Resting naïve CD4$^+$ T cells are massively infected and eliminated by X4-tropic simian–human immunodeficiency viruses in macaques

Yoshiaki Nishimura*, Charles R. Brown*, Joseph J. Mattapallil†, Tatsuhiko Igarashi*, Alicia Buckler-White*, Bernard A. P. Lafont*, Vanessa M. Hirsch*, Mario Roederer†, and Malcolm A. Martin*‡

*Laboratory of Molecular Microbiology and †Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

Contributed by Malcolm A. Martin, April 19, 2005

Unlike HIV-1 and simian immunodeficiency virus (SIV), which induce a slow, unrelenting loss of immune function spanning several years, highly pathogenic simian–human immunodeficiency viruses (SHIVs) induce a rapid, complete, and irreversible depletion of CD4$^+$ T lymphocytes in rhesus monkeys within weeks of infection, leading to death from immunodeficiency. We recently reported that, because these SHIVs exclusively use the CXCR4 coreceptor for cell entry, they target naïve CD4$^+$ T cells for depletion in infected monkeys, whereas SIVs, which use CCR5, not CXCR4, cause the selective loss of memory CD4$^+$ T lymphocytes *in vivo*. Here we show both by DNA PCR analyses and infectivity assays, using live sorted CD4$^+$ T lymphocyte subsets, that 30–90% of circulating naïve cells were productively infected by day 10 after inoculation. This result implies that direct cell killing, not bystander apoptosis, is responsible for the massive loss of CD4$^+$ T cells in the X4-tropic SHIV model. Furthermore, we directly demonstrate that >96% of virus producing cells did not express the Ki-67 proliferation marker on day 10 after inoculation using confocal microscopic analysis of lymph nodes samples. This finding is consistent with the prodigious levels of plasma viremia measured during acute X4-tropic SHIV infections of macaques being generated almost entirely by resting naïve CD4$^+$ T cells.

acute infection | animal model | CXCR4-tropic SHIV | HIV pathogenesis

During the past 4–5 years, highly pathogenic simian–human immunodeficiency viruses (SHIVs), which encode the HIV-1 envelope glycoprotein, have been extensively used as challenge viruses in vaccine experiments carried out in rhesus macaques. These SHIVs cause rapid and complete losses of CD4$^+$ T lymphocytes during the first 3–6 weeks of inoculation and death from immunodeficiency within 12–25 weeks of virus infection (1, 2). Nonetheless, despite their extremely aggressive and virulent pathogenic phenotype, SHIVs have proven to be relatively easy to control by a variety of vaccination regimens, most of which fail to protect monkeys from challenges with pathogenic strains of SIV (3, 4). In virtually all reported studies, vaccination and challenge of macaques with pathogenic SHIVs resulted in no or minimal losses of CD4$^+$ T cells, prompt and durable control of postpeak plasma viremia, and protection from disease development (5–9).

To investigate why the results of SHIV and simian immunodeficiency virus (SIV) vaccine experiments are so discordant, we and others initially examined chemokine coreceptor utilization properties of each virus during *ex vivo* infections of rhesus monkey peripheral blood mononuclear cells (PBMCs) and found that highly pathogenic SHIVs exclusively use the CXCR4 and SIVs use the CCR5 chemokine coreceptors during *ex vivo* infections of rhesus monkey PBMC (10–12). We also recently conducted side-by-side comparisons of SIV and SHIVs *in vivo* and found that each targets different CD4$^+$ T cell subsets for depletion in infected animals (11). Because of the differential expression of chemokine receptors on distinct CD4$^+$ T lymphocyte subsets, we observed that X4-tropic SHIVs caused the rapid elimination of both naïve and memory cells, whereas R5-tropic SIV induced the selective loss of only memory cells in the blood and lymphoid tissues of infected macaques. In the present study, we investigated whether the nonactivated naïve CD4$^+$ T cell subset, which were being completely and irreversibly depleted, were actually supporting the production of progeny SHIV *in vivo*. Sorted live CD4$^+$ T cell subsets in the peripheral blood, collected from rhesus monkeys on days 6 and 10 after X4-tropic SHIV inoculation, were examined by DNA PCR and *ex vivo* infectivity assays. The results obtained showed that an extraordinarily large fraction of circulating naïve cells were infected and released infectious SHIV. Furthermore, confocal microscopic analyses of lymph node samples directly demonstrated that resting naïve cells in lymphoid tissues were, in fact, the principal source of progeny SHIV production during the primary infection.

Methods

Virus and Animals. The origin and preparation of the tissue culture-derived $SHIV_{DH12R}$ stock have been described (1). Rhesus macaques (*Macaca mulatta*) were maintained in accordance with the guidelines of the Committee on Care and Use of Laboratory Animals (13) and were housed in a biosafety level 2 facility; biosafety level 3 practices were followed. Phlebotomies, i.v. virus inoculations [500–100,000 50% tissue culture infectious doses ($TCID_{50}$) of $SHIV_{DH12R}$], and euthanasias were performed as described (2, 14).

Plasma Viral RNA Quantitation. Viral RNA levels in plasma were determined by real-time RT-PCR (Prism 7700 Sequence Detection System, Applied Biosystems, Foster City, CA) as described (2).

Lymphocyte Immunophenotyping and Live Cell Sorting. EDTA-treated blood samples and lymphocytes isolated from mesenteric lymph nodes were stained for flow cytometric analysis as described (11) using combinations of the following fluorochrome-conjugated mAbs: CD3 [phycoerythrin (PE) or PE-Cy7], CD4 [PE, peridinin chlorophyll protein-Cy5.5 (PerCP–Cy5.5) or allophycocyanin (APC)], CD8 (PerCP or APC), CD28 (FITC or PE), CD95 (APC), CD69 (PE), HLA-DR (PE), and Ki-67 (FITC or PE). All antibodies were obtained from BD Biosciences Pharmingen and analyzed by four-color flow cytometry (FACSCalibur, BD Biosciences Immunocytometry Systems). Data analysis was performed by using CELLQUEST PRO (BD Biosciences) and FLOWJO (TreeStar, San Carlos, CA). For Ki-67 staining, cells were fixed with FACS Lysing Solution (Becton Dickinson), treated with FACS per-

Abbreviations: SHIV, simian–human immunodeficiency virus; SIV, simian immunodeficiency virus; PBMC, peripheral blood mononuclear cells; ISH, *in situ* hybridization.

‡To whom correspondence should be addressed. E-mail: malm@nih.gov.

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meabilization buffer (Becton Dickinson), and stained with Ki-67 mAb or a control isotype IgG1. Live cell sorting was performed by using a modified Becton Dickinson Digital Vantage SE in a BSL-3 laboratory. To analyze the SHIV viral DNA load in naïve ($CD3^+CD4^+CD95^{low}CD28^{high}$) or memory ($CD3^+CD4^+CD95^{high}CD28^{high}$ and $CD3^+CD4^+$-$CD95^{high}CD28^{low}$) $CD4^+$T cell populations, 5×10^5 to 1×10^6 cells were sorted into Eppendorf tubes. For quantitative virus isolation, live naïve or memory $CD4^+$T cells were sorted directly into 96-well plates at frequencies of 1, 3, 10, 30, 100, 300, 1,000, or 3,000 cells per well.

Cell-Associated DNA Levels in Naïve or Memory $CD4^+$ T Lymphocytes. Cell-associated viral DNA was measured by a quantitative PCR assay for SIV *gag* using an Applied Biosystems 7700 instrument as described (15) with SIV *gag* primers and probe as described by Lifson *et al.* (16). Briefly, T cells were lysed in proteinase K (100 μg/ml) (Boehringer, Indianapolis), and quantitative PCR was performed on 5-μl samples of the lysate for 45 cycles using Platinum Taq (Invitrogen) under conditions described in ref. 15. Cell numbers analyzed in each reaction was determined by simultaneous quantitative PCR of albumin genes. The rhesus macaque albumin primer and probe sequences were AlbF, TGCATGAGAAAACGCCAGTAA; AlbR, ATGGTCGCCT-GTTCACCAA; and AlbP, FAM-AGAAAGTCACCAAAT-GCTGCACGGAATC-QSY7. Plasmid standards were constructed for the absolute quantification of *gag* and albumin copy numbers and were validated with known numbers of FACS-sorted cells from a cell line containing a single integrated copy of SIV DNA (17). Duplicate reactions were run, and template copies were calculated by using ABI7700 software. The assay was sensitive and accurate down to a single copy of SIV *gag* DNA in a single FACS-sorted cell.

Quantitative Virus Isolation from Live Sorted Naïve or Memory $CD4^+$ T Lymphocytes. Live naïve or memory $CD4^+$ T cells were sorted directly into 96-well plates (three sets of quadruplicate dilutions for each sample) and immediately cocultivated with MT-4 cells (5×10^4 cells per well) for 4 weeks. Culture supernatants were assayed for reverse transcriptase (RT) activity to measure progeny virus production (18). The number of cells producing infectious $virus_{50}$ per 10^5 cells were calculated by the Reed and Muench method (19).

Immunohistochemistry. Formalin-fixed, paraffin-embedded tissues were stained for CD4, CD8, and Ki-67 as described (14). The samples were incubated in either CD4 (Novocastra) or CD8 (Novocastra) overnight at 4°C, and then goat-anti-mouse-IgG biotinylated secondary antibody (Vector Laboratories) and streptavidin–Alexa Fluor 633 (Invitrogen). After detection of CD4 or CD8, samples were then incubated with Ki-67 (DAKO A0047) and goat anti-rabbit-IgG-Alexa Fluor 488 (Invitrogen). The stained sections were then rinsed and coverslipped in Vectashield Hardset mounting media (Vector Laboratories) and photographed with a Leica confocal scanning microscope.

Combined *in Situ* Hybridization (ISH) and Immunohistochemistry. Formalin-fixed, paraffin-embedded tissues were stained for SHIV viral RNA by ISH as described with the addition of tyramide signal amplification (TSA Plus FITC, PerkinElmer, NEL741) (14, 20). After the ISH assay, the sections were incubated with a rabbit anti-human Ki-67 antibody (DAKO A0047), rinsed in Tris buffer, and then incubated with a goat anti-rabbit-IgG-Alexa Fluor 633 conjugated secondary antibody (Invitrogen). The stained sections were then rinsed, coverslipped in Vectashield Hardset mounting media (Vector Laboratories), and photographed with a Leica confocal scanning microscope. Negative ISH controls included (*i*) an antisense riboprobe with uninfected tissues; (*ii*) a sense riboprobe with infected tissues; and (*iii*) an antisense riboprobe with infected tissues without an incubation with SAD-HRP and anti-human Ki-67.

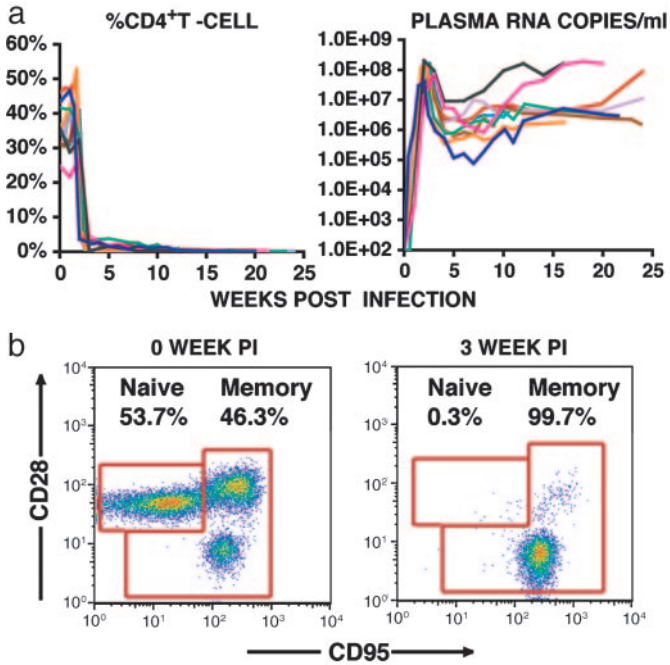

Fig. 1. Naive $CD4^+$ T cells are preferentially eliminated during the acute $SHIV_{DH12R}$ infection. (*a*) Peripheral blood $CD4^+$ T cell profiles and plasma viral RNA loads of 12 $SHIV_{DH12R}$-infected monkeys. Each animal was inoculated intravenously with >500 $TCID_{50}$ of the highly pathogenic $SHIV_{DH12R}$. Peripheral blood $CD4^+$ T cell percentages and plasma viral RNA levels were measured at the indicated times. $SHIV_{DH12R}$ preferentially targets the depletion of naïve $CD4^+$ T cells during the acute infection. (*b*) EDTA-treated samples of blood, collected at the indicated times from SHIV infected animals, were gated on $CD3^+CD4^+$ lymphocytes and analyzed for CD28/CD95, which distinguishes naïve and memory $CD4^+$ T cells (21). The percentage of total cells within each sector is indicated.

Results

Like other highly pathogenic SHIVs, $SHIV_{DH12R}$ induces rapid, systemic, and complete losses of $CD4^+$ T lymphocytes within weeks of inoculation, high and sustained levels ($>10^7$ copies per ml of plasma) of viral RNA, and death from immunodeficiency by 12–25 weeks after infection (14) (Fig. 1*a*). It has been reported that two highly pathogenic SHIVs ($SHIV_{DH12R}$ and $SHIV_{89.6P}$) exclusively use CXCR4 for infection of rhesus PBMC in *ex vivo* assays using small molecule competitors specific for CCR5 and CXCR4 (11, 12). We recently reported that, in contrast to SIV, both of these SHIV strains induce the elimination of naïve $CD4^+$ T cells (11) (the $CD95^{low}$ $CD28^{high}$ subset), which express CXCR4 but not CCR5. This is shown for a representative animal in Fig. 1*b*. The two SHIVs subsequently cause the depletion of $CD4^+$ memory T cell subsets, which express lower levels of CXCR4 (11). Thus, virtually all of the $CD4^+$ T lymphocytes are eliminated during the first 4–5 weeks in macaques inoculated with highly pathogenic X4-tropic SHIVs.

Naïve CD4 T Cells Are Productively Infected and Eliminated as a Result of Virus Infection. To ascertain whether the naïve $CD4^+$ T cells that were rapidly and irreversibly eliminated during acute SHIV infections were also productively infected and, therefore, were the source of the prodigious viremia observed in infected macaques, live cell sorting of naïve or memory $CD4^+$ T cells was carried out to identify the T cell subset(s) generating SHIV progeny. Three rhesus monkeys were challenged with a large

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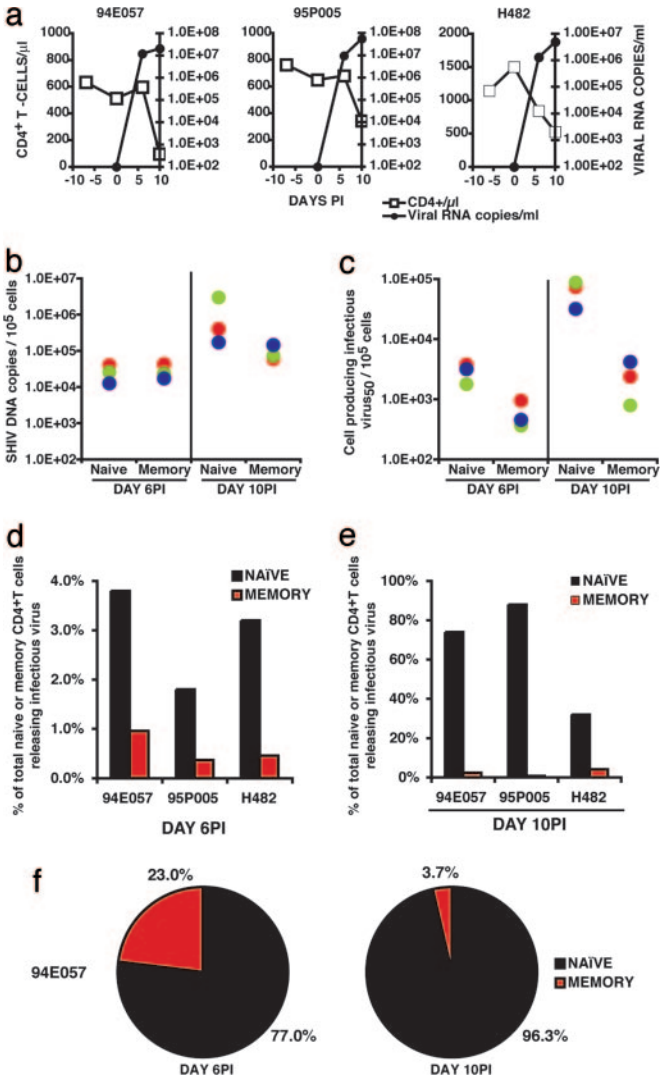

Fig. 2. A large fraction of circulating naïve CD4$^+$ T cells is productively infected with SHIV$_{DH12R}$ during the acute infection. (*a*) Three SHIV$_{DH12R}$-infected rhesus macaques experienced a rapid increase of plasma viremia and a marked but incomplete loss of peripheral CD4$^+$ T cells by 10 days after infection. (*b* and *c*) PBMC samples collected on days 6 and 10 after infection from rhesus monkeys 94E057 (red), 95P005 (green), and H482 (blue) were stained with a combination of anti-CD3, CD4, CD28, and CD95 mAbs to distinguish naïve or memory CD4$^+$ T cells. Live naïve or memory cells were sorted by FACS to determine the cell-associated viral DNA copies per 10^5 cells (*b*) or the frequencies of infectious virus production after cocultivation with MT4 cells, indicated as cell producing infectious virus$_{50}$ per 10^5 cells (*c*). The frequencies of total naïve or memory CD4$^+$ T cells releasing infectious virus were calculated based on the numbers of cells producing infectious virus$_{50}$ per 10^5 cells on days 6 (*d*) and 10 (*e*) after infection. (*f*) The source of infectious virus released from SHIV-infected CD4$^+$ cells from representative animal, RH94E057. The frequencies of productively infected naïve or memory cells in a total pool of infected cells on days 6 and 10 after infection are shown.

inoculum of SHIV$_{DH12R}$ and, as expected, experienced a rapid increase of plasma viremia, which on day 10 had reached levels of 5×10^6 to 5×10^7 viral RNA copies per ml; at this point in the acute infection, each of these macaques had already experienced a marked but incomplete loss of their peripheral blood CD4$^+$ T cells (Fig. 2*a*). PBMC collected from the infected monkeys on days 6 and 10 after inoculation were stained with a combination of anti-CD3, CD4, CD28, and CD95 mAbs to identify naïve or memory CD4 T cell subsets. Under these conditions, naïve CD4$^+$T cells exhibited a CD95^{low}CD28^{high} phenotype, whereas memory CD4 T cells were CD95^{high}CD28^{high} or CD95^{high}CD28^{low} in the CD3$^+$CD4$^+$ lymphocyte gate (21) (Fig. 5, which is published as supporting information on the PNAS web site). Live naïve and memory CD4$^+$ T cell subsets were sorted by FACS for cell-associated viral DNA determinations and the frequencies of infectious virus production after cocultivation with MT-4 cells.

Sorted circulating naïve and memory CD4$^+$ T cells, collected on days 6 and 10 after inoculation, were initially analyzed for the levels of SHIV *gag* DNA per 10^5 cells by quantitative DNA PCR. On day 6 after inoculation, 1.3 to 3.7×10^4 copies of *gag* DNA per 10^5 cells were amplified from both the naïve and memory CD4$^+$ T cells subsets collected from the three SHIV-infected monkeys (Fig. 2*b Left*). By day 10 after inoculation, the cell associated viral DNA loads had increased 20- to 100-fold in the naïve cells, reaching levels of 1.7×10^5 to 3×10^6 copies of SHIV DNA per 10^5 sorted naïve cells (Fig. 2*b Right*). This latter result suggested that an extremely large fraction of the naïve cells had been infected in all of the animals. On the other hand, only a modest increase in the frequency of infected memory CD4$^+$ T lymphocytes was observed on day 10.

Analyses of bulk DNA by PCR suffer from several shortcomings, including not being able to distinguish integrated from nonintegrated copies of viral DNA nor the frequencies of virus producing naïve and memory T cells in each CD4$^+$ T lymphocyte subset. In addition, because of the presence of replication defective copies of viral DNA in samples analyzed, it becomes difficult, if not impossible, to correlate the frequencies of intracellular viral DNA copies with the number of cells capable of releasing infectious virions. To circumvent these problems, decreasing numbers of live, sorted, naïve, or memory CD4$^+$ T cells from SHIV-infected monkeys were viably deposited by FACS into individual wells (3,000 cells to 1 cell per well) of a 96-well plate and immediately cocultivated with MT-4 T cells. Four weeks later, progeny virus production in the cocultures was monitored by the RT activity released into the medium and the frequencies of infected naïve and memory cells in replicate cultures were calculated by Poisson statistics as described by Reed and Muench (19). As shown in Fig. 2*c Left*, the frequency of sorted naïve cells producing infectious virus on day 6 after inoculation ranged from 1.6 to 3.8×10^3 cells per 10^5 cells; this corresponds to 1.6–3.8% of the total CD4$^+$ naïve T cells in peripheral blood at that time (Fig. 2*d*). The frequency of virus producing memory cells was significantly less on day 6 after inoculation (Fig. 2*c Left*), representing 0.37–0.96% of the total memory CD4$^+$ T lymphocytes in the blood (Fig. 2*d*). On day 10 after inoculation, extraordinarily high frequencies of productively SHIV-infected naïve CD4$^+$ T cells were detected (Fig. 2*c Right*), which corresponded to 32–88% of the total circulating naïve CD4$^+$ T cells (Fig. 2*e*). At this time point during the acute infection, naïve CD4$^+$ T lymphocytes comprised >96% of the virus producing cells in the blood in a representative animal, as monitored by cocultivation (Fig. 2*f*). In contrast, only 0.8–4.2% of the memory CD4$^+$ T lymphocytes in the blood of the three macaques on day 10 after inoculation were producing infectious virus. The extremely high fraction of SHIV-infected naïve CD4$^+$ T cells and their rapid elimination is consistent with direct cell killing of this T lymphocyte subset during the primary virus infection of rhesus monkeys.

CD4$^+$ T Cells in Blood and Lymph Nodes Are Not Activated During the Acute SHIV Infection of Rhesus Macaques. Because of the enormous number of SHIV-infected naïve CD4$^+$ T cells detected at day 10 of the acute infection, it became important to verify their activation status at various times during the primary infection. Not unexpectedly, an increase in the number of activated CD8$^+$ T cells in the peripheral blood was detected (22), as assessed by Ki-67 expression, in three animals evaluated (Fig. 3*a*). In contrast, the levels of Ki-67$^+$ CD4$^+$ T lymphocytes remained low in

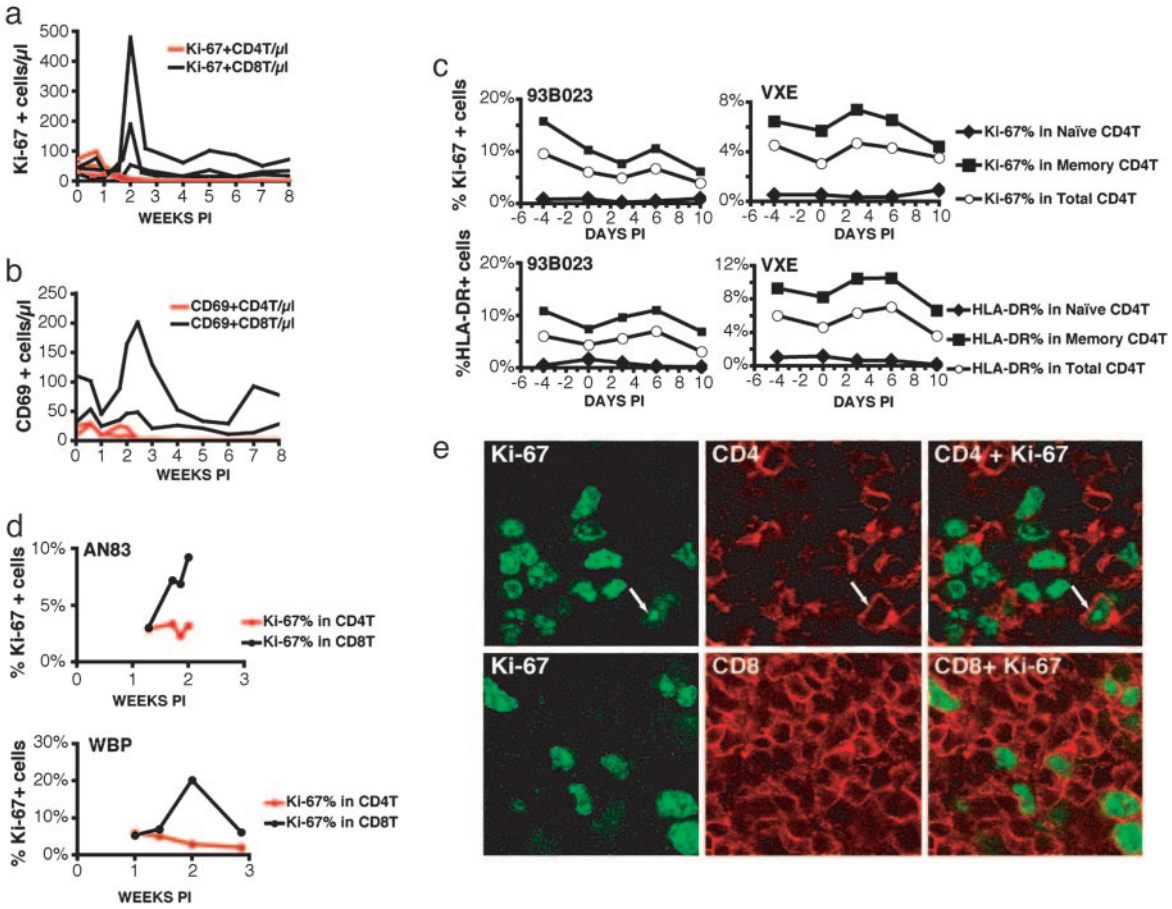

Fig. 3. CD4$^+$ T cells in blood and lymph node are not activated during the acute SHIV$_{DH12R}$ infection. (*a*) Longitudinal profiles of Ki-67$^+$ CD4$^+$ T cells (red) and Ki-67$^+$ CD8$^+$ T cells (black) in the peripheral blood of three rhesus monkeys (R46, R54, and WBP). (*b*) Longitudinal profiles of CD69$^+$ CD4$^+$ T cells (red) and CD69$^+$ CD8$^+$ T cells (black) in the peripheral blood of two rhesus monkeys (R46 and R54). (*c*) The activation status (*Upper*, Ki-67 expression; *Lower*, HLA-DR expression) of peripheral blood naïve and memory CD4$^+$ T cells in monkeys 93B023 and VXE during the acute SHIV infection. (*d*) The frequency of proliferating (Ki-67$^+$) CD4$^+$ and CD8$^+$T cells in inguinal and axillary lymph nodes, biopsied from two monkeys (AN83 and WBP) at the indicated times during the acute SHIV infection. (*e*) Lymph nodes collected from macaque AE56 on day 14 after infection were immunostained with the anti-Ki-67 (*Left*, visualized in green) and CD4 or CD8 (*Center*, visualized in red) mAbs and examined under conditions of UV enhancement. The superimposed images of Ki-67 and CD4 or CD8 are shown in *Right*. The white arrows indicate the single Ki-67$^+$ CD4$^+$ lymphocyte in the field.

these macaques both during and after the period of peak SHIV$_{DH12R}$ production. Similar results were obtained when circulating CD4$^+$ T cells from two of these monkeys were analyzed for expression of another marker of T cell activation, CD69, during this phase of the primary infection (Fig. 3*b*). When the activation status of circulating naïve and memory CD4$^+$ T cell subsets from two other acutely infected animals was monitored by expression of Ki-67 or HLA-DR, no appreciable change was observed during the period of peak virus production (Fig. 3*c*). CD4$^+$ and CD8$^+$ T cells in inguinal and axillary lymph nodes, biopsied from two of the macaques at four time points during the acute SHIV infection, exhibited activation profiles similar to that observed in the blood: increased Ki-67 expression was observed in CD8$^+$, but not in CD4$^+$, T cells (Fig. 3*d*).

In a final experiment assessing activation of T cell subsets during the primary infection, confocal microscopy was used to directly evaluate lymph node specimens collected on day 14 after infection, when the number of CD4$^+$ T lymphocytes had already started to decline. Staining with anti-Ki-67 and anti-CD4$^+$ or anti-CD8$^+$ mAbs revealed the presence of substantial numbers of Ki-67$^+$ CD8$^+$ cells, but only a rare Ki-67$^+$ CD4$^+$ cell in the paracortical region of this tissue section (Fig. 3*e*). In this lymph node specimen, virtually all of the cells staining with the anti-Ki-67 mAb were also CD8 positive.

Nonactivated Naïve CD4$^+$ T Cells Are the Principal Source of Progeny SHIV Production. Although the results presented in Figs. 2 and 3 support the conclusion that large numbers of quiescent naïve CD4$^+$ T lymphocytes are capable of releasing infectious virus, it could be argued that the production of progeny virus by naïve cells in the co-cultivation experiment (Fig. 2*c*) was an *ex vivo* artifact, possibly reflecting the activating effects of the monoclonal antibodies used for live cell sorting. To directly ascertain whether resting naïve CD4$^+$ T cells were actually productively infected *in vivo*, mesenteric lymph node samples were collected from two macaques (93B023 and VXE) at day 10 after inoculation with SHIV$_{DH12R}$ and were examined by confocal microscopy after ISH using SIV riboprobes and staining with an anti-Ki-67 mAb. These two animals had generated levels of plasma viremia and sustained depletions of their CD4$^+$ T cells similar to that seen in the three monkeys depicted in Fig. 2*a* (data not shown). No increase of Ki-67 expression in circulating CD4$^+$ T cell subsets was observed in these two animals on the day of lymph node collection (see Fig. 3*c*). In representative lymph node specimens from both monkeys, the vast majority of viral RNA-positive cells in paracortical regions were Ki-67 negative (Fig. 4). Occasional rare viral RNA-positive cells were observed that also exhibited intranuclear Ki-67 staining (Fig. 6, which is published as supporting information on the PNAS web site). Of

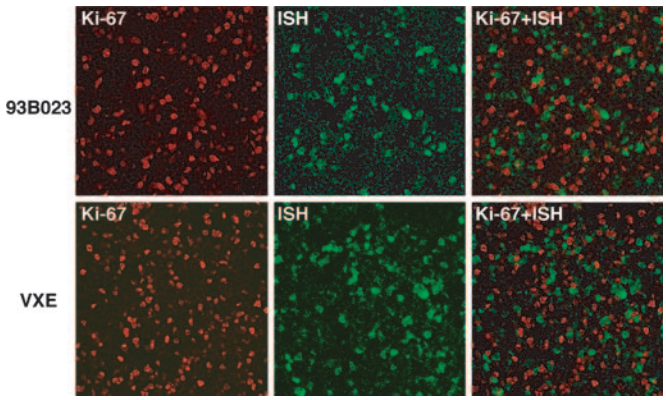

Fig. 4. Activation status of virus-producing lymph node cells on day 10 after infection. Mesenteric lymph node sections from two $SHIV_{DH12R}$-infected rhesus monkeys (*Upper*, 93B023; *Lower*, VXE) were subjected to combination immunostaining specific for Ki-67 (*Left*, visualized in red) and ISH, using a riboprobe recognizing SHIV sequences (*Center*, visualized in green). Superimposed images of Ki-67 and ISH are shown in *Right*.

1,329 ISH-positive cells counted in 24 fields from both animals, only 48 cells (3.6%) were Ki-67-positive, indicating that the vast majority of SHIV expressing cells also exhibited a nonactivated phenotype.

Discussion

Taken together, these results provide strong evidence that during the acute infections of rhesus monkeys with highly pathogenic X4 tropic SHIVs: (*i*) an extraordinarily large fraction (≈30–90%) of naïve $CD4^+$ T cells are infected at the time of peak viremia; (*ii*) high levels of plasma viremia are generated almost exclusively by naïve $CD4^+$ T cells; (*iii*) $CD4^+$ T cell subsets in the blood and lymph nodes remain in a nonactivated state during the period of peak virus production as monitored by Ki-67, CD69, and HLA-DR expression; and (*iv*) >96% of virus producing cells in lymph nodes do not express the Ki-67 marker associated with T cell activation.

The large number (≈30–90%) of naive $CD4^+$ T cells infected with SHIVs on day 10 after infection and the temporally associated systemic depletion of this T cell subset are consistent with a mechanism of direct cell killing rather than an indirect bystander effect. This massive loss of naïve $CD4^+$ T lymphocytes represents an irreversible insult to the immune system that abrogates any $CD4^+$ T cell renewal and is accompanied by a rapid downhill clinical course. In a sense, the SHIV/macaque model is a numbers game: if sufficient numbers of $CD4^+$ T cells (both naïve and memory) are eliminated within a specified short period, there will be inadequate total residual helper T cell activity and the infected animal is destined to succumb to immunodeficiency. In contrast, interventions that suppress SHIV replication, such as vaccination or antiretroviral therapy, and thereby preserve naïve $CD4^+$ T lymphocytes, are likely to result in benign clinical outcomes (2, 23). Acute SIV infections of rhesus monkeys and, by analogy, primary HIV-1 infections of human, may also cause the infection and loss of a substantial fraction of $CD4^+$ T lymphocytes (17, 24, 25), but because both of these viruses primarily target CCR5-positive memory cells, not CCR5-negative naïve T cells, the former continue to be replenished from the preserved pool of naïve cells for extended periods of time.

Nonetheless, there are features of X4-tropic SHIV infections of macaques that are also observed during HIV-1 infections. For example, in 50% of HIV-1-infected individuals, X4-tropic SI virus strains have been detected late in the course of their infections (26–29). It has been reported that naïve $CD4^+$ T lymphocytes from such patients are infected almost exclusively with X4-tropic HIV-1 strains. The emergence of X4 viruses in these individuals has been correlated with an accelerated rate of $CD4^+$ T lymphocyte depletion and a more rapid progression of their clinical course (30). On a purely speculative note, it is possible that the 50% of chronically infected persons harboring SIVs actually transmit X4-tropic HIV-1 strains at high frequencies to new recipients. However, as reported in a variety of experiments involving SHIV-infected macaques, such newly transmitted X4-tropic HIV-1 variants may easily be controlled and rapidly become "archival," yet reappear several years later when the immune system in infected individuals becomes increasingly dysfunctional. A previous report describing the parenteral transmission of X4 strains to two individuals and their subsequent suppression by R5 viral variants that emerged after the development of HIV-1-specific immunity is consistent with such a scenario (31). In addition, the failure to readily isolate HIV-1 coreceptor switch variants (R5 to X4) by serial passaging *in vitro* would also suggest a reemergence rather than a mutational model to explain the appearance of X4-tropic viral strains (32).

The capacity of lymphocytes lacking expression of surface molecules or nuclear antigens associated with the activated state to support productive HIV-1 and SIV infections is not presently understood. As suggested by others (33–36), the microenvironment within primate lentivirus-infected lymphoid tissues very likely promotes interactions between viral antigens, cytokines, chemokines, growth factors, and $CD4^+$ T lymphocytes, which together render an otherwise resistant population of quiescent cells permissive for virus replication. The delivery of virus to secondary lymphoid organs by dendritic cells, rather than as cell-free particles, and/or the rapid accumulation of large numbers of $HAM56^+$ macrophage in lymph nodes during the first week of $SHIV_{DH12R}$ infections, as reported (14), represent stimulatory events occurring *in vivo* that could promote virus replication in resting cells. Thus, although they are not cycling, as measured by using classical assays of T cell activation, naïve $CD4^+$ T cells in macaques experiencing overwhelming acute X4-tropic SHIV infections may be sufficiently stimulated by cytokines released in large quantities within lymphoid tissues and become able to generate high levels of plasma viremia.

When the results reported in this study are considered together with the large published literature describing the biology and natural history of primate lentivirus infections, the SIV/macaque system appears to be a better model for studies of HIV-1 pathogenesis and vaccine development, as has been recently suggested (37). The extremely rapid and complete elimination of $CD4^+$ T lymphocytes and onset of disease within weeks of virus inoculation seen in monkeys inoculated with X4-tropic SHIVs represent clinical features that are rarely observed in SIV or HIV-1 infections. Although SIV does cause modest $CD4^+$ T cell depletions spanning several months, the development of immunodeficiency does not require the total elimination of this T cell subset as is the case for the highly pathogenic SHIVs (38–40); clinical AIDS can emerge in SIV-infected monkeys at $CD4^+$ T lymphocyte levels of 200–300 cells per ml or higher (41). In this regard, the induction of disease by SIV also appears to be independent of inoculum size (0.3–30,000 50% monkey infectious doses inoculated intravenously) (42), whereas clinical AIDS caused by X4 SHIVs is dose dependent (2, 23). Thus, the partial depletions of $CD4^+$ T cells accompanying the administration of relatively small X4-tropic SHIV inocula to macaques is usually associated with prompt and durable control of viremia and an asymptomatic clinical course lasting several years (23). In these latter SHIV-infected monkeys, marked but transient depletions of naïve $CD4^+$ T lymphocytes may occur, but the pool of memory $CD4^+$ T cells is invariably preserved (Y.N., unpublished data). SIVs also share a critical virological property with HIV-1 variants present after the establishment of infection in humans: they both primarily use the CCR5 che-

mokine coreceptor *in vivo* and preferentially target CCR5-expressing $CD4^+$ memory T cells in nonlymphoid tissues for elimination. SIV-infected rhesus monkeys typically experience a profound loss of $CD4^+$ memory T lymphocytes in the gut-associated tissue, but only modest changes in the levels of $CD4^+$ T cells in the blood and lymph nodes during the primary infection (43). A similar marked depletion of $CD4^+$ T cells in the gastrointestinal tract has also recently been reported in HIV-1-infected individuals (24, 44). Therefore, it might be concluded that, because highly pathogenic X4-tropic SHIVs replicate so vigorously in naïve $CD4^+$ T lymphocytes during the acute infection, their use as challenge viruses in experiments modeling vaccine efficacy for HIV-1 would be inappropriate based on the $CD4^+$ T cells targeted by each virus during the initial weeks of the primary infection.

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