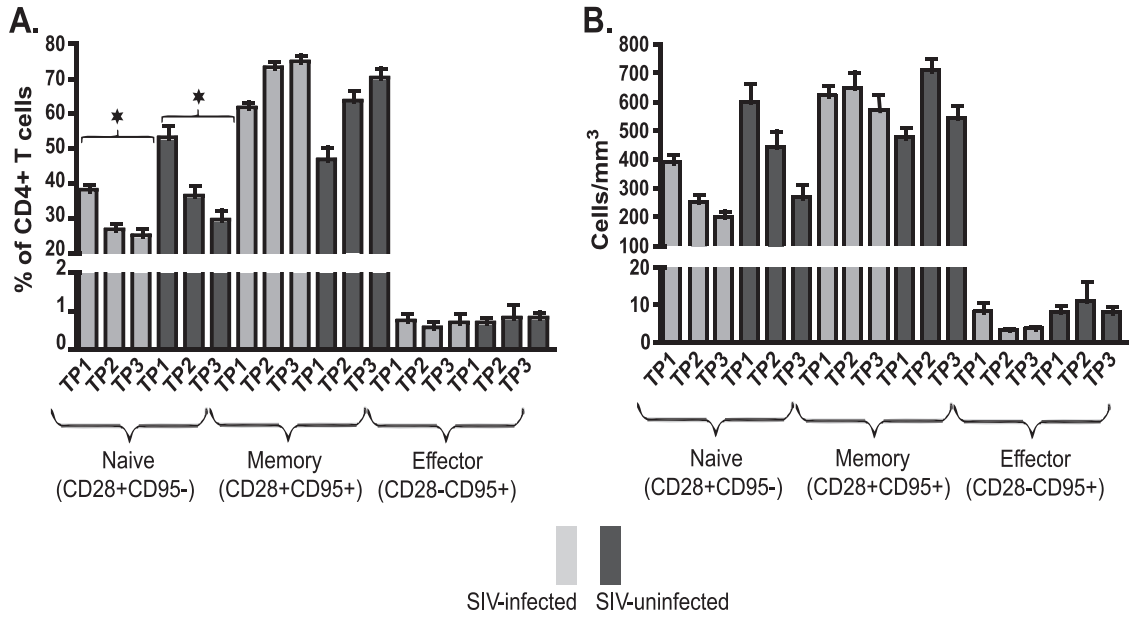

FIG. 3. Naïve, memory, and effector CD4+ T-cell dynamics in SMs naturally infected with SIV. Percentages (A) and absolute counts (B) of naïve, memory, and effector CD4+ T cells in 78 SIV-infected and 22 SIV-uninfected SMs at three time points were determined. Asterisks indicate *P* values of <0.001, as determined by linear mixed-effects models with Tukey's adjustments for multiple comparisons.

seems unlikely that natural SIV infection in SMs has any significant negative impact on CD4+ T-cell homeostasis.

SMs naturally infected with SIV show a progressive decline in naïve T cells with corresponding increases in memory and effector T cells. Pathogenic HIV infection in humans and SIV infection in macaques are associated with specific changes in the relative proportions of naïve, memory, and effector T cells (5, 16, 28, 35, 46). To investigate how natural SIV infection in SMs impacts these T-cell subpopulations, we longitudinally measured the fractions of CD4+ and CD8+ T cells expressing the naïve (CD28+ CD95−), memory (CD28+ CD95+), and effector (CD28− CD95+) phenotypes (47, 56). Note that this definition of memory cells includes both CD62L+ CCR7+ "central" memory T cells (Tcm) and CD62L− CCR7− "effector" memory T cells (Tem). Since the measurement of Tcm and Tem was conducted only at the second and third time points, we used the levels of total memory T cells in our analysis. As depicted in Fig. 3A, both SIV-infected and uninfected SMs experienced significant decreases in the percentages of naïve CD4+ T cells and concomitant increases in the percentages of circulating memory CD4+ T cells, with no change in the percentages of effector CD4+ T cells. As depicted in Fig. 3B, absolute counts of these CD4+ T-cell subsets showed that natural SIV infection in SMs was associated with declines in numbers of both naïve and effector CD4+ T cells, with constant numbers of memory CD4+ T cells. A similar trend of decline in the fraction of naïve cells and relative expansion of memory cells within the CD8+ T-cell pool was also observed (data not shown). Since the relative declines in naïve CD4+ T cell counts in SIV-infected and uninfected animals were of similar magnitudes, it is difficult to determine to what extent the decline in infected animals was caused by the infection as opposed to being simply related to aging.

Taken together, these data highlight a major difference from pathogenic SIVmac infection in rhesus macaques, which is associated with selective depletion of memory CD4+ T cells in peripheral blood (35, 46).

SMs naturally infected with SIV maintain persistently low levels of T-cell proliferation. While pathogenic HIV and SIV infections in humans and macaques are associated with a state of chronic, generalized immune activation (53), natural SIV infection in SMs is characteristically associated with low levels of immune activation during the chronic phase (6, 15, 21, 36, 42, 48, 52, 56, 60). In this longitudinal study, we sought to determine whether natural SIV infection in SMs is associated with any sign of increasing immune activation over time. To this end, we measured the percentages of CD4+ and CD8+ T cells expressing the proliferation marker Ki67 that is typically expressed on activated cells (49). As shown in Fig. 4, we found no significant change between TP1 and TP3 in the percentages of proliferating Ki67+ CD4+ and CD8+ T cells (3.1% of CD4+ Ki67+ T cells at TP1 and 3.9% at TP3 and 3.3% of CD8+ Ki67+ T cells at TP1 and 3.5% at TP3). This result indicates that persistently low-level immune activation is a key feature of the chronic phase of natural SIV infection in SMs and that the moderate decline in CD4+ T-cell counts observed in these animals does not appear to trigger any homeostasis-driven increase in CD4+ T-cell proliferation.

SMs naturally infected with SIV maintain low but stable levels of CD4+ CCR5+ T cells. Most strains of HIV and SIV use CCR5 as a coreceptor for virus entry and thus preferentially infect CD4+ CCR5+ T cells, which are rapidly depleted in the effector lymphoid sites of mucosal tissues (5, 14, 22, 26, 28, 45, 46, 58). However, the *in vivo* dynamics of the fraction of CD4+ CCR5+ T cells within the total pool of circulating CD4+ T cells appears to be more complex, with the fractions of these cells observed in HIV-infected humans and in SIV-infected rhesus macaques of Chinese origin (8, 33, 37) higher than

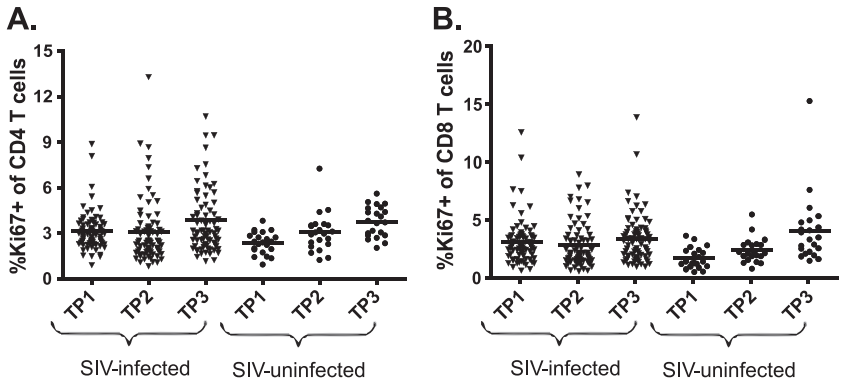

FIG. 4. SMs naturally infected with SIV maintain persistently low levels of T-cell proliferation. Percentages of $CD4^+$ $Ki67^+$ T cells (A) and $CD8^+$ $Ki67^+$ T cells (B) in 78 SMs naturally infected with SIV (▼) and 22 SIV-uninfected SMs (●) were measured at three time points.

those observed in SIV-infected rhesus macaques of Indian origin (27). As shown in Fig. 5, the percentages of $CD4^+$ $CCR5^+$ T cells in our cohort of SMs naturally infected with SIV (2.9% at TP1, 2.6% at TP2, and 2.3% at TP3) remained relatively stable over time, thus indicating a limited impact on the pool of circulating $CD4^+$ $CCR5^+$ T cells in this model of nonprogressive SIV infection.

Neither the level of viremia nor the magnitude of T-cell proliferation predicts the magnitude of decline in $CD4^+$ T-cell counts in SIV-infected SMs. The finding that natural SIV infection in SMs is associated with moderate but significant decline in peripheral blood $CD4^+$ T-cell counts prompted us to investigate what factors could predict the tempo of this decline. In HIV-infected humans, the main predictors of decline in $CD4^+$ T-cell counts are the viral load (29, 30) and the level of immune activation (12, 17, 24, 54). To determine if a similar relationship is present in SMs naturally infected with SIV, we performed a series of analyses of correlations between the change in $CD4^+$ T-cell counts from TP1 to TP3 (hereinafter referred to as ΔCD4) and the viral load, the levels of proliferating (i.e., $Ki67^+$) $CD4^+$ and $CD8^+$ T cells, and the fraction of memory ($CD95^+$ $CD28^+$) $CD4^+$ T cells at TP1. As shown in Fig. 6, these analyses revealed that ΔCD4 is not correlated with the viral load, the percentages of proliferating $CD4^+$ and $CD8^+$ T cells, or the percentage of memory $CD4^+$ T cells as measured at TP1. In all, these results indicate that, in contrast to the decline in $CD4^+$ T-cell counts observed in HIV-infected humans, the decline in $CD4^+$ T-cell counts observed in SMs naturally infected with SIV does not appear to be consistently predicted by the levels of viremia or immune activation.

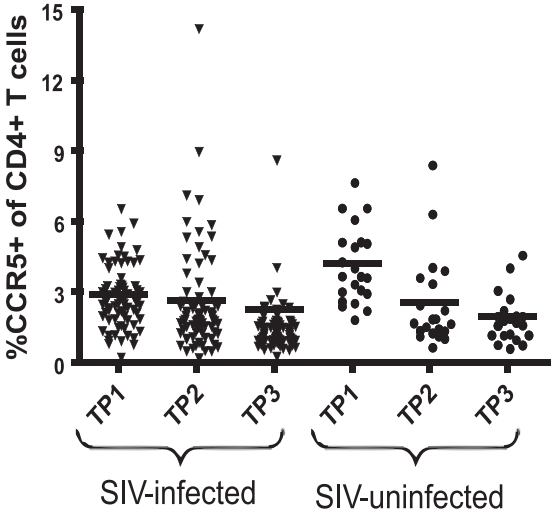

FIG. 5. SMs naturally infected with SIV maintain low but stable levels of $CD4^+$ $CCR5^+$ T cells. The percentages of $CD4^+$ $CCR5^+$ T cells in 78 SMs naturally infected with SIV (▼) and 22 SIV-uninfected SMs (●) were measured at three time points.

DISCUSSION

Over the past several years, we and others have intensively studied the virologic and immunologic features of natural SIV infection in SMs. These studies led to some basic conclusions regarding this model of infection that can be summarized as follows: (i) the infection is usually not associated with progression to simian AIDS, (ii) the set-point level of viremia typically ranges between 10^4 and 10^6 SIV RNA copies/ml of plasma, (iii) 80 to 90% of animals maintain $CD4^+$ T-cell counts comparable to those observed in uninfected SMs, (iv) the level of T-cell activation is significantly lower than those detected in humans and macaques with pathogenic HIV and SIV infections, respectively, (v) the breadth and magnitude of antiviral cellular immune responses are similar to, if not lesser than, those found in pathogenic HIV and SIV infections, and (vi) the infection is associated with a short *in vivo* life span of productively infected cells (41, 51). Of note, all these studies were conducted either as cross-sectional analyses of naturally infected animals (9, 18, 25, 52, 56, 59) or as longitudinal analyses of animals experimentally infected with SIV (10, 14, 25, 31, 32, 34, 50). Here, we report the results of a longitudinal virologic and immunologic analysis of a cohort of 78 SMs naturally infected with SIV and 22 uninfected SMs from which samples were obtained at three different time points over a 5-year period. To the best of our knowledge, this work represents the first comprehensive longitudinal assessment of SIV infection in the large group of naturally infected animals housed at the Yerkes Center.

The main finding of this study is that the natural history of SIVsmm infection in SMs is characterized by a triad of stable viral loads, slowly declining $CD4^+$ T-cell counts, and persis-

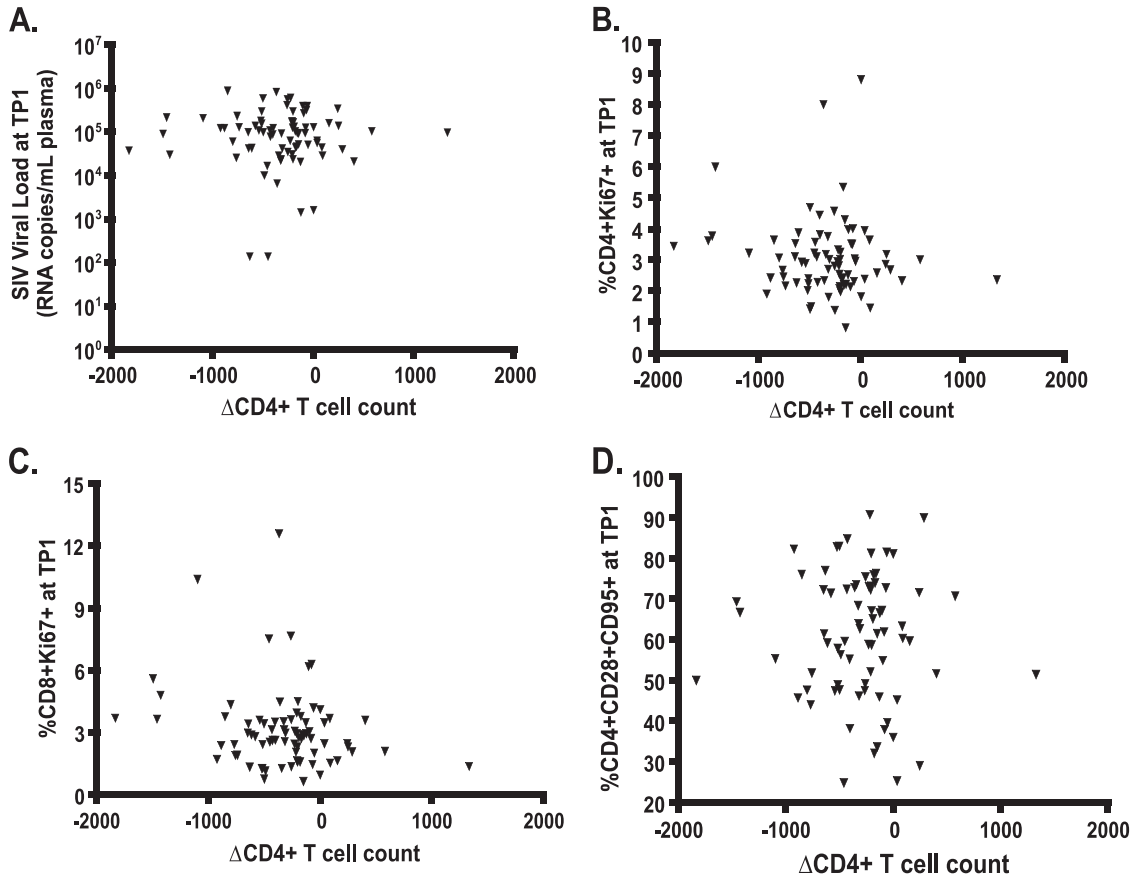

FIG. 6. Neither the level of viremia nor the magnitude of T-cell proliferation predicts the tempo of decline in $CD4^+$ T-cell counts in SIV-infected SMs. Scatter plots depict the relationships between ΔCD4 and the viral load (A), the percentage of $CD4^+$ $Ki67^+$ T cells (B), the percentage of $CD8^+$ $Ki67^+$ T cells (C), and the percentage of memory ($CD28^+$ $CD95^+$) $CD4^+$ T cells (D) at TP1 for 78 SMs naturally infected with SIV.

tently low levels of immune activation. These results identify a previously unrecognized difference from the natural history of HIV infection in humans, in which declining $CD4^+$ T-cell counts are associated with increasing levels of both viremia and immune activation. Of note, a decline in $CD4^+$ T-cell counts in uninfected SMs had a level of significance similar to that in uninfected SMs, suggesting that, at least in part, the loss of $CD4^+$ T cells in SIV-infected SMs may be simply an effect of aging. It should also be noted that the interpretation of these results is somewhat complicated by the fact that in this study the SMs naturally infected with SIV were significantly older than the uninfected ones (17.2 ± 0.5 years of age versus 12.4 ± 0.7 years of age [$P < 0.0001$; unpaired t test]). Thus, it is difficult to directly compare the effects of aging on $CD4^+$ T-cell counts in these two groups of SIV-infected and uninfected animals. Previous cross-sectional studies of SIV-infected SMs revealed lower $CD4^+$ T-cell counts than those in uninfected animals (18, 52). However, these results should be approached with caution as the cross-sectional design of these studies precludes direct comparisons with the longitudinal analysis in our present study.

The fact that SMs naturally infected with SIV tend to significantly deplete their pool of peripheral $CD4^+$ T cells over time is not entirely unexpected, as several previous studies indicated that this model of nonprogressive infection can be associated with perturbations of $CD4^+$ T-cell homeostasis. First, a previous study from our group showed that a subset of 5 to 10% of SMs naturally infected with SIV may experience profound $CD4^+$ T-cell depletion, and in two cases the $CD4^+$ T-cell count dropped below 50 cells/mm^3 (56). Second, a virus with expanded coreceptor tropism emerged in a group of experimentally SIV-infected SMs, which subsequently manifested extreme $CD4^+$ T-cell depletion in both blood and mucosal tissues (32). Third, a study of the levels of $CD4^+$ T cells in mucosal tissues (those of the rectum and lung) in SIV-infected SMs revealed that these cells are depleted in the majority of animals (14). Collectively, the results of these studies indicate that, in SMs, SIV infection is not immunologically silent but rather has a clearly discernible effect on the host immune system, particularly in determining the size of the pool of mucosal (and, to a lesser extent, circulating) $CD4^+$ T cells. While this effect is predominantly subclinical, with animals typically living an apparently normal life span in captivity, we suggest that this infection is better defined as nonprogressive rather than nonpathogenic. The recent finding that chimpanzees naturally infected with SIVcpz have an ~16-fold increase in mortality compared to uninfected animals (19) emphasizes

how the clinical spectrum of SIV infection in African nonhuman primates is far from being fully appreciated.

The observation of a slow but progressive decline in $CD4^+$ T-cell counts in SMs naturally infected with SIV must be reconciled with the fact that these animals do not appear to show any increased morbidity or mortality. One possibility is that this $CD4^+$ T-cell depletion is so slow that the SMs simply die of other causes (i.e., natural death) prior to developing clinically significant immunodeficiency. Of note, we previously observed that SIV infections in SMs do not progress to AIDS even when the animals experience severe systemic and mucosal $CD4^+$ T-cell depletion (32, 56). This puzzling finding may be explained by postulating that the immune system of SMs has evolved to be less dependent on the function of $CD4^+$ T cells, with other cell types providing immunological help *in vivo*. Another possibility is that at some point in the infection, T helper cells in SMs stop expressing the CD4 molecule in a pattern similar to what has been described recently for African green monkeys (3). A third potential explanation is that in a "resting," non-chronically activated immune environment, the need for $CD4^+$ T-cell help is much smaller and that even very low levels of $CD4^+$ T cells are sufficient to avoid AIDS. Further studies are needed to fully elucidate the role of $CD4^+$ T cells in SMs as well as other natural SIV hosts.

In this study, we sought to identify predictors of the magnitude of decline in $CD4^+$ T-cell counts in SMs naturally infected with SIV by performing a series of statistical analyses in which we investigated potential correlations between parameters measured at TP1 of our longitudinal analysis and ΔCD4. Interestingly, we found that neither the viral load nor the level of immune activation (measured as the fractions of $CD4^+$ $Ki67^+$ and $CD8^+$ $Ki67^+$ T cells, as well as the fraction of memory $CD4^+$ T cells) predicts the magnitude of decline in $CD4^+$ T-cell counts. Similarly, we did not find evidence for an effect of baseline total $CD4^+$ T-cell counts or naïve $CD4^+$ T-cell counts (data not shown). Based on these results, we favor the possibility that the depletion of $CD4^+$ T cells in SMs naturally infected with SIV may reflect insufficient regeneration of these cells that is not directly related to the amount of virus replication or the prevailing level of immune activation.

While this study is clearly descriptive in nature, we believe that it is nonetheless of interest as it is the first ever conducted with such a large group of nonhuman primates naturally infected with SIV over an extended period of observation. In particular, differences between the results described here and findings from studies of the natural history of HIV infection in humans provide clues as to the pathogenic mechanisms that are likely to be central to the development of clinically significant immunodeficiency after infection with a primate lentivirus. Further follow-up with the colony of SMs naturally infected with SIV housed at the Yerkes National Primate Research Center will provide additional clarification of the natural history of this model of nonprogressive infection.

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