Depletion of CD8$^+$ Cells in Sooty Mangabey Monkeys Naturally Infected with Simian Immunodeficiency Virus Reveals Limited Role for Immune Control of Virus Replication in a Natural Host Species[1]

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SIV infection of sooty mangabeys (SMs), a natural host species, does not cause AIDS despite high-level virus replication. In contrast, SIV infection of nonnatural hosts such as rhesus macaques (RMs) induces an AIDS-like disease. The depletion of CD8$^+$ T cells during SIV infection of RMs results in marked increases in plasma viremia, suggesting a key role for CD8$^+$ T cells in controlling levels of SIV replication. To assess the role that CD8$^+$ T cells play in determining the virologic and immunologic features of nonpathogenic SIV infection in SMs, we transiently depleted CD8$^+$ T cells in SIV-infected and uninfected SMs using a CD8α-specific Ab (OKT8F) previously used in studies of SIV-infected RMs. Treatment of SMs with the OKT8F Ab resulted in the prompt and profound depletion of CD8$^+$ T cells. However, in contrast to CD8$^+$ cell depleted, SIV-infected RMs, only minor changes in the levels of plasma viremia were observed in most SIV-infected SMs during the period of CD8$^+$ cell deficiency. Those SMs demonstrating greater increases in SIV replication following CD8$^+$ cell depletion also displayed higher levels of CD4$^+$ T cell activation and/or evidence of CMV reactivation, suggesting that an expanded target cell pool rather than the absence of CD8$^+$ T cell control may have been primarily responsible for transient increases in viremia. These data indicate that CD8$^+$ T cells exert a limited influence in determining the levels of SIV replication in SMs and provide additional evidence demonstrating that the absence of AIDS in SIV-infected SMs is not due to the effective control of viral replication by cellular immune responses. ***The Journal of Immunology,*** **2007, 178: 8002–8012.**

Experimental SIV infection of rhesus macaques (RMs),[5] a nonnatural host species, represents the best-characterized and most widely studied animal model for the study of AIDS pathogenesis (1). Similarities between SIV infection in RMs and HIV infection in humans include the targeting of CD4$^+$ T cells for infection, the development of progressive CD4$^+$ T cell depletion and attendant immunodeficiency, the clinical spectrum of diseases observed following infection, and the inverse correlation seen between levels of chronic virus replication and rates of disease progression (1). However, in contrast to the substantial pathogenic consequences of SIV and HIV infections of nonnatural host species (Asian macaques and humans, respectively), primate species that are natural hosts for SIV infection (such as sooty mangabey monkeys (SM), Sykes monkeys, mandrills, and African green monkeys) remain healthy following SIV infection and do not experience CD4$^+$ T cell depletion, immunodeficiency, neuropathology, or the wasting syndromes characteristic of AIDS (2). SMs (*Cercocebus torquatus atys*), Old World primates indigenous to Central and Western Africa that are often naturally infected with SIV in the wild, represent one of the best-studied models of natural, nonpathogenic SIV infection (3–7). Importantly, the majority of SIV-infected SMs maintain normal CD4$^+$ T cell numbers despite prolonged SIV infection and levels of chronic virus replication that are as high or higher than those observed in pathogenic HIV infections in humans and experimental SIV infections of RMs (4–6).

Although the reasons underlying the lack of immunodeficiency in SIV-infected SMs remain incompletely understood, we have found that SIV-infected SMs display no evidence of the chronic generalized immune activation that typifies pathogenic SIV and HIV infections (6). In pathogenic SIV and HIV infections, chronic immune activation plays a central role in driving CD4$^+$ T cell depletion through precipitation of the substantial loss of uninfected T cells (both CD4$^+$ and CD8$^+$ T cells) to activation-induced cell death and the compromise of T cell regenerative capacity. Of particular predictive importance, increased levels of CD8$^+$ T cell activation have been shown to directly correlate with accelerated progression to AIDS and death in HIV-infected humans and SIV-infected RMs (8–14). However, in contrast to pathogenic SIV and HIV infections, levels of CD8$^+$ T cell activation and proliferation are not significantly increased in SMs during chronic SIV-infection—suggesting that the overall host cellular immune response to

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[5] Abbreviations used in this paper: RM, rhesus macaque; SM, sooty mangabey.

SIV infection is attenuated in SMs (6, 7). Indeed, although SIV-specific $CD8^+$ T cell responses can be detected (by precursor frequency analyses, ELISPOT assays, and intracellular cytokine staining methods) in most SIV-infected SMs, their magnitude and breadth, when seen, are substantially lower than what is observed in HIV-infected humans (15–17). Furthermore, no correlation is seen between the presence, absence, or level of SIV-specific responses measured ex vivo with in vivo levels of virus replication (15–17). Given the absence of increased levels of $CD8^+$ T cell proliferation and activation in vivo and the limited or absent SIV Ag-specific $CD8^+$ T cell responses measured ex vivo, it is unclear whether $CD8^+$ T cell responses in SIV-infected SMs exert any control over the levels of SIV replication in infected SMs. Based on these observations, we have proposed that the absence of chronic aberrant immune activation (especially the generalized activation of $CD8^+$ T cells) may protect SIV-infected SMs from bystander immunopathology that contributes to accelerated $CD4^+$ T cell destruction and impaired $CD4^+$ T cell regeneration during pathogenic HIV and SIV infections (6, 18). Should this hypothesis be true, nonpathogenic SIV infections may reflect the relative absence, rather than a greater representation of, persistently activated antiviral $CD8^+$ T cell responses.

In both the HIV infection of humans and the SIV infection of RMs the antiviral properties of HIV/SIV-specific $CD8^+$ T cells, in particular the CTLs directed against virus-infected $CD4^+$ T cells, are thought to play an important role in determining the level of viral replication during chronic infection (19–21). However, the role of $CD8^+$ T cell-mediated CTL activity in the control of virus replication and the amelioration of disease progression in HIV-infected humans is still incompletely understood. A number of studies show a lack of correlation between the breadth and magnitude of HIV-specific CTL responses and both the levels of virus replication and the rates of disease progression (17, 22, 23) when virus-specific $CD8^+$ T cell responses are assessed by virus peptide-driven cytokine production (e.g., IFN-γ) assays. Among the most influential evidence supporting the hypothesis that $CD8^+$ T cells exert important antiviral effects in vivo comes from studies performed in acutely and chronically SIV-infected RMs in whom the experimental depletion of $CD8^+$ cells using CD8α-specific mAbs has been shown to result in significant and rapid increases in viral replication in $CD8^+$ cell-depleted animals, with rapid return to the baseline levels of viremia once circulating $CD8^+$ T cells are restored (21, 24–27).

Seeking to directly discern potential antiviral effects of $CD8^+$ T cells in the modulation of SIV replication in infected SMs, we performed a series of studies using a well-characterized anti-CD8α mAb (OKT8F) to induce a prompt, near complete and transient depletion of $CD8^+$ cell populations in vivo. In notable contrast to similar $CD8^+$ T cell depletion studies previously performed in SIV-infected RMs, only minor changes in the levels of plasma viremia were observed in the majority of treated animals during the period of marked $CD8^+$ T cell depletion, suggesting that $CD8^+$ T cells and NK cells exert limited, if any, direct immune control of SIV replication in SIV-infected SMs.

Materials and Methods

Animals

Six naturally infected, two experimentally infected, and two uninfected SMs were used in this study. Eight of the 10 animals tested positive for anti-CMV Abs. The experimentally infected SMs were infected with an aliquot of plasma obtained from a naturally infected SM (7) containing highly diverse viral quasispecies (28) ~3 years before depletion. All of the SIV-infected animals were asymptomatic and free of any signs of immunodeficiency at the time of depletion. Eight of the animals (four naturally infected, two experimentally infected, and two uninfected) received the anti-CD8α mAb OKT8F depleting Ab. Two naturally infected animals received the isotype matched (i.e., IgG2a) nondepleting mAb OKT3. All animal housing, care, and research were in accordance with the guidelines for the care and use of laboratory animals from the National Research Council (Washington, D.C.), and protocols were approved by the Emory University Institutional Animal Use and Care Committee.

Depletion of $CD8^+$ T cells in vivo

The depletion of $CD8^+$ cells was performed using the CD8α-specific OKT8F mAb (provided by the R. W. Johnson Pharmaceutical Research Institute) that has been shown to effectively deplete $CD8^+$ cells in vivo in RMs (24, 27). As in previous RM studies, the nondepleting isotype-matched OKT3 mAb (provided by the R. W. Johnson Pharmaceutical Research Institute), which is directed against human CD3 and does not cross-react with SM CD3 or any other identifiable SM cell surface molecule, was used as a negative control (24, 27). Dr. R. S. Mittler (Emory University, Atlanta, GA), provided the sterile Ab solutions used in this study. To prepare the sterile Ab solutions, hybridomas were grown in 40-liter bioreactors in RPMI 1640 (Invitrogen Life Technologies) containing L-glutamine, penicillin/streptavidin, Fungizone, and 5% low Ig FBS (HyClone). Culture supernatants are then spun down to remove cells and debris in a continuous flow centrifuge (Beckman Coulter) and concentrated to <1 liter using a tangential flow concentrator (Pall). The supernatant was diluted 1/1 with binding buffer and applied to a protein G-Sepharose column. The column was washed free of unbound protein using binding buffer, and the bound IgG was eluted by lowering the pH of the buffer to pH 3.0. The eluted protein was collected in 2 M Tris buffer (pH 8.0) to neutralize the pH of the eluted Ab. This preparation was concentrated, dialyzed against PBS, and filter sterilized and protein concentration was determined. Levels of endotoxin were measured using the standard *Limulus* amoebocyte agglutination assay (Cambrex). All Ab preparations were endotoxin free to the limit of detection of 0.06 endotoxin units/ml. For these studies we used two different regimens of OKT8F Ab treatment. According to one depletion protocol, OKT8F was infused at 1 mg/kg i.v. (in 50 ml 0.9% saline; B. Braun Medical) for 4 consecutive days. In a second protocol, OKT8F mAb was infused for three consecutive days at the higher dose of 4 mg/kg i.v. per day (also diluted in 50 ml 0.9% saline; B. Braun Medical). Control animals were treated in an identical manner with the nondepleting OKT3 mAb that does not bind to any identifiable SM cell subset (data not shown). Blood was collected in acid citrate dextrose Vacutainer tubes (Becton Dickinson) at baseline (i.e., two weeks before infusion) and on days 0, 1, 2, 4, 7, 10, 14, 21, 28, 42, and 56 of the study for viral load and immunophenotypic analyses. Lymph node biopsies were performed at baseline and on days 4 and 10 of the study. Bone marrow aspirates in heparin were drawn at baseline and then again on days 7 and 21 of the study.

Immunophenotypic analysis of lymphocyte subsets

An extensive four-color flow cytometric panel was used to immunophenotypically define the expression of markers on lymphocytes and NK cells in peripheral blood, bone marrow, and lymph node samples. For all samples, lymphocytes were separated using a lymphocyte separation medium (ICN Pharmaceuticals) and extensively washed to remove any anti-mouse Abs produced in response to the infusions. The mAbs used included anti-CD3 allophycocyanin (provided by Dr. C. Ibegbu, Emory Vaccine Center, Atlanta, GA), anti-CD4 (PE and allophycocyanin), anti-CD20 PerCP, anti-CD25 PE, anti-CD28 PE, anti-CD62L PE, anti-CD69 PE, anti-CD95 PE, Ki67 FITC (all from BD-Pharmingen), anti-CD16 PC5 (Beckman Coulter), and anti-CD45RA-TC (Invitrogen Life Technologies). All these Abs have been shown to be cross-reactive with SM cells in previous studies (6). The anti-CD8 Ab OKT8 (provided by Dr. R. S. Mittler, Emory University, Atlanta, GA) was used to determine the amount of $CD8^+$ cells remaining after depletion as it binds to an epitope of the CD8 molecule that is not masked by preincubation with OKT8F. In these experiments, $0.5–1 \times 10^6$ PBMCs were incubated with titered amounts of mAbs in 100 ml PBS (Mediatech) for 20 min at 4°C in the dark and then washed with PBS containing 0.5% BSA (Sigma-Aldrich). Cells were then fixed and permeabilized with FACS permeabilizing solution (BD Pharmingen), washed, and stained intracellularly with an appropriate isotype control or an Ab that recognizes the MIB-1 epitope of the proliferation-associated Ag Ki-67 (BD Pharmingen). After further washes, cells were fixed in 1% formaldehyde (Polysciences). At least 50,000 gated events were acquired on a FACSCalibur flow cytometer powered by CellQuest (BD Biosciences). Further analysis was performed using FlowJo flow cytometry analysis software (Tree Star).

Viral load analysis

Levels of SIVsm RNA in plasma samples were quantified in duplicate with a sensitive and reliably quantitative two-step, real-time RT-PCR assay as

previously described (6). RNA copy number was determined by comparison with an external standard curve consisting of in vitro transcripts representing bases 216–2106 of the SIVmac239 genome (HIV Sequence Database at http://hiv-web.lanl.gov/seq-db.html). The sensitivity of this assay is ≤160 RNA copies per milliliter of plasma, a linear dynamic range of 10^2 to 10^8 RNA copies, and a coefficient of variation of 10–25%. CMV DNA was quantified in duplicate from plasma using real-time PCR. Primers and probe for amplification of rhesus CMV DNA sequences by real-time PCR were designed using Primer Express software (PerkinElmer). The forward primer (5′-GTTTAGGGAACCGCCATTCTG-3′) corresponds to residues 719–739, the reverse primer (5′-GTATCCGCGTTCCAATGCA-3′) corresponds to residues 826–808, and the probe (5′-FAM-TCCAGCCTC CATAGCCGGGAAGG-TAMRA-3′) corresponds to residues 784–806 (29). The real-time PCR was performed on an ABI Prism 7700 sequence detection system (PerkinElmer) using a TaqMan Gold kit under the following conditions: the 50-μl reactions contained 5 μl of 10× TaqMan buffer A, 4 mM MgCl2, 200 μM dNTPs, a 300 nM concentration of each primer, a 125 nM concentration of the fluorogenic probe, and 1.25 U of AmpliTaq Gold DNA polymerase). Thermal cycling conditions consisted of 10 min at 95°C followed by 45 cycles of 15 s at 95°C and 1 min at 60°C. Standard DNA templates based on a pSP72 plasmid encoding 9.2kb of *Macaca mulatta*-derived CMV were provided by Dr. C. Larsen (Emory University). The sensitivity of the assay was 150–300 copies/ml.

Statistical methods

Repeated measures analyses for $\log_{10}$ viral load, proliferation of CD4$^+$ T cells (log Ki67), and activation of CD4$^+$ T cells (log CD69) were analyzed with a means model using SAS PROC MIXED software (version 8) providing separate estimates of the means by time on study and treatment group (OKT8F antibody). A compound symmetry variance-covariance form among the repeated measurements was assumed for each outcome and estimates of the SE values of parameters were used to perform statistical tests. The model-based means are unbiased with unbalanced and missing data as long as the missing data are noninformative (missing at random). Mean changes over time within a treatment group were tested for a linear trend. Statistical tests were two-sided. Values of $p \leq 0.05$ was considered statistically significant. The rate of increase of a $\log_{10}$ viral load between days 4 and 10 was obtained using a mixed effects model specifying that the $\log_{10}$ viral load follows a linear regression over log Ki67 with a random intercept for each animal.

Results

Study rationale and design

Given that rates of virus replication are equivalent in pathogenic and nonpathogenic SIV infections (Refs. 6 and 30–32 and our unpublished results), substantial depletion of CD8$^+$ T cells should result in prompt increases in viremia in nonpathogenic infections of SMs—just as they do in pathogenic infections of RMs (21, 24–27)—if CD8$^+$ cells play a significant role in containing SIV replication in SMs. To effect CD8$^+$ T cell depletion in SIV infected and uninfected SMs, we used a well-characterized Ab that had been used in previous studies in RMs so that the results obtained in these two different species could be compared directly. Toward this end we selected the CD8α-specific murine mAb OKT8F that efficiently binds to SM CD8$^+$ cells (data not shown) and is known to enable prompt and near-complete depletion of CD8$^+$ cells in RMs (24, 27). Because the majority of nonhuman primates available for study in captivity, including SMs and RMs, are infected with other viruses (e.g., CMV, alpha-herpes (HSV-like), and gamma-herpes (EBV-like) viruses) (33, 34) whose level of replication is known to be controlled by CD8$^+$ T cell and NK cell responses (29, 35–42), we reasoned that a mAb that results in near-complete but transient depletion of CD8$^+$ cells would be preferable to the use of a mAb that results in prolonged CD8$^+$ cell depletion (such as the humanized anti-CD8α mAb cM-T807) (21, 25, 43). In particular, we wanted to avoid confounding variables resulting from the reactivation of latent herpesvirus (or other) infections that would cause an unintended immune CD4$^+$ T cell activation that could, in turn, lead to increased levels of SIV replication irrespective of CD8$^+$ T cell effects on SIV itself.

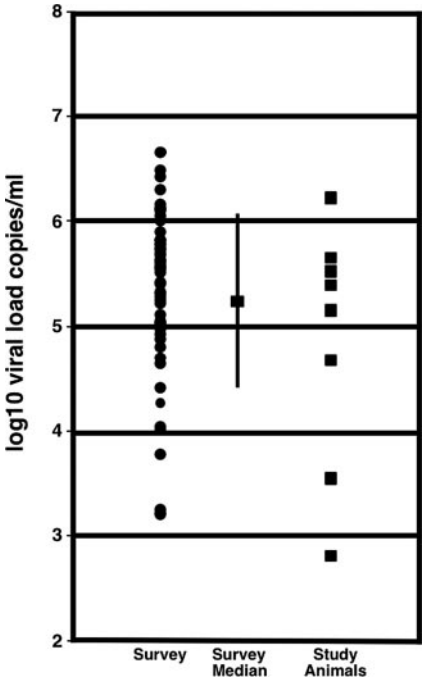

FIGURE 1. Cross-sectional viral load. A survey of 54 naturally infected sooty mangabeys to determine viral load is shown (●). The animals used in this study are shown in comparison (■). The SD is shown with the survey median.

Ten SMs were used in this study; six had become naturally infected with SIV around the time of their attainment of sexual maturity, two were experimentally infected with SIV ~3 years before initiation of the study, and two were uninfected. The naturally SIV-infected SMs used in this study were selected as representative of the relatively wide range of viremia (Fig. 1) following natural SIV infection of SMs with average levels that are at least as high as those observed in HIV infection (4–6). The range of starting viral loads for the infected animals was $\sim 1 \times 10^3$ to 1.7×10^6 (Fig. 1). In a survey of 54 naturally SIV-infected SMs, the range of set point viral loads was 1.5×10^3 to 4.5×10^6 with a median of 3.2×10^5 (Fig. 1). Extensive sampling of peripheral blood and serial lymph node and bone marrow biopsies at baseline and at sequential times after Ab administration allowed us to carefully monitor lymphocyte changes in multiple anatomic compartments, as well as variations in plasma viremia induced by the treatment. This design made it possible to do the following: 1) determine the effects of CD8$^+$ cell depletion on the level of SIV replication; 2) determine whether SIV-infected and uninfected SMs differ in the extent and kinetics of CD8$^+$ T cell depletion and recovery; and 3) evaluate whether the maintenance of T cell homeostasis in SIV-infected and uninfected SMs is predicated on total CD3$^+$ T cell numbers (CD8$^+$ plus CD4$^+$) or upon levels of lineage-specific (CD8$^+$ and CD4$^+$) T cells. The inclusion of two CD8-depleted, SIV-uninfected SMs allowed us to evaluate potential effects that preexisting SIV infection exerts on the changes in the proportion and properties of lymphocyte subsets following CD8 depletion. In addition, given that the xenogeneic mAbs used in CD8 depletion studies also act as foreign Ags that elicit immune responses (43), the inclusion of two SIV-infected SMs who were "mock-depleted" with the murine OKT3 mAb (of the same isotype as the depleting OKT8F mAb) that does not bind to SM CD8α (and does not react with any identifiable cell surface molecules in SMs; data not shown) allowed for the assessment of potential nonspecific effects of Ab administration on the level of SIV replication

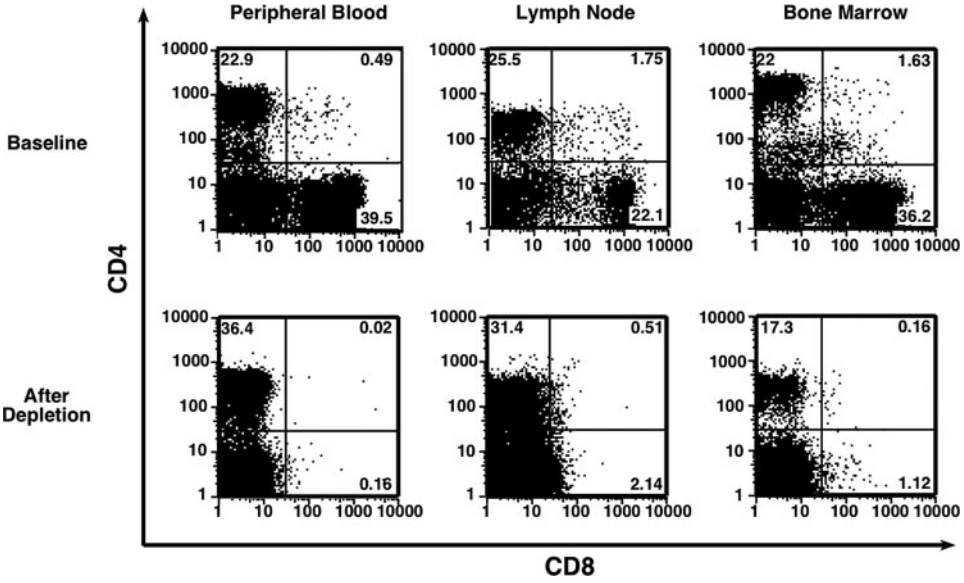

FIGURE 2. Effect of OKT8F infusion on $CD4^+$ and $CD8^+$ T cells in peripheral blood, lymph node, and bone marrow. Flow cytometric plots are shown from a representative SIV-infected animal at baseline and at 2–5 days after the last infusion of the CD8-depleting Ab. The lymph node sample was obtained 2 days after the last infusion, and bone marrow and peripheral blood samples were obtained 5 days after the last infusion. Samples were first gated on lymphocytes by side scatter vs forward scatter gating and then gated on $CD3^+$ T cells. Flow cytometric plots show $CD4^+$ vs $CD8^+$ T cells.

in vivo, as well as on lymphocyte numbers, distributions, and immunophenotypes.

Although the levels of SIV-specific CD8 T cell responses were not determined before the initiation of the CD8 depletion protocol in this study, SIV-specific CD8 T cell responses were subsequently quantified following CD8 T cell reconstitution as part of a comprehensive survey of SIV-specific CD8 T cell responses in SIV-infected SMs residing at the Yerkes National Primate Research Center (Atlanta, GA) (14). Consistent with the other SMs studied, the SIV-infected SMs included in the current study also exhibited low or undetectable levels of SIV-specific CD8 T cell responses.

To accurately determine the level of $CD8^+$ T cell depletion achieved in animals treated with the depleting Ab OKT8F, it was first necessary to identify a second Ab whose binding is not blocked by prior OKT8F binding. Toward this end, we identified an anti-CD8 Ab that efficiently binds $CD8^+$ cells both before and after preincubation with OKT8F. We determined that preincubation with OKT8F at concentrations of up to 40 μg/ml did not interfere with the staining of the same cells with OKT8, which was then used throughout this study for the measurement of $CD8^+$ cells in various tissues (data not shown). As expected, incubation of the same cells with an isotype-matched Ab (OKT3) that does not recognize any SM

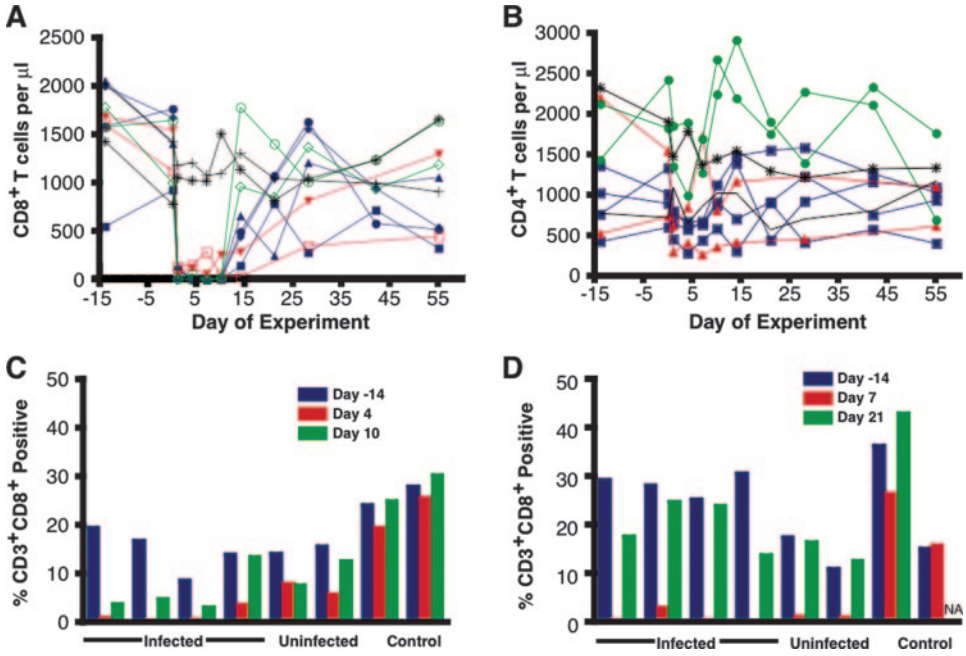

FIGURE 3. Graphs of effects of depletion on $CD3^+CD8^+$ and $CD3^+CD4^+$ T cells over time in peripheral blood and on $CD3^+CD8^+$ cells in lymph node and bone marrow. In *A* and *B* animals receiving control Ab OKT3 are designated with a black line, SIV-infected animals receiving depleting Ab OKT8F at 4 mg/kg/day are denoted by blue lines, SIV-infected animals receiving OKT8F at 1 mg/kg/day are denoted by a red lines, and uninfected animals are denoted by green lines. Symbols for individual animals are as follows: ■, FNg; ▲, FZo; ●, FLn; ♦, FCo; □, FVl; ▼, FDh; ◇, FQk; ○, FUr; ∗, FOo; and +, FWk. *A*, $CD3^+CD8^+$ T cells per microliter of peripheral blood were determined throughout the study. *B*, $CD3^+CD4^+$ T cells per microliter of peripheral blood were determined as in *A*. *C*, Percentage of $CD3^+CD8^+$ T cells in lymph node biopsies were determined by flow cytometric analysis at baseline (blue bar), at day 4, which is 2 days after the last infusion (red bar), and at day 10 (green bar). *D*, Percentage of $CD3^+CD8^+$ T cells in bone marrow aspirates were determined by flow cytometric analysis at baseline (blue bar), at day 7, which is 5 days after the last infusion (red bar), and at day 21 (green bar).

molecule did not interfere with the binding of OKT8. In contrast to the results obtained with OKT8, OKT8F inhibited and, at a concentration of 40 μg/ml, completely abrogated the binding of mAb clone SK3 (Leu 2a; BD Biosciences) to SM CD8$^+$ cells (data not shown). Interestingly, when similar ex vivo studies were performed using peripheral blood cells from RMs (data not shown) we also observed that preincubation with OKT8F blocks the staining of CD8$^+$ cells with SK3, even though SK3 has been used to assess the extent of CD8$^+$ cell depletion in some previously published studies of CD8$^+$ T cell depletion (24, 27).

OKT8F infusion is followed by CD8$^+$ cell depletion in peripheral blood, lymph node, and bone marrow

To determine the CD8$^+$ cell-depleting effect of the infusion of OKT8F or the control Ab OKT3 on the relative proportion of various lymphoid subsets in mononuclear cells isolated from the peripheral blood, lymph nodes and bone marrow, we performed a detailed sequential analysis of the immunophenotype of these cells in all treated animals. None of the animals receiving either the CD8-depleting or control mAbs showed any discernible side effects from the Ab infusion. Fig. 2 shows staining with anti-CD4 and anti-CD8 mAbs of peripheral blood-, lymph node-, and bone marrow-derived mononuclear cells from a representative SIV-infected SM treated with 4 mg/kg OKT8F for 3 days, at baseline, and at 2 days after the last Ab infusion for the lymph node sample and 5 days after the last Ab infusion for the bone marrow and peripheral blood samples. As shown in Fig. 3*A*, treatment with either the high dose (4 mg/kg $\times$ 3 days) or the low dose (1 mg/kg $\times$ 4 days) of OKT8F induced a dramatic decline in circulating CD8$^+$ T cells in all SMs within 24 h of the first infusion, with further decreases seen following two subsequent rounds of Ab infusion for the animals receiving the high-dose depletion regimen. Achievement of a real depletion of CD8$^+$ T cells in the OKT8F-treated animals, as opposed to a mere masking of the CD8 molecule, was also confirmed by the observation that at the time of the absence of measurable CD8$^+$ T cells the vast majority of the remaining CD3$^+$ T cells were CD4$^+$ (data not shown). As shown in Fig. 3*A*, the nadir of CD8 depletion in the high-dose animals was usually reached at day 7 of the study (5 days after the last infusion), when >99% of circulating CD8$^+$ T cells had been depleted. The animals receiving the lower dose of OKT8F for 4 days never achieved >97% depletion of CD8$^+$ T cells and rebounded quickly. Importantly, similar levels of CD8$^+$ T cell depletion were observed in the lymph node and bone marrow of both naturally and experimentally infected animals after receiving the high-dose depletion regimen, suggesting that a significant depletion of CD8$^+$ cells throughout the body had been achieved with the 4 mg/kg $\times$ 3 days experimental protocol (Fig. 3, *C* and *D*). The levels of depletion in the bone marrow of the animals receiving the lower-dose depletion regimen of 1 mg/kg OKT8F for 4 days were similar to the depletion levels seen in the blood and less complete (maximum of 80%) in the lymph nodes (data not shown). Interestingly, lymph node depletion of CD8$^+$ cells was substantially less complete in SIV-uninfected animals treated with the low dose of OKT8F in which the maximum percentage of depletion was 56 and 36.2% (Fig. 3*C*). Consistent with this incomplete level of CD8$^+$ cell depletion from the OKT8F-treated SIV-uninfected SMs, these mangabeys were the first to return to levels of circulating CD8$^+$ cells similar to those observed before the treatment (Fig. 3*A*). The levels of CD8 depletion in the bone marrow of the high-dose OKT8F-treated animals were similar to levels of depletion in the blood and, in contrast to the lymph node findings, no differences were seen in the extent of bone marrow CD8 depletion between infected and uninfected animals (Fig. 3*D*).

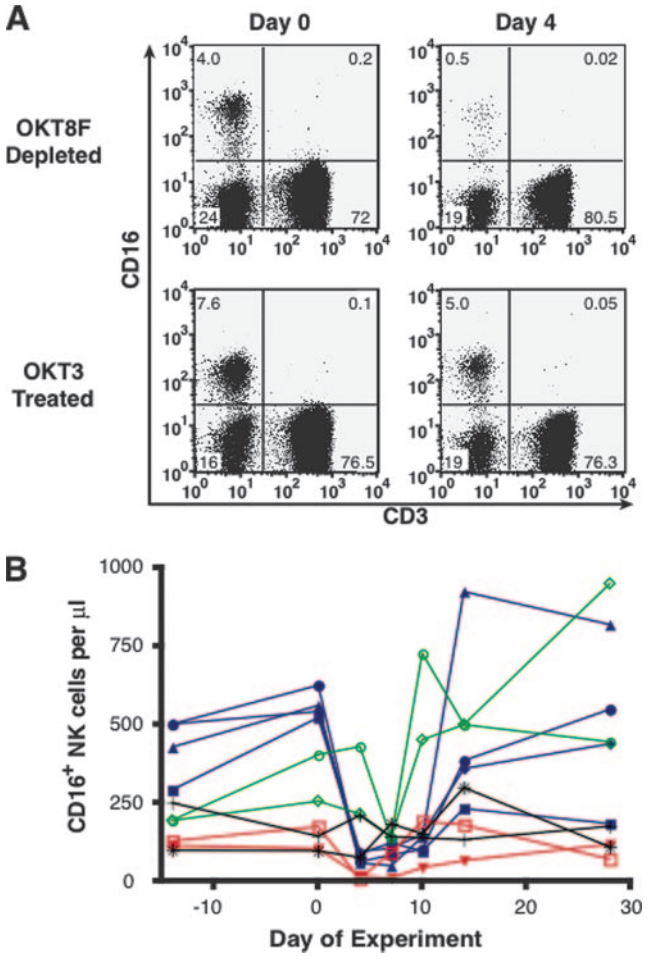

FIGURE 4. Depletion of CD8$^+$CD16$^+$ NK cells by OKT8F in SIV-infected SMs. Populations were determined by flow cytometric analysis. *A*, Representative OKT3- and OKT8F-treated animals are shown at baseline and at day 4, 2 days after the last Ab infusion. *B*, Longitudinal determinations of NK cells per microliter in peripheral blood. NK cells were determined by flow cytometric analysis by first gating on lymphocytes by side scatter vs forward scatter and then gating on CD8$^+$CD16$^+$ cells. SIV-infected animals receiving OKT8F at 4 mg/kg/day are denoted with blue lines. SIV-infected animals receiving OKT8F at 1 mg/kg/day are plotted with red lines. Uninfected animals receiving OKT8F at 4 mg/kg/day are represented by green lines. OKT3-treated (control) animals are denoted by black lines. Symbols for individual animals are as follows: ■, FNg; ▲, FZo; ●, FLn; ♦, FCo; □, FVl; ▼, FDh; ◇, FQk; ○, FUr; ∗, FOo; and +, FWk.

Peripheral CD4$^+$ T cell levels were also transiently affected by the infusion of either OKT8F or OKT3 Ab. Although the increased percentage of total lymphocytes staining for CD4 during depletion was clearly an expected result of the removal of CD8$^+$ cells, the absolute number of circulating CD4$^+$ T cells per milliliter in both SIV-infected and uninfected, high- and low-dose OKT8F-treated SMs showed a 22–49% decrease during the first 4 days of the study (Fig. 3*B*). Decreases in circulating CD4$^+$ T cells of up to 50% have been previously reported during OKT8F depletion of RMs (24, 27). The fact that this decrease in CD4$^+$ T cells is observed in both SIV-infected and uninfected SMs suggests that an anatomic redistribution of CD4$^+$ T cells rather than increased levels of viral replication may be responsible for this phenomenon. Furthermore, the observation that the decrease in CD4$^+$ T cells is also seen in animals treated with the nondepleting control Ab OKT3 indicates that this CD4$^+$ T cell decline is not caused by specific binding of the depleting OKT8F Ab to CD4$^+$ T cells.

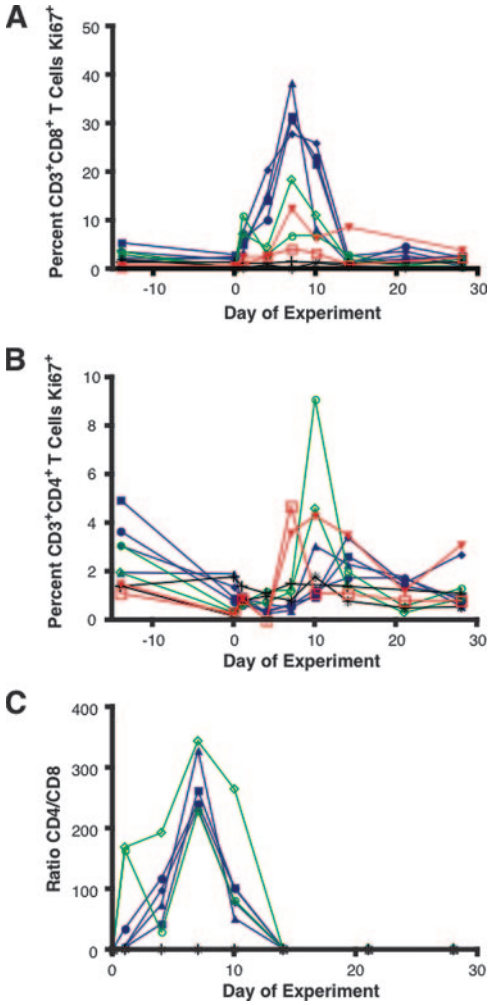

FIGURE 5. Homeostatic proliferation of T cell subsets in peripheral blood. SIV-infected control animals receiving OKT3 are denoted with a black line. SIV-infected animals receiving OKT8F at 4 mg/kg/day are denoted with a blue line, SIV-infected animals receiving OKT8F at 1 mg/kg/day with a red line, and uninfected OKT8F-depleted (4 mg/kg/day) animals with a green line. Symbols for individual animals are as follows: ■, FNg; ▲, FZo; ●, FLn; ♦, FCo; □, FVl; ▼, FDh; ◇, FQk; ○, FUr; ∗, FOo; and +, FWk. Proliferation of T cells was determined in all samples by Ki67 staining throughout the study in peripheral blood. *A*, Proliferation of $CD3^+CD8^+$ T cells. *B*, Proliferation of $CD3^+CD4^+$ T cells. *C*, The ratio of absolute $CD3^+CD4^+$ to $CD3^+CD8^+$ T cells per microliter in peripheral blood of animals receiving either the control Ab (OKT3) or OKT8F at 4 mg/kg/day (depleting Ab) is shown throughout the first month of the study.

As expected, $CD16^+$ NK cells expressing the CD8α molecule were also depleted following OKT8F infusion. High-dose OKT8F-treated SMs lost an average of 85.8 ± 3.2% (range 82%–89%) of $CD16^+$ cells, whereas animals receiving OKT3 did not experience any significant loss of NK cells (Fig. 4). The two animals receiving the low-dose depletion regimen for 4 days lost 91 and 94% of $CD8^+CD16^+$ NK cells. The greater degree of depletion of $CD16^+$ NK cells observed in the low-dose OKT8F-depleted animals, as compared with high-dose depleted animals, may be the result of the longer period of treatment (4 vs 3 days of infusions) used in the low-dose depleted animals. The less complete depletion of $CD3^-CD8^+$ NK cells than of $CD3^+CD8^+$ T cells, regardless of depletion regimen, suggests that the lower level of CD8 expression observed on this cell population may confer some resistance to the depleting Ab treatment (data not shown). Recent data from our group (our unpublished observations) has shown that a low level of

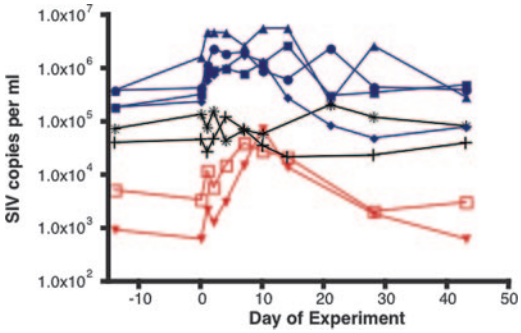

FIGURE 6. Viral load analysis of all infected animals. Sooty mangabeys that received the control Ab, OKT3, are designated with a black line, SIV-infected animals receiving the depleting Ab OKT8F at 4 mg/kg/day are denoted by a blue line, and SIV-infected animals receiving OKT8F at 1 mg/kg/day are denoted by a red line. Symbols for individual animals are as follows: ■, FNg; ▲, FZo; ●, FLn; ♦, FCo; □, FVl; ▼, FDh; ◇, FQk; ○, FUr; ∗, FOo; and +, FWk. Plasma viral RNA copy number was determined by a real-time RNA PCR assay.

CD8α is expressed at low levels on SM dendritic cells of both plasmacytoid and myeloid lineage. Therefore, although the levels of dendritic cells were not determined in our study, it is possible that dendritic cells were also partially depleted in our experimental system. In all, this set of data indicates that OKT8F treatment (but not OKT3 treatment) was able to specifically deplete $CD8^+$ cells of both T and NK lineage with a more significant magnitude and duration of the depletion in SIV-infected SMs as compared with the uninfected SMs. Treating animals with the higher dose of OKT8F for fewer days resulted in a more complete depletion of $CD8^+$ cells in blood, lymph node, and bone marrow.

Homeostatic proliferation of $CD8^+$, but not $CD4^+$, T lymphocytes is observed early following $CD8^+$ cell depletion

The rapid repopulation of the $CD8^+$ T cell pool in $CD8^+$ cell depleted SMs, both SIV-infected and uninfected, suggests that a burst of homeostatic proliferation of T cells promptly follows the OKT8F-induced $CD8^+$ cell depletion. To study the kinetics of $CD8^+$ T cell repopulation, we performed a sequential analysis of the immunophenotype of proliferating T cells in the peripheral blood and lymphoid tissues of SMs treated with OKT8F or OKT3. Proliferating cells were identified as expressing the Ki67 nuclear proliferation-associated Ag, a widely used technique that has been shown to yield results similar to those obtained using techniques based on the direct labeling of proliferating cells, such as BrdU or [^{2}H]glucose labeling (44, 45).

A marked but transient increase in the fraction of $CD8^+Ki67^+$ T cells was observed in all OKT8F-treated SMs (but not in OKT3-treated SMs), regardless of infection status or depletion regimen (Fig. 5*A*). The animals receiving the higher dosage of OKT8F (and achieving more complete $CD8^+$ T cell depletion) exhibited higher levels of $CD8^+$ T cell proliferation during the recovery phase. The percentage of proliferating $CD8^+$ T cells in the peripheral blood peaked at day 7, i.e., 4 days after the last infusion and at the time of the nadir of $CD8^+$ T cells. As would be expected for a homeostatic-driven proliferation in response to marked contraction of the $CD8^+$ T cells population size, the percentage of $CD8^+Ki67^+$ T cells returned to baseline levels after the repopulation of $CD8^+$ T cell compartment was complete. A small but consistent increase in the level of $CD4^+$ T cell proliferation was observed between day 10 and day 14 in all animals regardless of treatment (Fig. 5*B*), with the largest increase seen in the uninfected and low dose-depleted

Table I. *Description of all study animals detailing study regimen, CMV status, and viral load characteristics*

Animal Name	Depletion Status	Depletion Dose	CMV Seropositivity	CMV Reactivation	Day 0 Viral Load	Fold Increase in SIV Viral Load
FNg	Yes	4 mg/kg	Positive	No	3.3×10^5	8.3
FZo	Yes	4 mg/kg	Positive	Yes	1.7×10^6	3.5
FLn	Yes	4 mg/kg	Negative	No	4.4×10^5	5.5
FCo	Yes	4 mg/kg	Positive	No	2.4×10^5	7.5
FVl	Yes	2 mg/kg	Positive	No	3.5×10^3	11.5
FDh	Yes	2 mg/kg	Positive	Yes	6.4×10^2	116
FWk	No	2 mg/kg	Positive	No	4.7×10^4	2.6
FOo	No	4 mg/kg	Positive	No	1.4×10^5	1.5

infected animals. As shown in Fig. 5*C*, by day 14 after the depletion the CD4:CD8 ratio has returned to the same level observed at baseline, indicating that the homeostatic stimulus for CD8$^+$ T cell proliferation does not induce an "over-proliferation" of CD8$^+$ T cells.

CD8$^+$ cell depletion is followed by minor increases in plasma viral load in SIV-infected SMs

Depletion of CD8$^+$ T and NK cells is associated with a marked increase in the level of virus replication in acute and chronic SIV infection of RMs (21, 24–27). In a similar study in RMs during chronic SIV infection, viral replication increased 20- to 2500-fold during the period of CD8 depletion (24). To determine the role played by CD8$^+$ cells in determining the level of prevailing viral replication in SIV-infected SMs, we sequentially measured plasma viremia by using a real-time RT-PCR method (6) before and at sequential times after treatment with the depleting or control Abs. As shown in Fig. 6 (blue lines), the SIV-infected SMs ($n = 4$) that had been treated with 4 mg/kg of OKT8F for 3 days and had the greatest degree of CD8$^+$ cell depletion exhibited a significant yet modest and transient increase in viral load, with a magnitude ranging from 3.5- to 8.3-fold and an average increase of 6-fold. Log_{10} viral load in the two study groups (CD8 depleted and controls) changed in significantly different ways over time ($p = 0.005$; test for the interaction between time on study and Ab treatment group). Mean $\log_{10}$ viral load was similar in both Ab-treated groups on day 0, but there was a linear increase between day 0 and day 10 in the CD8 depleted group ($p < 0.001$). The mean changes in $\log_{10}$ viral load from day 0 to days 1, 2, 4, 7, and 10 were 0.47, 0.45, 0.59, 0.66, and 0.86 (SE of 0.18). No change in mean $\log_{10}$ viral load was identified in the two animals not treated with OKT8F.

A small increase in viral load (between 1.5- and 2.6-fold) was observed in animals receiving the control mAb OKT3 (Fig. 6, black lines), thus further emphasizing the limited magnitude of the changes observed after CD8$^+$ cell depletion in SIV-infected SMs. As also shown in Fig. 6, the two SMs treated with an alternative regimen of four doses of 1 mg/kg OKT8F (red lines) who experienced less complete CD8$^+$ cell depletion also coincidentally had lower levels of baseline SIV viremia than is typically seen in naturally infected SMs (Fig. 1). Among this group, one OKT8F-treated animal displayed a transient 11.5-fold increase in plasma SIV RNA levels, whereas another SM exhibited the greatest increase in viremia (116-fold). Interestingly, this animal (FDh) had the lowest baseline SIV viral load of <1000 copies/ml (see Table I).

Increases in SIV replication in CD8-depleted SMs are associated with increased CD4$^+$ T cell activation and/or CMV reactivation

We sought to understand why one of the six SIV-infected SMs displayed a significant increase in SIV viremia while the others had variable but substantially lower changes following CD8 depletion. The fact that an increase of viremia in CD8$^+$ cell depleted animals, when seen, peaked at some time after the nadir of CD8$^+$ cells was reached suggests than mechanisms other than the direct effects of loss of SIV Ag-specific CD8$^+$ T cells might be involved in the modest (<1 log) increase of viremia observed in most OKT8F-treated, SIV-infected SMs. To determine whether there is

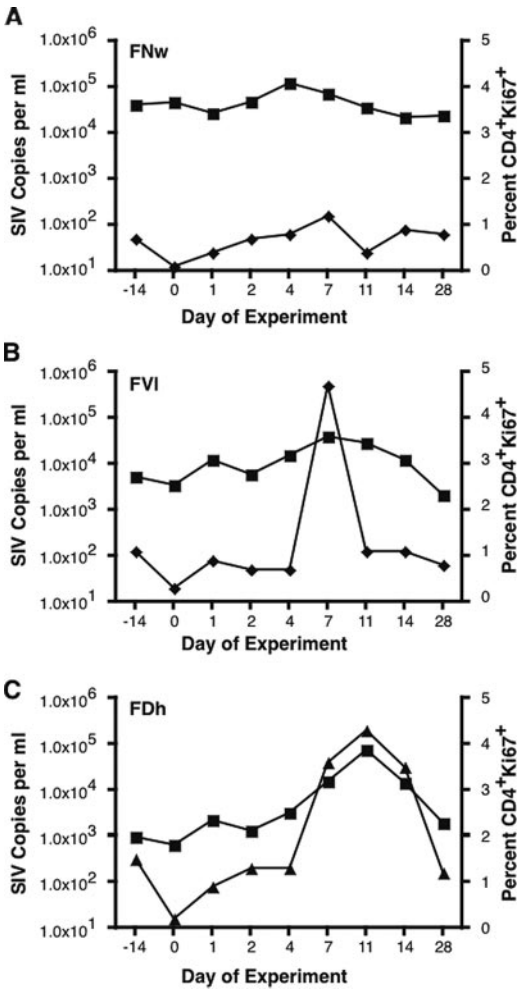

FIGURE 7. Temporal correlation of CD3$^+$CD4$^+$ T cell proliferation with increases in viral load. One control mAb treated animal (FWk) (*A*) and two experimentally depleted animals (*B* and *C*) are shown. Viral load is denoted by the squares (■). The percentage of proliferating CD3$^+$CD4$^+$ T cells, as measured by Ki67 staining, is denoted by the diamond points (◆). The animal shown in *B* (FVl) is typical of CD8-depleted SMs. In *C* the animal FDh is atypical, exhibiting low starting SIV viremia and prolonged CD4$^+$ T cell proliferation (possibly due to CMV reactivation.).

a relationship between the level of $CD4^+Ki67^+$ T cells and viral load, we assessed the linear regression equation for these two parameters in the six treated animals during the first 10 days of treatment (for a total of 18 observations). This analysis revealed a statistically significant direct correlation between increases in $\log_{10}$ viral load and increases in the $\log_{10}$ of $CD4^+Ki67^+$ ($p = 0.013$) (data not shown).

Three distinct profiles of the relationship between viral load and $CD4^+$ T cell proliferation are shown in Fig. 7. Animals receiving the control Ab OKT3 (Fig. 7*A*) show minimal fluctuations in viral load and $CD4^+$ T cell proliferation, whereas most CD8-depleted SMs exhibit modest changes in viral load coincident with a transient increase in levels of $CD4^+$ T cell proliferation (assessed by Ki67 expression) (Fig. 7*B*). Finally, Fig. 7*C* shows that a prolonged period of $CD4^+$ T cell proliferation is observed at the time of a marked increase in SIV viral load in the animal designated FDh. Increased levels of SIV viremia and $CD4^+$ T cell proliferation observed in this animal also coincided with a reactivation of latent CMV infection as determined by quantitative CMV DNA real-time PCR after $CD8^+$ cell depletion (Table I). The combination of an atypically low starting set point SIV viral load and the increased number of activated $CD4^+$ T cells (quite possibly generated by CMV reactivation) that can function as targets for SIV replication may underlie the larger fold increase (but lower absolute peak viral load) of plasma viremia observed after CD8 depletion in this animal. In all, these results indicate that $CD8^+$ cells (which include both $CD8^+$ CTL and $CD8^+$ NK cells) do not play a major role in determining the level of set point viremia in naturally SIV-infected SMs.

Discussion

The primary objective of this study was to explore, via targeted Ab-mediated depletion, the role that $CD8^+$ T cells and $CD8^+$ NK cells play in determining the set point level of SIV replication and protection from immunodeficiency disease in SIV-infected SMs. Depletion of $CD8^+$ lymphocytes in both SIV-infected and uninfected SMs also provided the opportunity to gain insights into primate lymphocyte homeostasis, including the anatomic location, phenotype, and kinetics of repopulating cells and the integrity of these homeostatic mechanisms in infected vs uninfected animals. Additional study objectives included evaluation of the possible confounding effects of the Ab intervention itself, including the potential effects of the infusion of a foreign immunogenic protein (the antibody) and the effects of the resulting Ab-mediated depletion of $CD8^+$ cells on other relevant lymphocyte populations as well as on the immune control of latent infections extant in treated SMs. Given their important role in serving as targets for HIV/SIV replication (18, 46), we were particularly interested in treatment effects on the frequency, activation state, and proliferation status of $CD4^+$ T cells.

To detect and quantitate the true extent of $CD8^+$ lymphocyte depletion achieved by the mAb treatment, we first validated that the detection Ab used for our studies was not masked by the depleting Ab. To ascertain the extent of lymphocyte depletion in important anatomic sites for SIV replication and lymphocyte generation, we sampled lymph node and bone marrow compartments in addition to peripheral blood. Finally, to better characterize the extent of lymphocyte depletion and repopulation, as well as the lymphocyte activation state following Ab treatment, we performed extensive multiparameter immunophenotyping of multiple lymphocyte populations at several time points during the treatment and cellular recovery phases.

The murine mAb we used in this study was employed to effectively deplete $CD8^+$ cells in previous studies of the effects of $CD8^+$ T cells on SIV replication in rhesus macaques (24, 27). As with all other CD8 depletion studies published to date, the Ab depletes $CD8^+$ T cells, NK cells, and possibly dendritic cells due to the fact that these cell types express CD8α. However, previous studies using either murine or humanized anti-CD8α Abs reported the effects of Ab treatment only on $CD8^+$ T cells, with only one study (27) acknowledging (but ascribing to nonspecific effects of depletion) an effect on the NK cell, an important potential antiviral effector cell. Because nonhuman primates used in AIDS pathogenesis studies commonly harbor a number of latent virus infections (e.g., CMV, rhadinovirus (EBV-like), and other HSV-like viruses (33, 34)) whose replication is known to be controlled by $CD8^+$ T cells and NK cells, we reasoned that transient depletion was preferable to prolonged depletion so as to minimize confounding effects of herpesvirus reactivation on lymphocyte activation and SIV replication (known to be linked to the state of activation of $CD4^+$ T cells). We therefore used the murine anti-CD8α mAb OKT8F, which is expected to have a shorter half-life in primates and consequently a shorter duration of depletion than the humanized mAb cM-T807 that results in prolonged $CD8^+$ cell depletion in treated animals (43). To determine the potentially immune-activating effects of infusion of a foreign protein itself (e.g., a murine Ab) on lymphocyte activation, particularly the activation of $CD4^+$ T target cells, we carefully monitored the effects of isotype control Ab on these parameters.

In the current study we achieved rapid and effective depletion (97–99%) of $CD8^+$ T cells. Interestingly, the depletion of $CD8^+$ T cells in the lymph nodes was more efficient in SIV-infected than uninfected animals, suggesting higher baseline levels of $CD8^+$ T cell activation in the infected SMs (6). In addition to efficient depletion of $CD8^+$ T cells, NK cell levels were markedly reduced (by 86%) by day 4 of treatment. Other cell populations were not significantly affected by the CD8 cell-depleting Ab or the isotype control Ab. A transient diminution of the peripheral $CD4^+$ T cell counts was observed in both depleted and isotype control-treated animals, suggesting that a temporary redistribution of $CD4^+$ T cells occurs in response to the mAb infusion. Overall, we observed rapid and significant depletion not only of $CD8^+$ T cells but also of NK cells in the blood, lymph nodes, and bone marrow, with only limited effects on other cell populations.

Previous studies of $CD8^+$ cell depletion in SIV-infected macaques observed rapid increases in the level of SIV replication in the range of 20- to 10,000-fold coincident with the loss of $CD8^+$ cells (21, 24–27). As the kinetics of viral replication in SIV-infected SMs (Refs. 6 and 7 and our unpublished results) are similar to those of SIV-infected macaques during both acute and chronic infection (4, 30–32), it was expected that substantial increases in virus replication would promptly follow the depletion of $CD8^+$ cells if either $CD8^+$ T cells or NK cells played a major role in the recognition and clearance of SIV-infected cells in SMs. Although profound $CD8^+$ cell depletion was achieved in our study, the observed increases in plasma viral load were much more limited (albeit statistically significant when compared with baseline levels of viral load) than those reported following CD8α cell depletion in SIV-infected RMs ranging from 3.5 to 11.5 in seven of eight SMs, with a single treated SM exhibiting a 116-fold increase in viremia (3.5- to 116-fold; overall median of 8-fold). For comparison, the control mAb-treated SMs exhibited ~2- to 3-fold increases in viremia.

Because activated and proliferating $CD4^+$ T cells serve as important targets for SIV replication (47), we carefully monitored the effects of control mAb infusion and mAb-mediated $CD8^+$ lymphocyte depletion on $CD4^+$ T cell proliferation. As expected, $CD8^+$ T cells clearly exhibited the most dramatic proliferation in

response to CD8 depletion. However, modest but detectable blood CD4 proliferation was also observed at days 7–14 in all SIV-infected and uninfected CD8 depleted SMs, but not in control mAb-treated SMs. The presence of CD4$^+$ T cell proliferation in SIV-negative, CD8-depleted SMs indicates that SIV replication is not required for CD4$^+$ T cell proliferation to occur; rather it appears to be an indirect effect arising from the CD8$^+$ cell depletion protocol. The fact that increased CD4$^+$ T cell proliferation was not observed in isotype control-treated SMs indicates that the infusion of the foreign protein Ag itself did not provide a significant stimulus for CD4$^+$ T cell activation. Instead, increased CD4$^+$ T cell proliferation may have been induced by a proinflammatory signal related to the loss of large numbers of CD8$^+$ cells in lymphoid tissues. It has recently shown that, in both SIV-infected and uninfected RMs, Ab-mediated depletion of CD8$^+$ cells results in a marked increase in the level of proliferation of circulating CD4$^+$CCR5$^+$ effector memory T cells (L. Picker, personal communication).

CD8$^+$ T cells and NK cells are known to play a key role in the control of many viruses, including herpesviruses (29, 34, 39–42). In previous studies we observed CMV reactivation in nonhuman primates following an in vivo blockade of CD28 and CD40 T cell costimulation pathways (our unpublished observations), whereas others have observed EBV reactivation following mAb-mediated manipulation of T cell function in humans (48). SMs are often seropositive for simian CMV Abs and can harbor other herpesviruses including alpha-herpesviruses (33). We therefore wanted to analyze herpesvirus reactivation following CD8$^+$ cell depletion in this species. However, the lack of mangabey-specific sequence information for the diverse herpesviruses extant in SM populations made it difficult to perform an exhaustive survey of possible herpesvirus reactivation. Nonetheless, probing for evidence of CMV reactivation by using PCR primers designed for rhesus macaque CMV sequences demonstrated clear increases in plasma CMV DNA levels in two of seven CD8-depleted CMV-seropositive SMs, suggesting that, similar to observations in other species (29, 35–42), such depletion can be associated with herpesvirus reactivation in mangabeys. The T cell response to CMV typically involves a large percentage of memory CD4$^+$ T cells, and CMV reactivation has been associated with an expansion of CMV-specific CD4$^+$ T cells (49). Therefore, CMV reactivation in CD8-depleted animals would be expected to be associated with the expansion of activated CMV-specific CD4 T cells serving as targets for SIV infection. Likewise, the reactivation of other latent viruses (such as other herpesviruses or other retroviruses (like STLV-I) known to be prevalent in SMs) that were not assayed for in the current study would also be expected to result in increased levels of CD4$^+$ T cell activation. The link between in vivo CD4$^+$ T cell activation and increased HIV replication has been well documented in a number of settings, such as the effects of active TB and other coinfections (50–53) and of immunizations on HIV replication (54–56). We have previously found that exposure to a relatively modest antigenic stimulus (inactivated influenza vaccine) can give rise to significant transient viral load increases in HIV-infected people (up to 100-fold fold or more), with the largest increases often observed in individuals with the highest CD4 counts and the lowest baseline viral load (54). In the current study we observed the largest fold increases in SIV replication (12- and 116-fold) following CD8 depletion in the two SMs that had the lowest initial SIV set points ($\sim 10^3$ SIV RNA copies/ml). Interestingly, these two SMs also had the lowest baseline CD4$^+$ T cell proliferation (1–1.5% Ki67$^+$ CD4$^+$ T cells) before CD8 depletion (perhaps a reflection of their atypically low baseline viremia). Following CD8 cell depletion, these two animals manifested the most rapid and pronounced CD4 T cell proliferation among all SIV-infected SMs, with Ki67$^+$CD4$^+$ T cells increasing to 4–5% by 1 wk after the first Ab infusion. In these animals, SIV replication increases temporally coincided with increased CD4$^+$ T cell proliferation. The SM with the largest (2-log) increase in SIV RNA levels also displayed the most prolonged NK cell depletion and clear evidence of CMV reactivation. Although it is possible that CD8$^+$ cells contributed to the atypically low set point viremia in this animal, the correlation between the timing and magnitude of increased levels of CD4$^+$ T cell proliferation and SIV replication suggests that treatment-induced changes in CD4$^+$ T cell target availability can provide an equally plausible explanation for increased SIV replication, particularly when the levels of activated CD4$^+$ T cell targets for virus replication were also unusually low before study initiation. These studies demonstrate that changes in activated CD4$^+$ target cell availability and the effects on resident infections (e.g., herpesviruses) other than SIV must be carefully monitored in studies using the depletion of CD8$^+$ T cell populations to probe the mechanisms of host immune control of CD4$^+$ T cell-tropic viruses like SIV.

An additional notable finding of this study was that, in both infected and uninfected SMs, the depletion of CD8$^+$ T cells was followed by a rapid activation of homeostatic mechanisms enabling the prompt reconstitution of baseline levels of CD8$^+$ T cells. CD8$^+$ T cell recovery occurred promptly following a nadir at day 7 and returned to baseline levels by day 28 after study initiation (and day 26 following the last mAb infusion). The reestablishment of the baseline CD8$^+$ T cell pool was obtained through a marked increase in the level of CD8$^+$ T cell proliferation (tracked by expression of the Ki67 proliferation-associated marker) that was first evident on day 4 following study initiation and was particularly pronounced in bone marrow samples. Consistent with the possibility that this increased CD8$^+$ T cell proliferation is homeostasis driven, the attainment of CD8$^+$ T cell numbers similar to the baseline level is followed by a rapid decline of the fraction of proliferating CD8$^+$ T cells, with no overabundance of CD8$^+$ T cells observed. The reconstitution of the CD8$^+$ T cell pool was slightly faster in uninfected than in SIV-infected SMs (average time of 14 days for uninfected and 26 days for infected), a finding that could be explained by the fact that, in uninfected animals, a larger pool of CD8$^+$ T cells remained in the lymph nodes following OKT8F treatment. However, the main features of CD8$^+$ T cell homeostasis (i.e., rate of proliferation and percentage of repopulation) were similar between SIV-infected and uninfected animals, providing further evidence for the integrity of the SM immune system function in the setting of SIV infection. NK cell levels also rebounded promptly following a nadir at day 4, returning to normal levels by day 14. Overall, in our experimental system the homeostasis of CD8$^+$ T cells appeared to be, by and large, lineage specific and thus regulated separately from that of CD4$^+$ T cells. The finding of a lineage-specific proliferation of CD8$^+$ T cells after Ab-mediated CD8$^+$ T cell depletion argues against the presence of a "blind" (i.e., incapable of distinguishing between CD4$^+$ and CD8$^+$ T cells) homeostasis of T cells in primates and mice (57–59). However, the fact that the homeostasis of CD4$^+$ and CD8$^+$ T cells is regulated separately in primates will need to be confirmed in future studies in which CD4$^+$ T cells are depleted with anti-CD4 Abs and the reconstitution of the CD4$^+$ T cell pool is followed over time.

Additional studies will be required to determine to what extent the CD4$^+$ T cell proliferation observed in the CD8-depleted SMs is due to an immune response to herpesvirus reactivation (29), a response to the partially lymphopenic state (60), or both. Similarly, the mechanisms by which rare SMs maintain low set point levels of SIV replication and the extent to which host CD8$^+$ T cell or NK

cell responses are responsible for restriction virus replication in these animals are still to be determined. In any case, the results of the current study highlight the often unappreciated complexity of CD8 depletion studies performed in nonhuman primates that are, unlike the mice commonly used in other experimental immunologic analyses, neither free of other defined infectious agents or housed in especially clean environments. With a virus such as SIV, the replication of which is closely associated with levels of CD4 activation, the depletion of $CD8^+$ cells (including $CD8^+$ T cells, NK cells, and dendritic cells) results in a multitude of direct and indirect effects that, contrary to earlier assumptions, make it difficult to ascribe changes in viral load to a single identifiable factor.

Overall, our observations indicate that $CD8^+$ T and NK cells do not play a major role in determining set point viremia in SIV-infected mangabeys. Rather, set point levels of virus replication in SMs appear to depend on target cell availability rather than the levels of adaptive ($CD8^+$ T cell) or innate (NK) immune effectors. The lack of a major impact of $CD8^+$ cell depletion on SIV replication in SMs supports the accumulating evidence that an attenuation of potentially proinflammatory immune responses rather than an active and effective SIV-specific cellular immune response is the primary determinant of the preservation of immune function in natural host species for SIV infection (6). Supporting data suggesting a subdued SM cellular immune response to SIV include the consistent observation of high viremia in the absence of disease, the lack of significant $CD8^+$ T cell proliferation and activation during chronic SIV infections (4–6), an observed low frequency of SIV-Ag specific precursors (61), and levels of SIV-specific effector $CD8^+$ T cells that are substantially lower than those seen in HIV-infected humans (15, 16). Our recent studies seeking to define when in the course of infection SMs achieve a limited $CD8^+$ T cell response to SIV have demonstrated that $CD8^+$ T cell activation and proliferation are also attenuated during primary SIV infection in SMs relative to the high level and persisting $CD8^+$ T cell activation and proliferation seen in RMs infected at the same time with an identical virus inoculum (7). Given that $CD8^+$ T cell proliferation during acute SIV infection of RMs is highly correlated with the induction of adaptive antiviral $CD8^+$ T cell responses and the extent of postpeak diminution in viremia (62, 63), the more limited and transient $CD8^+$ T cell proliferation in SIV-infected SMs supports the suggestion that they fail to mount significant SIV-specific $CD8^+$ T cell responses during primary infection and that an attenuated antiviral response persists throughout the chronic infection period. In more recent studies, we have found that the divergence in early host responses to SIV infection between SMs and RMs extends to include innate immune responses, with markedly attenuated dendritic cell and NK cell activation responses seen during acute infection of SMs (our unpublished observations). Overall, the absence of chronic proinflammatory host innate and adaptive immune anti-SIV immune responses may protect natural hosts from the bystander damage that represents a central feature in pathogenic SIV and HIV infections. Thus, the preservation of health in SIV-infected SMs may be the manifestation of less intense host innate and adaptive immune responses rather than more.

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Disclosures

The authors have no financial conflict of interest.

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