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Author(s):	E. Shudo, Z # 194289, T-10/T-Division R.M. Ribeiro, Z # 171295, T-10/T-Division A.S. Perelson, Z # 082011, T-10/T-Division
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Depletion of CD8+ T Cells Does Not Affect the Lifespan of Productively Infected Cells During Pathogenic SIVmac239 Infection of Rhesus Macaques

Nichole R. Klatt[1,2], Emi Shudo[3], Alex M. Ortiz[1], Jessica C. Engram[1], Mirko Paiardini[1], Benton Lawson[2], Michael D. Miller[4], James G. Else[2], Joern E. Schmitz[5], Ruy M. Ribeiro[3], Alan S. Perelson[3], Guido Silvestri[1,2]

[1]*Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA, 19104, USA.*

[2]*Yerkes National Primate Research Center, Emory University, Atlanta, GA, 30329 USA.*

[3]*Los Alamos National Laboratory, Los Alamos, NM, 87545 USA.*

[4]*Gilead Sciences, Inc., Foster City, California, 94404 USA.*

[5]*Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, 02115 USA.*

Abstract

While CD8+ T cell responses are clearly important in anti-viral immunity during HIV/SIV infection, the mechanisms by which CD8+ T cells induce this effect remain poorly understood, as emphasized by the failure of the Merck adenovirus-based, cytotoxic T lymphocyte (CTL)-inducing AIDS vaccine in a large phase IIb clinical trial. In this study, we measured the in vivo effect of CD8+ lymphocytes on the lifespan of productively infected cells during chronic SIVmac239 infection of rhesus macaques by

treating two groups of animals (i.e., CD8+ lymphocyte-depleted or controls) with antiretroviral therapy (PMPA and FTC). The lifespan of productively infected cells was calculated based on the slope of the decline of SIV plasma viremia using a well-accepted mathematical model. We found that, in both early (i.e., day 57 post-inoculation) and late (i.e., day 177 post-inoculation) chronic SIV infection, depletion of CD8+ lymphocytes did not result in an increased lifespan of productively infected cells in vivo. This result indicates that direct killing of cells producing virus is unlikely to be a major mechanism underlying the anti-viral effect of CD8+ T cells during SIV infection. These results have profound implications for the development of AIDS vaccines.

Twenty-five years after the discovery of HIV, an effective AIDS vaccine has yet to be developed. This is largely due to our lack of understanding of the complex interactions between HIV and the host immune system. The recent failure of the first cytotoxic T lymphocyte (CTL)-based candidate AIDS vaccine (i.e., the Merck human adenovirus 5-vectored AIDS vaccine-V520) in a large, highly publicized clinical trial has emphasized the need for a better understanding of the role of CD8+ T cells in controlling HIV replication[1]. This knowledge is crucial to determine the most appropriate method of inducing a CD8+ T cell-mediated anti-viral immune response that will protect from HIV infection and/or disease progression.

Several lines of evidence indicate that HIV/SIV-specific CD8+ T cells play an important role in anti-viral immunity. First, CD8+ T cells can inhibit HIV and SIV replication in vitro[2]. Second, there is a strong association between specific major histocompatibility

alleles and rates of disease progression during HIV and SIV infection (reviewed in[3]). Third, CD8+ T cell escape mutants consistently arise during both acute and chronic HIV/SIV infections, indicating selective immune pressure on the virus population (reviewed in[4]). Fourth, there is a temporal association between post-peak decline of acute viremia and emergence of CD8+ T cell responses[5,6]. However, the most direct evidence supporting a key role of CD8+ T cells in controlling virus replication is demonstrated by the dramatic increase in viremia observed after CD8+ lymphocyte depletion in SIV-infected rhesus macaques[7-10]. While this observation is very clear, the mechanisms by which CD8+ T cells exert anti-viral effects in vivo are still poorly understood. Conceivably, these mechanisms can be summarized into two major categories: (i) CD8+ T cells may reduce production of virions on a per cell basis by either direct killing of infected cells (thus decreasing the average lifespan of infected cells) or via inhibition of (or decreasing) the rate of virus production by non-cytolytic mechanisms. And/or, (ii) CD8+ T cells may reduce the number of productively infected cells; this could be due to an inhibition of the spread of infection (i.e., via production of β-chemokines or other cytokines) or by limiting the number of targets (i.e., activated CD4+ T cells) available for infection.

In this study, we sought to better understand the role of CD8+ T cells during SIV/HIV infection by determining how the presence (or absence) of CD8+ T cells affects the lifespan of productively SIV-infected cells in vivo. We treated two groups of SIVmac239-infected rhesus macaques (RM) with potent antiretroviral therapy (ART) either alone (control) or after depletion of CD8+ lymphocytes with the OKT8F mAb.

Several previous studies have demonstrated that analysis of changes in viral load after initiation of ART provides substantial insight into the dynamics of HIV and SIV infection[11-18]. This is possible because reverse transcriptase inhibitors block de novo infections while not affecting productively infected cells; thus all measurable virus comes from cells that were infected prior to treatment. As these cells die, plasma viral loads decrease, and mathematical modeling can be used to determine the lifespan (or death-rate) of productively infected cells in vivo based on the rate of viral decay[15]. We applied this experimental/modeling approach and used two potent reverse transcriptase inhibitors, 9-R-(2-phosphonomethoxypropyl)adenine (PMPA) and beta-2',3'-dideoxy-3'-thia-5-fluorocytidine (FTC) immediately after CD8+ lymphocyte depletion (or alone in the control animals). All RMs were given two cycles of ART for 28 days, during early and late chronic infection; group A (n=5) RMs were depleted of CD8+ lymphocytes during the early chronic phase of infection (day 57) immediately prior to treatment with ART, while group B (control, n=5) was treated with ART alone. Conversely, during the late chronic phase of infection (day 177), group B (n=4) was CD8+ lymphocyte-depleted prior to ART, while group A (control, n=3) was treated with ART alone. In all cases, the lifespan of productively infected cells in vivo (in the presence and absence of CD8+ T cells) was estimated using a well-accepted mathematical model that includes both short and long-lived productively infected cells[15].

Results

CD8+ T cells are efficiently depleted in peripheral and mucosal tissues

As expected, we observed a very rapid and near complete depletion of CD8+ T cells from both peripheral and mucosal tissues in all RMs treated with intravenous administration of the OKT8F monoclonal antibody. During the first phase of this experiment (early chronic infection, i.e., day 57 post-inoculation), in group A animals, CD8+ T cells were depleted by (average ± s.d.) 99.97 ± 0.01% in peripheral blood (Fig. 1A, 1B), 99.29 ± 0.50% in rectal biopsies (Fig. 1C, 1D), and 99.23 ± 1.07% in bronchoalveolar lavage (data not shown) as measured by flow cytometry. During the second phase of this experiment (late chronic infection, i.e., day 177 post inoculation), in group B animals, CD8+ T cells were depleted by an average of 99.95 ± 0.03% in peripheral blood (Fig. 1B) and 98.07 ± 1.54% in rectal biopsies (Fig. 1D) as measured by flow cytometry. The extent of depletion in mucosal tissues was corrected for non-CD8+ T cell fluctuations (as described in[19]). In all cases, and consistent with previous studies[8], CD8+ T cells were depleted very rapidly (>98% depletion after 24 hours) and CD8+ T cell depletion was sustained for 8-13 days with nadir depletion occurring between 5-6 days after the first infusion.

ART effectively suppresses SIV replication

Similar to previous studies in which CD8+ T cells were depleted during pathogenic SIV infection[7-10], we observed an initial increase in viremia between 0.7-2.2 logs (Fig. 2). This increase in viremia, however, is circumvented by ART treatment beginning three days after the last OKT8F infusion. During both phases of the study, ART effectively suppressed virus replication in all RMs by at least 0.5 $\log_{10}$ (and in 16 out of 17 instances of treatment by at least 1.5 $\log_{10}$) within a week after initiation of therapy (Fig. 3).

Previous studies[11,12,15,17] demonstrated that there are two phases of viral decay; an initial rapid, exponential decline of 1-2 logs, in which productively infected short-lived cells are lost, followed by a second phase, which is characterized by a slower decline, where long-lived infected cells are lost. In order to quantify the contribution of CD8+ T cells to the lifespan of productively infected cells in vivo, we measured this parameter in the presence or absence of CD8+ T cells by analyzing the viral decline after initiation of ART using the equation:

$$V(t)=V_0\,(A\,\exp(-\delta t) + C\,\exp(-\mu t) + (1-A-C)\,\exp(-ct)) \quad (1)$$

where $A=(NkT_o)/(c-\delta)$, $C=(c-NkT_o)/(c-\mu)$ and V_0 is the initial viral load, kT_0 is a parameter quantifying the rate of infection before therapy starts, N is the viral burst size, δ is the death rate of short-lived productively infected cells, μ is the death rate of long-lived productively infected cells, and c is the rate of virion clearance[15]. By fitting the natural logarithm of $V(t)$ given by equation (1) to the natural logarithm of the measured SIV RNA between initiation and termination of therapy, we were able to estimate δ and μ, the death-rate of short-lived and long-lived productively infected cells, for each animal either in the presence or absence of CD8+ T cells. However, for monkeys RAj7 and RMm6, we could not fit a second phase decline (due to too few data points or a flat second phase), thus for these animals we used a monophasic decline model[20] by making $C=0$ in (Eq. 1), i.e., setting $NKT_0=c$, and estimated δ but not μ. For monkey RPp6 in early infection the second phase was flat and thus we set $\mu=0$, but estimated δ. The virion clearance rate c was fixed at 23 day^{-1} a value previously estimated[21].

Absence of CD8+ T cells does not increase the lifespan of productively infected cells

During both the early and late phases of this study, we found that the lifespan of short-lived productively infected cells ($1/\delta$) is similar regardless of the presence or absence of CD8+ T cells. Specifically, during the early phase, the mean lifespan for group A (CD8+ lymphocyte-depleted) was 1.10 ± 0.38 days (median=0.87), while the mean lifespan for group B (control) was 1.05 ± 0.35 days (median=0.93) (p=0.83) (Fig. 4). Of note, two RMs from group A and one from group B were sacrificed after the first cycle of ART due to severe weight loss. During the late phase, the mean lifespan for group A (control) was 0.87 ± 0.21 days (median=0.86), while the mean lifespan for group B (CD8+ lymphocyte-depleted) was 0.89 ± 0.28 days (median=0.98) (p=1.0) (Fig. 4). These data indicate that CD8+ T cells do not affect the lifespan of short-lived productively infected cells in vivo during pathogenic SIV infection of RMs. Moreover, the estimated lifespans of long-lived infected cells ($1/\mu$) were also not different between CD8+ lymphocyte-depleted and not depleted animals in either early or late chronic infection (9.6 ± 10.5 vs. 8.8 ± 6.1 days, respectively, p=0.71, with both treatment periods analyzed together). An interesting observation, however, is that of the 7 macaques that participated in both the early and late phase studies, 5 had a shorter lifespan of productively infected cells in the later phase (average of 36% shorter), thus suggesting that the cytolytic anti-viral activity of CD8+ T cells does not progressively decline during chronic infection. This finding is somewhat unexpected and indicates that immune correlates of CD8+ T cell-mediated control of virus replication might be much more complex than previously appreciated.

Perturbations to steady state do not significantly affect estimates of δ and μ

A caveat to this analysis is that the mathematical model (Eq. 1) used to determine the death-rate of infected cells is based on the assumption that the virus and the target cells are at their set-point or steady state levels upon the initiation of therapy and that therapy is 100% effective in blocking new infections[15]. However, CD8+ lymphocyte depletion causes two perturbations to the steady state: (i) an increase in viremia prior to ART treatment (Fig. 2), and (ii) an increase in the level of activated CD4+ T cells, thus expanding the target cell population for virus replication (Supplementary Fig. 3). To determine the effect of changes in viremia after CD8+ lymphocyte depletion, surrogate data for SIV kinetics with virus not in steady state were created by Eq. 3 with a known value of δ and then fit using equation (1) to assess if, and to what extent, viral load increases before the start of therapy altered estimated δ values (Supplementary Note 1, Supplementary Fig. 1). To take into account the possibility that significant changes in the activation state of CD4+ T cells occurs after CD8+ lymphocyte depletion, surrogate data were created that include changes in target cells (Supplementary Note 2, Supplementary Fig. 2). Finally, the above analyses were repeated with various drug effectiveness less than 100% to study the influence of this factor on our estimate of δ (Supplementary Note 2). All three analyses demonstrated that errors due to lack of steady-state viremia, due to changes in target cell pools after CD8+ lymphocyte depletion as well as to drug effectiveness < 100% lead to an underestimation of both δ and μ (Supplementary Notes 1 and 2). Furthermore, when the drug effectiveness was high, i.e. 99%, the maximum error in estimating δ and μ was < 3.5%. This analysis shows that the actual values of δ and μ in systems with CD8+ lymphocyte depletion may be even higher than we estimate (thus

errors would result in a shorter lifespan), and further emphasizes that CD8+ T cells are not required for the rapid loss of productively infected cells.

Discussion

These studies provide the first assessment of the impact of CD8+ T cells on the longevity of SIV-infected cells in vivo. Perhaps surprisingly, our data indicate clearly that CD8+ T cells do not affect the lifespan of productively infected cells during SIVmac239 infection of rhesus macaques. It should be noted that the current study does not imply, in any way, that SIV-specific CD8+ T cells are less important than previously believed in anti-viral immunity. In fact, our experiments confirmed in all circumstances that in vivo CD8+ lymphocyte depletion is associated with a marked and consistent increase in viral load. However, our experiments do challenge the widely assumed paradigm that a major anti-viral effect of CD8+ T cells is related to the direct killing of productively infected CD4+ T cells and thus acts by reducing the amount of time in which infected cells are able to produce virions. However, as noted by Klenerman et al.[22], our analysis of the effects of drug therapy does not preclude that CD8+ T cells kill infected cells before they begin producing virus. While CD8+ T cells may be playing an early cytotoxic role, this study rules out the possibility that CD8+ T cells act via direct killing once a cell is productively infected. Instead, these results are consistent with a model wherein the effect of CD8+ T cells in SIV infection is related to block of virus spread and entry (possibly via production of chemokines such as CCL3, CCL4 and CCL5) and/or decreased production of virus from infected cells by means of non-cytolytic mechanisms. Our data are also

consistent with the possibility that the increase in viral load after CD8+ lymphocyte depletion is caused by increased CD4+ T cell activation. However, in this experiment, the observed increase in CD4+ T cell activation that occurred in all tissues followed, rather than preceded, the increase in plasma viral load (Supplementary Fig. 3), thus supporting a more direct role for CD8+ T cells in maintaining the steady-state of viral load. It should also be noted that all SIV-infected RMs in this study show high levels of virus replication; as such, these experiments did not rule out the possibility that direct killing of infected cells is a key mechanism of protection in animals with extremely low viremia (i.e., elite controllers).

Interestingly, the possibility that non-cytolytic mechanisms are responsible for the bulk of the anti-viral activity of CD8+ T cells in vivo is consistent with several previous observations. First, a prominent role of factors produced by CD8+ T cells that can block virus dissemination or the level of virus entry is consistent with the known anti-viral activity of CCL3, CCL4 and CCL5[23], as well as the protective effect of increased gene copies of CCL3L1[24]. Second, a non-cytolytic anti-viral effect of CD8+ T cells would be consistent with the classical observations by Levy et al. of CD8+ T cell-mediated anti-viral factor(s)[25],[26]. Third, our results are in agreement with theoretical predictions on the in vivo role of CD8+ T cells that were made based on the kinetics of viral increase observed post CD8+ T cell depletion[8].

In conclusion, this study demonstrates that during chronic SIV infection of RMs with high virus replication, the anti-viral effect of CD8+ T cells is due to mechanisms that do not affect the in vivo longevity of infected cells (i.e., non-cytolytic). This result provides a significant improvement in our understanding of the correlates of CD8+ T cell-

mediated protection from HIV/SIV replication and may have important implications for the design of candidate AIDS vaccines.

Materials and Methods

Animals

Ten rhesus macaques of Indian origin (of which 6 were MamuA*01, equally distributed 3/group) were infected with 3000 TCID50 of SIVmac239 i.v. for this study. All animals were housed at the Yerkes National Primate Research Center and maintained in accordance with NIH guidelines. RMs belonging to the two groups were age and weight matched. A power analysis (Supplementary Note 3) showed that ten animals were sufficient to detect the expected difference in productively infected cell lifespan if CD8+ T cells acted through cytotoxic activity. These studies were approved by the Emory University and University of Pennsylvania Institutional Animal Care and Use Committees.

CD8+ lymphocyte depletion

RMs were treated with 4mg/kg/ day i.v. of OKT8F mAb for three consecutive days. Depletion efficiency in blood was calculated based on flow cytometric analysis and complete blood cell counts; depletion efficiency in tissues other than blood (where absolute number calculations were not available) was calculated based on flow cytometry, as fraction of the baseline percent of CD8+ T cells.

Antiretroviral Therapy

Reverse transcriptase inhibitors 9-*R*-(2-phosphonomethoxypropyl)adenine (PMPA; tenofovir) and beta-2,3-dideoxy-3-thia-5-fluorocytidine (FTC; emtricitabine) provided by Gilead were administered to each animal i.m. (30mg/kg/animal/day each) for 28 days during both early and late phases of the study (ART began at days 63 and 182 post-SIV infection, respectively).

Sample collection and processing

Peripheral blood mononuclear cells were isolated by gradient centrifugation (ficoll). Procedures for lymph node biopsies, rectal biopsies, and bronochoalveolar lavage as well as isolation of lymphocytes form the obtained samples were performed as previously described[19].

Immunophenotyping and flow cytometry

Multicolor flow cytometric analysis was performed on whole blood or isolated cells according to standard procedures using human mAbs that crossreact with RMs. Predetermined optimal concentrations were used of the following antibodies: anti-CD3-Alexa700 (clone SP34-2, BDPharmigen), anti-CD8-PacOrange (clone RPA-T8, BDPharmigen), anti-CD8-PE-TR (clone RPA-T8, Caltag/Invitrogen), anti-CD4-PE-Cy5.5 (clone OKT4, eBioscience), anti-CD4-PerCP-Cy5.5 (clone L200, BDPharmigen), anti-CD4-PacBlue (clone OKT4, eBioscience), anti-Ki67-FITC (clone B26, BDPharmigen), anti-CCR5-PE (clone 3A9, BDPharmigen), anti-CD69-PE-Cy7 (clone FN50, BDPharmigen), anti-HLA-DR-PE-Cy5 (clone L243, BDPharmigen), Aqua Live/Dead amine dye-AmCyan (Invitrogen). All samples were fixed and permeabilized using CytoFix/Perm Kit (BDPharmigen) and intracellularly stained to detect Ki67. Flow cytometric acquisition was performed on at least 100,000 lymphocytes on an LSRII cytometer driven by the FACS DiVa software (version 5.2; BDPharmigen). Analysis of the acquired data was performed using FlowJo software (version 8.7.1; TreeStar). For all analysis of specific cell subsets, we used a threshold of 200 collected events.

Plasma viral loads

SIVmac239 plasma viremia was measured by RT-PCR as previously described[27].

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Author Contributions

N.R.K. contributed to the experimental design and coordinated animal studies, as well as performing tissue processing, cell isolation and flow cytometry, performed data analysis, and contributed to the writing of the manuscript. E.S. contributed to mathematical modeling and analysis of lifespans. A.M.O., J.C.E. and M.P. contributed to tissue processing and intellectual input. B.L. quantified plasma viremia. M.D.M. contributed PMPA and FTC as well as intellectual contribution. J.G.E. contributed coordination of animal studies. J.E.S. contributed to the experimental design and intellectual input. R.M.R. performed statistical analysis and contributed to mathematical modeling. A.S.P. contributed to mathematical modeling and analysis, and experimental design. G.S. directed the study and wrote the manuscript.

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Figures

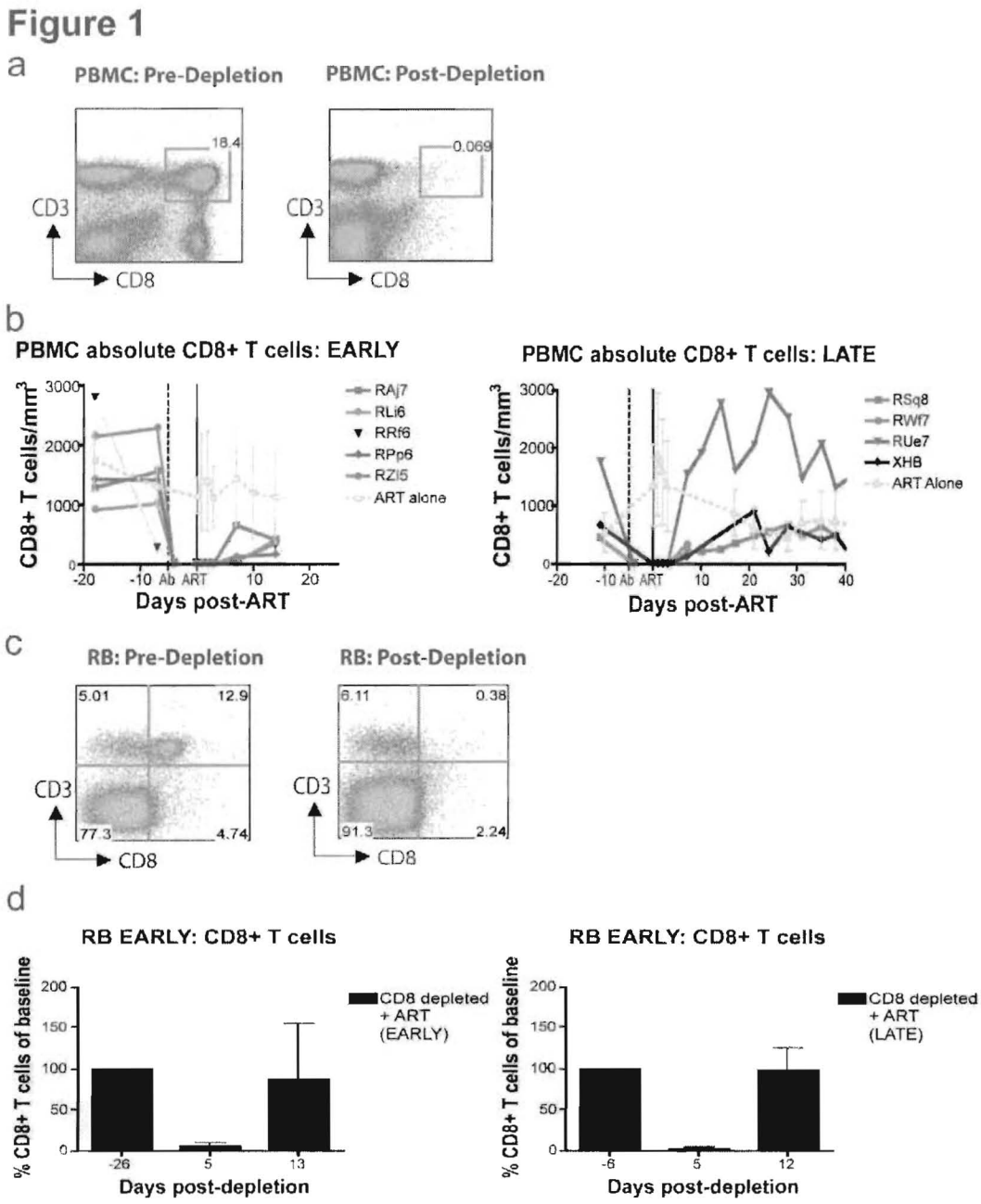


Figure 1
a
PBMC: Pre-Depletion
PBMC: Post-Depletion
18.4
0.069
CD3
CD8
b
PBMC absolute CD8+ T cells: EARLY
PBMC absolute CD8+ T cells: LATE
CD8+ T cells/mm3
Days post-ART
RAj7
RLi6
RRf6
RPp6
RZl5
ART alone
RSq8
RWf7
RUe7
XHB
ART Alone
c
RB: Pre-Depletion
RB: Post-Depletion
5.01
12.9
77.3
4.74
6.11
0.38
91.3
2.24
d
RB EARLY: CD8+ T cells
RB EARLY: CD8+ T cells
% CD8+ T cells of baseline
Days post-depletion
CD8 depleted + ART (EARLY)
CD8 depleted + ART (LATE)

Figure 2

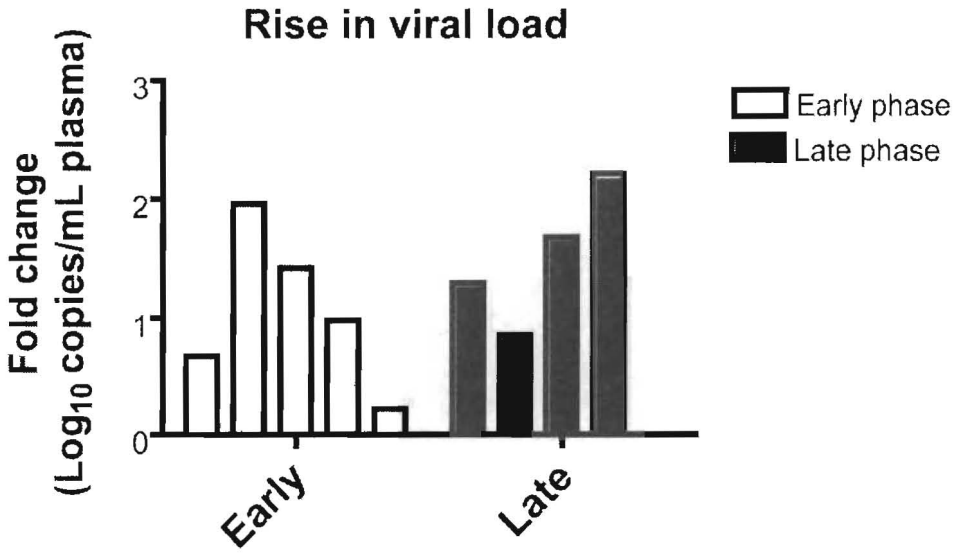

Rise in viral load
Fold change
(Log10 copies/mL plasma)
3
2
1
0
Early
Late
Early phase
Late phase

Figure 3

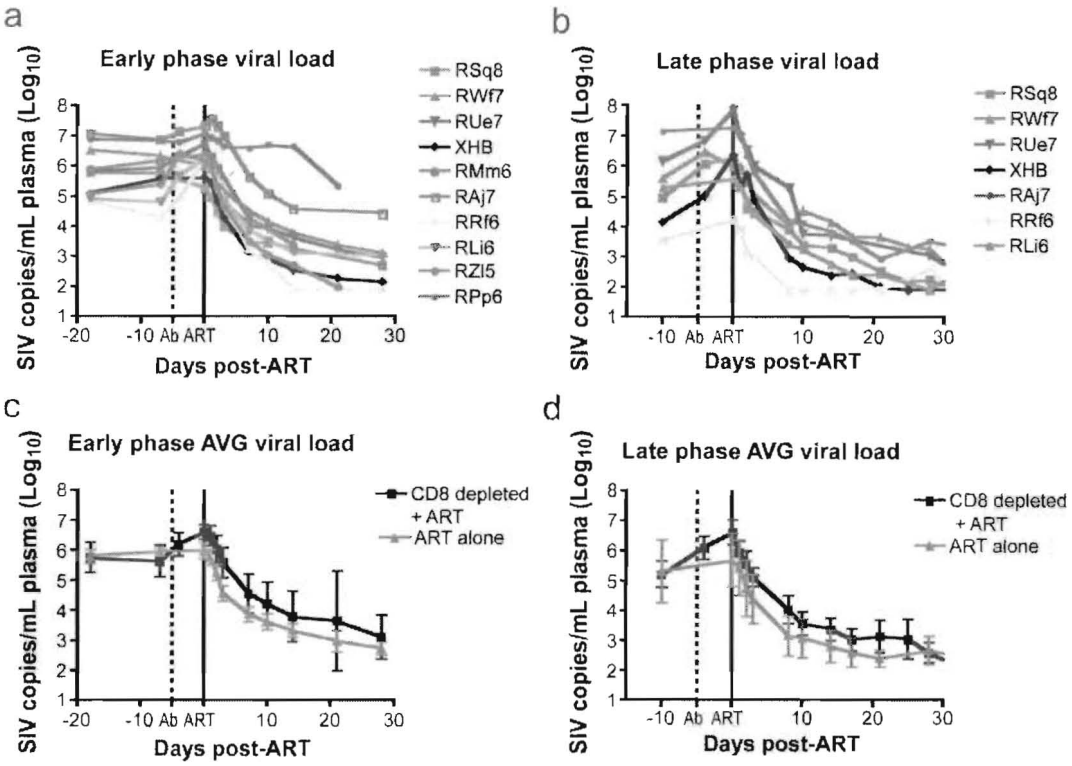

a
Early phase viral load
SIV copies/mL plasma (Log10)
Days post-ART
-20
-10 Ab ART
10
20
30
RSq8
RWf7
RUe7
XHB
RMm6
RAj7
RRf6
RLi6
RZl5
RPp6
b
Late phase viral load
SIV copies/mL plasma (Log10)
Days post-ART
RSq8
RWf7
RUe7
XHB
RAj7
RRf6
RLi6
c
Early phase AVG viral load
SIV copies/mL plasma (Log10)
Days post-ART
CD8 depleted + ART
ART alone
d
Late phase AVG viral load
SIV copies/mL plasma (Log10)
Days post-ART
CD8 depleted + ART
ART alone

Figure 4

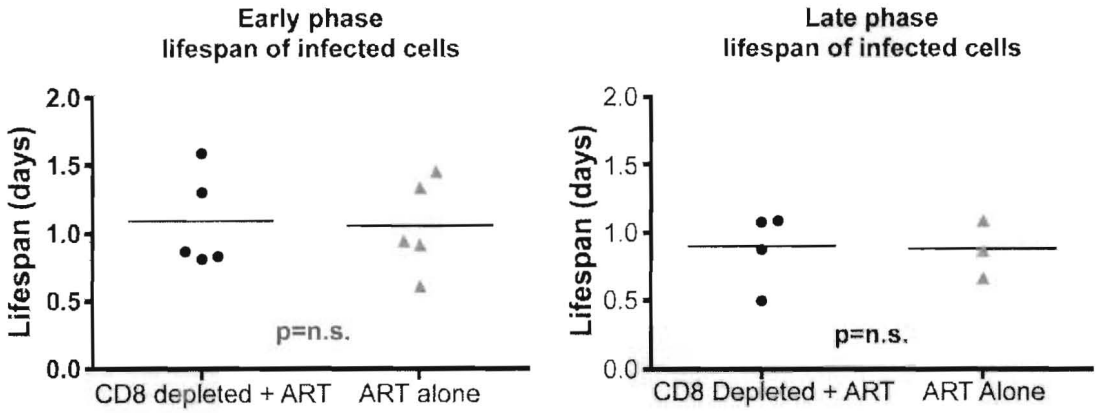

Early phase
lifespan of infected cells
Lifespan (days)
2.0
1.5
1.0
0.5
0.0
p=n.s.
CD8 depleted + ART
ART alone
Late phase
lifespan of infected cells
Lifespan (days)
2.0
1.5
1.0
0.5
0.0
p=n.s.
CD8 Depleted + ART
ART Alone

Figure Legends

Figure 1. Administration of OKT8F results in near complete depletion of CD8+ lymphocytes. (**a**) Representative flow cytometry plots (x-axis, CD8; y-axis, CD3) demonstrating CD8+ lymphocyte levels in blood (left, 7 days before depletion; right, 6 days after depletion). CD8+ T cells previously gated on live lymphocytes. (**b**) Longitudinal assessment of the absolute number of CD8+ T cells in peripheral blood for each animal during early chronic phase (left) or late chronic phase (right). Each colored line indicates an individual animal (CD8+ lymphocyte-depleted). Gray lines indicate the average CD8+ T cell number in non-depleted (ART alone) RMs. Dotted vertical line indicates the first day of depleting Ab treatment, solid vertical line indicates the first day of ART (**c**) Representative flow cytometry plots (x-axis, CD8; y-axis, CD3) demonstrating CD8+ lymphocyte depletion in rectal biopsies (left, 10 days before depletion; right, 6 days after depletion). CD8+ T cells previously gated on live lymphocytes. (**d**) Longitudinal assessment of the percent of CD8+ T cells (compared to baseline) in rectal biopsies during early chronic phase (left) or late chronic phase (right). Bars represent average of treated animals.

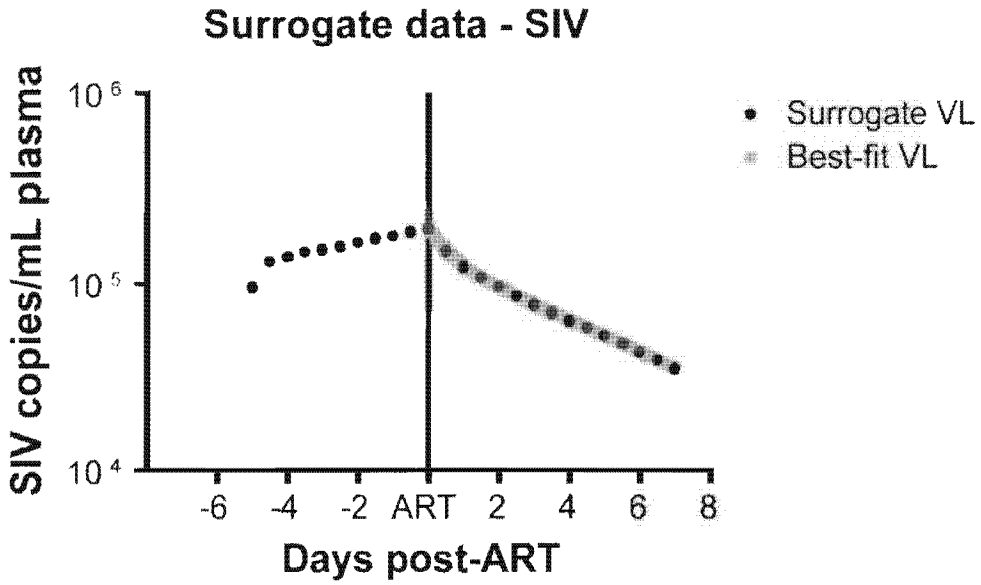
Figure 2. CD8+ lymphocyte depletion results in a 0.7-2.2 $\log_{10}$ rise in viral load. Fold change of viral load for each individual animal during early chronic phase (white bars, left) or late chronic phase (black bars, right).

Figure 3. Treatment with PMPA and FTC effectively suppresses virus replication in SIVmac239-infected RMs. (**a,b**) Plasma viral load ($\log_{10}$) measured longitudinally for each individual animal during (**a**) early chronic phase or (**b**) late chronic phase. (**c,d**) Average plasma viral load ($\log_{10}$) for each group (black, CD8+ lymphocyte-depleted; red, control) during (**c**) early chronic phase or (**d**) late chronic phase. Error bars represent standard deviation. Dotted vertical line indicates the first day of depleting Ab treatment, solid vertical line indicates the first day of ART

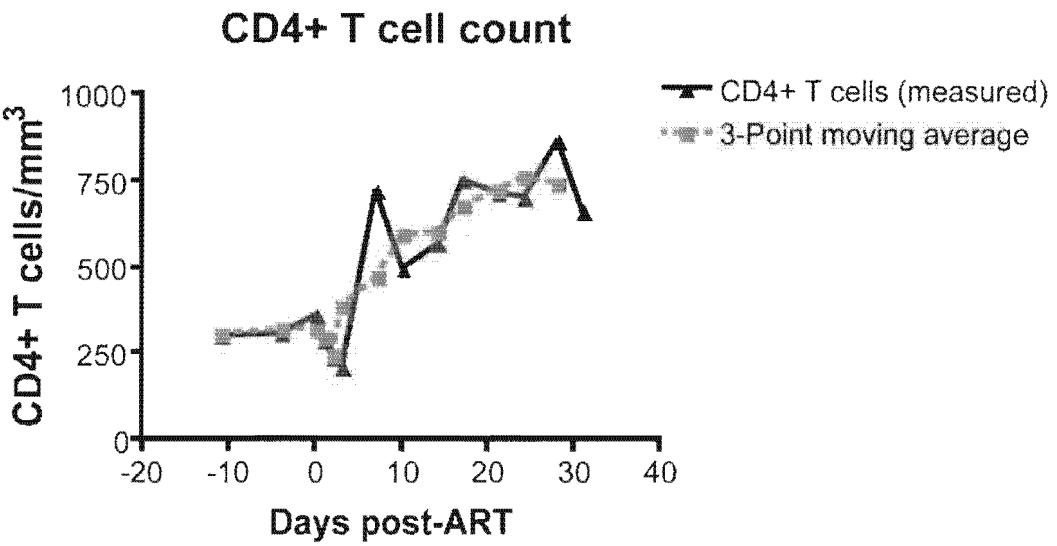
Figure 4. CD8+ lymphocyte depletion does not affect the lifespan of infected cells during SIV infection. The estimated lifespan of productively infected cells, $1/\delta$, for each animal; CD8+ lymphocyte-depleted (black) and control (red) during early chronic phase (left) or late chronic phase (right). P=n.s.

Supplementary Figures

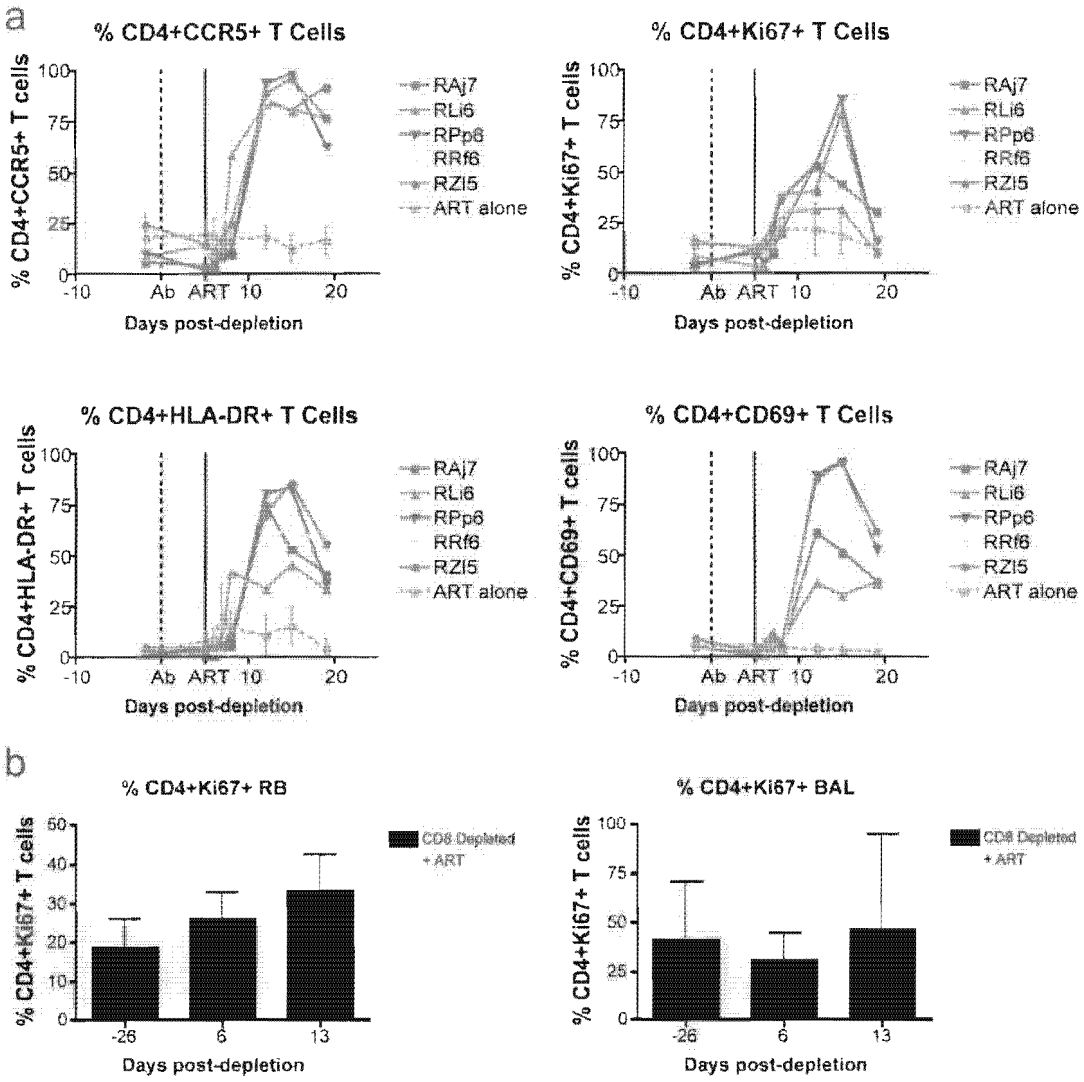
Supplementary Figure 1



Supplementary Figure 2



Supplemental Figure 3



Supplementary Figure Legends

Supplementary Figure 1. Surrogate data for SIV kinetics with virus not in steady state. Representative surrogate data (black dots) was created to account for changes in viremia caused by CD8+ lymphocyte depletion in order to determine the effect of rise in viremia on the analysis by Eq. 1, which was derived assuming pre-treatment the viral load was at its set-point. This data was chosen to agree with the change in viremia for monkey Rsq8. The best-fit data with Eq. 1 is shown by the orange squares. Here the drug effectiveness was chosen as 99% and the errors in estimating δ and μ were negligible (<3.5%).

Supplementary Figure 2. CD4+ T cell data used to estimate the change in target cells after CD8+ lymphocyte depletion. Measured CD4+ T cell values for Rsq8 in late chronic infection, (black line) and data smoothed by using a 3 point moving average (purple line). The 3-point moving average was then fit using linear regression to obtain the parameters α and T_0 used in the supplemental text to define the T cell increase during CD8+ lymphocyte depletion. Analysis of the surrogate SIV RNA data indicates that the effect of changes in CD4+ T-cells and SIV RNA due to CD8+ lymphocyte depletion has a negligible (<3.5%) effect on the estimates of δ and μ when the drug effectiveness is high (~99 %).

Supplementary Figure 3. CD8+ lymphocyte depletion results in a rise in activated CD4+ T cells. (**a**) Longitudinal assessment (individual animals from CD8+ lymphocyte-depleted group and mean and s.d. from control group) of the percent of CD4+CCR5+ (top left), CD4+Ki67+ (top right), CD4+HLA-DR+ (bottom left), and CD4+CD69+ (bottom right) T cells during early chronic infection. (**b**) Longitudinal assessment of the mean (and s.d.) percent of CD4+Ki67+ T cells in rectal biopsies (left) and bronchoalveolar lavage (right).

Supplementary Notes

Supplementary Note 1. Surrogate data was created to determine the effects of changes in steady-state viremia after CD8+ lymphocyte depletion by simulating the long-lived infected cell model given by equations:

$$dT^*/dt = (1-\varepsilon(t))\, k\, V T_o - \delta I \qquad (3a)$$

$$dM^*/dt = (1-\varepsilon(t))\, k\, V M_o - \mu M^* \qquad (3b)$$

$$dV/dt = N(t)\delta I + p(t)M - cV \quad (3c)$$

where T^* and M^* are the densities of short-lived and long-lived productively infected cells, respectively, V is the virus (SIV RNA) concentration, T_0 and M_0 are the steady state (baseline) levels of short-lived and long-lived target cells (assumed here to be constant) and $\varepsilon(t)$ is the effectiveness of ART in blocking reverse transcription. If $\varepsilon=1$ then the therapy is 100% effective and all new infections are blocked. The parameter k is the rate of viral infection, N is the viral burst size from short-lived infected cells, and $p(t)$ is the rate of viral production per long-lived infected cell. As shown in[1] if $\varepsilon=1$ and treatment is started at steady state, then $V(t)$ is given by equation (1) where t is the time on therapy. To simulate the effects of CD8+ lymphocyte depletion, we assume that CD8+ T cells release a factor that acts to decrease viral production. Thus, depleting CD8+ lymphocytes will increase virus production as observed. Other mechanisms could also be used to increase virus production, but here our main interest is simply to study the effect of increased virus before initiation of ART. The first CD8+ lymphocyte-depleting antibody was given 5 days before the start of ART, and by day 8 after the start of ART, CD8+ T cells have reappeared. Thus, choosing $t=0$ as the time therapy is started, we assume that between days -5 and day 8, viral production per infected cell is elevated. For simplicity, this rate was chosen the same for the short and long-lived infected cell populations, i.e. $N\delta = p$, although this need not be the case. Further, we assume that before CD8+ lymphocyte depletion, the system is in steady state and that no antiretroviral drugs are present, i.e. $\varepsilon=0$. At the pre-treatment steady state $M_0= \mu\,(c/(p_s k)-T_0/\delta)$ and $T^*=(KV_sT_0)/\delta$ and $M^* = kV_s\,(c/(p_s k)-T_0/\delta)$, where V_s, the pre-treatment viral load, was chosen as 9.62×10^4 copies/ml (to represent monkey RSq8's data in late chronic infection

before CD8+ lymphocyte depletion) and $T_0 = 10^6$/ml (average of CD4+ T cell count in all monkeys and in both phases before therapy). The other parameters were c=23 day^{-1}; $p=p_s$ =100 day^{-1}, δ = 1.99 day^{-1}, ε =0.99 (during therapy i.e. for $0 \le t \le 28$), μ=0.2 day^{-1}, $k=4\times10^{-7}$ ml day^{-1}. Lastly, we assumed that during CD8+ lymphocyte depletion, i.e. for $-5 \le t < 8$ that p was increased and took on values of either 110, 130, 150, or 200 day^{-1}. After day 8, p was returned to its pre-treatment steady state value p_s=100 day^{-1}. Simulating this modified system of equations, with parameters as given above, we generated sets of surrogate data that were then fitted with equation (1) in the text. The surrogate data was sampled every 12 hours for 7 days after initiation of therapy to generate the datasets for fitting (Supplementary Fig. 1). For all four values of increased viral production we found that with ε=0.99, δ and μ were both underestimated and within 3.5% of their true values. For example, although δ=1.99 day^{-1} was used to generate the surrogate data, δ was estimated at 1.96 and 1.92 day^{-1}, with p=110 and 200 day^{-1}, respectively. Similarly, μ=0.2 day^{-1} was used to generate the surrogate data and then was estimated as μ=0.199 and 0.196 day^{-1}, with p=110 and 200 day^{-1}, respectively

Supplementary Note 2. To determine the impact of increased CD4+ T cell pools as well as increased viremia during CD8+ lymphocyte depletion, we assumed that T rather than being held constant at T_0 in eqn (2a) is variable, i.e., we replaced the term $(1-\varepsilon)kVT_0$ by $(1-\varepsilon)kVT$, where T was given by $dT/dt = \alpha(t)$ with $\alpha(t)= 0$ for $t < 0$, $T(0)= 275 \times 10^3$ /ml, and α =27.3 x 10^3/(ml/day) ($0 \le t \le 7$). This choice of $T(0)$ and α gives rise to a CD4+ T cell increase similar to that observed in monkey Rsq8 (Supplementary Fig. 2). Simulating this modified system of equations, with parameters as given above, and p varied as above generated a set of surrogate data that was then fit with equation (1) in the

text. As in the case of fixed target cell levels, for all four values of increased virus production we found that with ε=0.99, δ and μ were again underestimated and within 3.5% of their true values. This is not surprising since with ε close to 1 the impact of changing levels of target cells should be negligible since these levels only enter in the model in terms of the form $(1-\varepsilon)kVT$. We thus also studied the effects of assuming lower efficacy of therapy.

Equation (1) was derived assuming ε=1[1]. When ε <1, and eqn, (1) is used to fit data, δ and μ are is always underestimated because in reality more killing of infected cells is needed to counterbalance their continuing generation by de novo infection[2]. Thus, if we generate surrogate data with lower values of ε and fit the surrogate data with equation (1) we will obtain lower estimated values of δ and μ, irrespective of whether or not the system is originally in steady state. We also confirmed this by using the procedure given above for non steady-state viral levels and increased numbers of target cells. Thus, these simulations showed that the actual values of δ and μ in systems with CD8+ lymphocyte depletion may be even higher than we estimate. For example, the underestimate of δ caused by CD4+ T cell increase and ε=0.8 was 6.2 % (w/o CD8+ lymphocyte depletion) and 15.6 % (w/ CD8+ lymphocyte depletion, p_L=200 day^{-1}) respectively, while under the same conditions the underestimates of μ were 19 % (w/o CD8+ lymphocyte depletion) and 42 % (w/ CD8+ lymphocyte depletion). This larger underestimate of μ with CD8+ lymphocyte depletion may be the reason that the estimated lifespan of long-lived cells ($1/\mu$) cited in the main text is larger with CD8+ lymphocyte depletion than without it (9.6 vs 8.8 days). However, these underestimates do not change our conclusion in the main text that CD8+ T cells are not required for the rapid loss of productively infected cells.

Supplementary Note 3. In order to determine whether the number of animals used were adequate to detect a difference in infected cell lifespans with CD8+ lymphocyte depletion, a power analysis was completed. The average lifespan of short-lived productively infected cells for all treatment periods taken together was 1.0 day, with a standard deviation of 0.3 days, which is consistent with a standard deviation of 0.4 days found in a smaller previous study[3]. If we assume that CD8+ T cells are responsible for the loss of productively infected CD4+ T cells, then we expect that CD8+ lymphocyte depletion would increase the lifespan of these cells to that of an activated (possibly effector) CD4+ T cell of several days. In mice this lifespan has been estimated to be at least ~5 days[4]. Thus, we need to be able to identify an increase in lifespan of 4 days. Given the small standard deviations observed, it is intuitive that we should be able to find such a difference very reliably with our study. Indeed, a priori power calculation indicated that with 10 animals, with a standard deviation of lifespan of 0.4 days, we should be able to identify in this crossover study a difference of 0.7 days with more than 90% probability. After the study analysis, using Schuirmann's two one-sided tests to assess equivalence[5, 6] indicates that we can reject the hypothesis that the life spans with and without CD8+ lymphocytes are not equivalent at the 0.05 level, if we define equivalence as the life spans not differing by more than 0.25 days. Thus, these results give us confidence that depletion of CD8+ lymphocytes indeed does not increase substantially the lifespan of productively infected cells.

Supplementary References

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