

Profound CD4⁺/CCR5⁺ T cell expansion is induced by CD8⁺ lymphocyte depletion but does not account for accelerated SIV pathogenesis

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Depletion of CD8⁺ lymphocytes during acute simian immunodeficiency virus (SIV) infection of rhesus macaques (RMs) results in irreversible prolongation of peak-level viral replication and rapid disease progression, consistent with a major role for CD8⁺ lymphocytes in determining postacute-phase viral replication set points. However, we report that CD8⁺ lymphocyte depletion is also associated with a dramatic induction of proliferation among CD4⁺ effector memory T (T_{EM}) cells and, to a lesser extent, transitional memory T (T_{TrM}) cells, raising the question of whether an increased availability of optimal (activated/proliferating), CD4⁺/CCR5⁺ SIV "target" cells contributes to this accelerated pathogenesis. In keeping with this, depletion of CD8⁺ lymphocytes in SIV⁻ RMs led to a sustained increase in the number of potential CD4⁺ SIV targets, whereas such depletion in acute SIV infection led to increased target cell consumption. However, we found that the excess CD4⁺ T_{EM} cell proliferation of CD8⁺ lymphocyte-depleted, acutely SIV-infected RMs was completely inhibited by interleukin (IL)-15 neutralization, and that this inhibition did not abrogate the rapidly progressive infection in these RMs. Moreover, although administration of IL-15 during acute infection induced robust CD4⁺ T_{EM} and T_{TrM} cell proliferation, it did not recapitulate the viral dynamics of CD8⁺ lymphocyte depletion. These data suggest that CD8⁺ lymphocyte function has a larger impact on the outcome of acute SIV infection than the number and/or activation status of target cells available for infection and viral production.

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Abbreviations used: APC, allophycocyanin; PID, postinfection day; RM, rhesus macaque; SIV, simian immunodeficiency virus; T_N cell, naive T cell; T_{TrM} T cell, transitional memory T cell.

In the initial weeks of HIV infection of humans and pathogenic simian immunodeficiency virus (SIV) infection of Asian macaques, viral replication peaks, then declines to a quasiequibrated set point of ongoing viral production and clearance, the level of which plays a major role in determining the subsequent tempo of disease progression (Mellors et al., 1996; Staprans et al., 1999). Outcomes range from an inability to substantially restrain viral replication from peak levels, leading to early immunological

collapse and rapid progression to AIDS, to control of viral replication to undetectable levels and long-term nonprogression (Farzadegan et al., 1996; Picker et al., 2004; Deeks and Walker, 2007; Goulder and Watkins, 2008). However, the vast majority of infections manifest viral replication set points and progression rates between

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these two extremes (Munoz et al., 1989; Okoye et al., 2007). The mechanisms responsible for these different outcomes have not been precisely defined, although differences in adaptive immunity, innate immunity, and CD4⁺, CCR5⁺ target cell availability, susceptibility to infection, productivity (viral yield per infected cell), and dynamics have all been implicated (Goldstein et al., 2000; Seman et al., 2000; Zhang et al., 2004; Alter et al., 2007; Goulder and Watkins, 2008; Lehner et al., 2008; Mahalanabis et al., 2009).

The HIV/SIV-specific CD8⁺ T cell response has been widely accepted as a major, if not dominant, contributor to this heterogeneity of outcomes based on the observations that (a) the appearance of these responses is temporally coordinated with the postpeak fall in viral replication (Koup et al., 1994), (b) vaccines that elicit strong CD8⁺ T cell responses can lower viral replication set points compared with unvaccinated controls (Wilson et al., 2006; Liu et al., 2009), (c) particular class I MHC alleles and their associated CD8⁺ T cell responses are strongly associated with postpeak control of viremia (Goulder and Watkins, 2008), (d) viral mutations facilitating escape from CD8⁺ T cell recognition can be associated with either loss of virologic control or a fitness cost that handicaps replication of escaped virus (Barouch et al., 2002; Goulder and Watkins, 2008), and (e) treatment of rhesus macaques (RMs) with depleting anti-CD8⁺ mAbs at the outset of SIV infection, transiently depleting CD8⁺ lymphocytes from blood and secondary lymphoid tissues, typically results in unrestrained viral replication and rapid disease progression (Matano et al., 1998; Schmitz et al., 1999; Kim et al., 2008; Veazey et al., 2008). On the other hand, there is considerable circumstantial evidence suggesting that the availability, susceptibility to infection, and cumulative per cell virus production of HIV/SIV target cells may also play a major role in determining acute-phase viral dynamics and subsequent viral load set points. In early acute SIV infection, the primary target cells are small, resting CD4⁺, CCR5⁺ T_{EM} and transitional memory T (T_{TM}) cells in tissues; massive infection and destruction of these cells corresponds to the initial peak of viral replication and its subsequent decline (Picker et al., 2004; Li et al., 2005; Mattapallil et al., 2005). With the destruction of resting CD4⁺ target cells and the onset of infection-associated inflammation, the infection shifts to predominant replication in activated, proliferating CD4⁺ T_{EM} and T_{TM} cells (Zhang et al., 2004; Haase, 2005). These observations suggest that in typical SIV infections, plateau-phase viral replication might depend on both the rate of new target cell production and the enhanced per cell virus production of activated target cells. Consistent with this, it has been well documented that both coinfection with other pathogens and other modes of immune activation in acute infection, which increase the number of activated target cells, are associated with increased levels of viral replication and rapid disease progression (Folks et al., 1997; Zhou et al., 1999; Sequer et al., 2002; Garber et al., 2004; Cecchinato et al., 2008).

Determination of the interplay between cellular immune effector responses and target cell dynamics in the regulation of acute-phase HIV/SIV replication will be crucial to understand

the potential impact of vaccines and other immunomodulators on HIV/SIV pathogenesis. However, experimental dissection of these processes in vivo is complicated by the fact that primary viral targets—CD4⁺ memory T cells—are an intrinsic and essential part of the adaptive immune response and are almost invariably coregulated with CD8⁺ lymphocyte-mediated effector responses. In this paper, we report on the use of CD8⁺ lymphocyte depletion in RMs, which effectively eliminates CD8⁺ effector lymphocyte responses in acute SIV infection (Matano et al., 1998; Schmitz et al., 1999; Kim et al., 2008; Veazey et al., 2008), to determine the importance of target cell expansion and activation on viral dynamics in acute SIV infection. We document that CD8⁺ lymphocyte depletion induces massive selective proliferation of CD4⁺, CCR5⁺ memory T cell targets, leading to profound expansion of this population in uninfected RMs and their increased consumption in acute SIV infection. Furthermore, we identify IL-15 as the major mediator of this proliferative response. Significantly, however, in vivo inhibition of IL-15 by administration of a neutralizing anti-IL-15 mAb during CD8⁺ lymphocyte depletion of acutely SIV-infected RMs blocked induction of CD4⁺ target cell proliferation, but did not reverse either the high viral replication or rapid disease progression in CD8⁺ lymphocyte-depleted, acutely infected animals. Moreover, administration of exogenous IL-15 during acute SIV infection in RMs with an intact CD8⁺ lymphocyte compartment stimulated CD4⁺ target cell proliferation with kinetics similar to CD8⁺ lymphocyte depletion, but did not recapitulate the early viral dynamics of CD8⁺ lymphocyte depletion. Collectively, these data provide compelling evidence that the relative effectiveness of adaptive and/or innate immune function of CD8⁺ lymphocytes plays a considerably more significant role in determining outcome of acute SIV infection than differences in target cell number and/or activation status.

RESULTS

CD8⁺ lymphocyte depletion during acute SIVmac239 infection abrogates the postpeak decline in viral replication and results in rapid disease progression

i.v. administration of highly pathogenic, CCR5-tropic SIV (SIVmac239 or SIVmac251) to Indian-origin RMs results in a characteristic pattern of plasma viremia arising from the massive, systemic infection of CD4⁺ memory T cells (Picker et al., 2004; Mattapallil et al., 2005; Grossman and Picker, 2008). As shown in Fig. 1, acute-phase plasma viral loads peak at postinfection day (PID) 10 at a mean value of $\sim 3 \times 10^7$ SIV RNA copy equivalents/ml, and in typical infections, those destined for AIDS-free survival for >200 d ("normal" progressors; Picker et al., 2004), plasma viral loads drop by a mean of 1.85 logs in the subsequent 32 d to a stable plateau-phase level. In RMs administered the depleting, CD8 α -specific mAb cM-T807 concomitant with SIV infection, mean plasma viral loads are identical to control infections through PID 10, but in these CD8⁺ lymphocyte-depleted RMs (see also Fig. 5 A), plasma viral loads continue to rise from PID 10 to 14 by a mean of 0.3 logs, and thereafter fall only 0.21 logs to plateau-phase

plasma viral loads $>4 \times 10^7$ copy equivalents/ml (Fig. 1). In keeping with these high viral replication rates, all seven of the CD8⁺ lymphocyte-depleted RMs studied in this experiment manifested rapid disease progression, with a median time to overt AIDS of 92 d (range = 42–161 d).

CD8⁺ lymphocyte depletion induces profound proliferation and expansion of CD4⁺, CCR5⁺ memory T cells in SIV⁻ RMs

These plasma viral load patterns indicate that the effect of CD8⁺ lymphocyte depletion on acute-phase SIV replication manifests quite early, between PID 10 and 14. Although abrogation of developing CD8⁺ T cell effector responses could certainly account for the accelerated viral replication of CD8⁺ lymphocyte-depleted RMs, it is significant that this time period also corresponds to the peak of the massive, direct or indirect destruction of viral targets (Li et al., 2005; Mattapallil et al., 2005), raising the possibility that a limitation in target cell availability may also restrict subsequent viral production (Haase, 2005). Given the overlap between regulatory factors controlling CD4⁺ and CD8⁺ population dynamics (Moniuszko et al., 2004; Picker et al., 2006; Boyman et al., 2007), CD8⁺ lymphocyte depletion might set in motion a homeostatic reaction that would spur production and/or activation of CD4⁺, CCR5⁺ memory T cells, and thereby provide additional and/or more productive targets for the maintenance of high level viral production.

To investigate this latter possibility, we initially assessed the effect of CD8⁺ lymphocyte depletion on CD4⁺ T cell dynamics in healthy, SIV-uninfected RMs. As shown in Fig. 2 A, cM-T807 administration to such RMs completely depleted CD8⁺ T cells from the circulation for ~ 14 d, followed by a slow and incomplete recovery over the period of follow up.

Except for a transient decline immediately after the first cM-T807 dose, the absolute counts of CD4⁺ naive T (T_N) cells in blood remained steady after CD8⁺ lymphocyte depletion. In contrast, the absolute counts of total CD4⁺ memory T cells in these RMs progressively increased after day 14, peaking, on average, at more than twice their initial levels at days 42–70 before declining back toward baseline. This memory expansion suggests the development of a CD4⁺ memory T cell “homeostatic” response to CD8⁺ lymphocyte depletion, and in keeping with this, starting at day 3 and peaking at day 14 we observed a profound but highly asymmetric increase in the proliferative fraction of certain CD4⁺ memory T cell subsets (Fig. 2 B). Although both CD4⁺ T_N and CD4⁺, CD28⁺/CCR7⁺ T_{CM} T cells showed no or little proliferative response to CD8⁺ lymphocyte depletion, the CD4⁺, CD28⁺/CCR7⁻ T_{TM} and the CD4⁺, CD28⁻, CCR7⁻ T_{EM} cell subsets manifested moderate and very strong proliferative responses, respectively.

The proliferative asymmetry among CD4⁺ memory T cell subsets in response to CD8⁺ lymphocyte depletion was further explored in two additional SIV-uninfected RMs (Fig. 3). In these RMs, CD4⁺ memory T cell subsets were defined by expression patterns of CD28, CCR7, and CCR5, allowing precise definition of progressive memory differentiation from CD28⁺/CCR7⁺/CCR5⁻ T_{CM} cells to the earliest transitional effector memory subset (CD28⁺/CCR7⁺/CCR5⁺ T_{TM-1}), to more mature transitional effector memory cells (CD28⁺/CCR7⁻/CCR5⁺ T_{TM-2}), and, finally, to fully differentiated T_{EM} cells (CD28⁻/CCR7⁻/CCR5^{dim+}; Picker et al., 2006; Grossman and Picker, 2008). As shown in Fig. 3 A, the CD4⁺ memory T cell proliferative response to CD8⁺ lymphocyte depletion followed this differentiation process

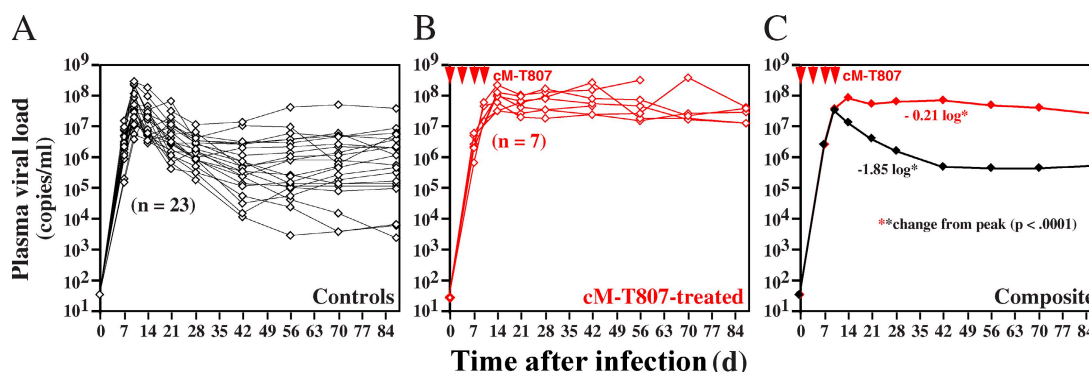


Figure 1. CD8⁺ lymphocyte depletion abrogates the postpeak decline in viral replication rates during acute SIVmac239 infection.

(A and B) Comparison of log-transformed, acute-phase plasma viral load profiles of (A) 23 SIVmac239-infected, untreated normal progressors versus (B) 7 RMs treated with 10, 5, 5, and 5 mg/kg of the depleting anti-CD8 α mAb cM-T807 at days 0, 3, 7, and 10 of SIVmac239 infection, respectively. Normal progression was defined as SIV-infected RMs that either manifested disease-free survival for >200 d without antiretroviral treatment or that manifested the typical immunological characteristics of normal progression through a minimum of 100 d of untreated infection (Picker et al., 2004; Okoye et al., 2007). None of the cM-T807-treated RMs expressed class I MHC alleles (*Mamu* B*08 and B*17) associated with spontaneous SIV control (Goulder and Watkins, 2008). The effects of cM-T807 treatment on CD8⁺ T cell numbers in the blood of these seven RMs are shown in Fig. 5. (C) The means of the log-transformed plasma viral loads at each time point for each group are shown together. For each RM, the log viral load numbers from days 42–84 were averaged to define plateau-phase levels, and the differences between these levels and the viral load peaks were determined so as to define the mean “change from peak” for the CD8⁺ lymphocyte-depleted and control groups. The significance of the difference in these values in the CD8⁺ lymphocyte-depleted and control groups was determined by an unpaired Student’s *t* test, with the *p*-value shown.

precisely, with no response among T_{CM} cells and an increasing response magnitude from T_{TrM-1} to T_{TrM-2} to T_{EM} cells. Changes in the absolute counts of these subsets in blood were in keeping with this pattern of proliferation: $CD4^+ T_{CM}$ and T_{TrM-1} cells were, on a fold-change basis, largely stable over the course of $CD8^+$ lymphocyte depletion (although showing some random fluctuation); $CD4^+ T_{TrM-2}$ cells were stable in one RM, and showed a modest posttreatment increase in the other; and $CD4^+ T_{EM}$ cell numbers increased dramatically (approximately ninefold) in both RMs. Strikingly, the $CD4^+ T_{EM}$ cell proliferative response in these RMs lasted for

30–40 d after the onset of cM-T807 treatment, and the absolute $CD4^+ T_{EM}$ cell counts peaked still later (\sim day 50), indicating that the homeostatic mechanism induced by $CD8^+$ lymphocyte depletion has prolonged effects.

It is noteworthy that the frequency of actively proliferating (Ki-67⁺) effector site $CD4^+$ T cells (T_{TrM} and/or T_{EM} cells in lung airspace and small intestinal lamina propria) was not increased at day 7 after $CD8^+$ lymphocyte depletion compared with baseline (unpublished data). These data indicate that the stimulation of $CD4^+$ T cells by $CD8^+$ lymphocyte depletion primarily affects the cells recirculating among secondary lymphoid tissues, either via induction or maintenance of proliferation in preexisting or developing $CD4^+ T_{TrM}$ and T_{EM} cells, and/or the simultaneous induction of proliferation and effector memory differentiation among $CD4^+ T_N$ or $CD4^+ T_{CM}$ cells. However, because induction of proliferation among circulating $CD4^+ T_{TrM}$ and T_{EM} cells is associated with extensive migration of their postproliferative progeny into effector sites (Picker et al., 2006; Grossman and Picker, 2008), it is highly likely that the expansion of $CD4^+ T_{TrM}$ and T_{EM} cell populations in response to $CD8^+$ lymphocyte depletion does include the extralymphoid effector site compartment.

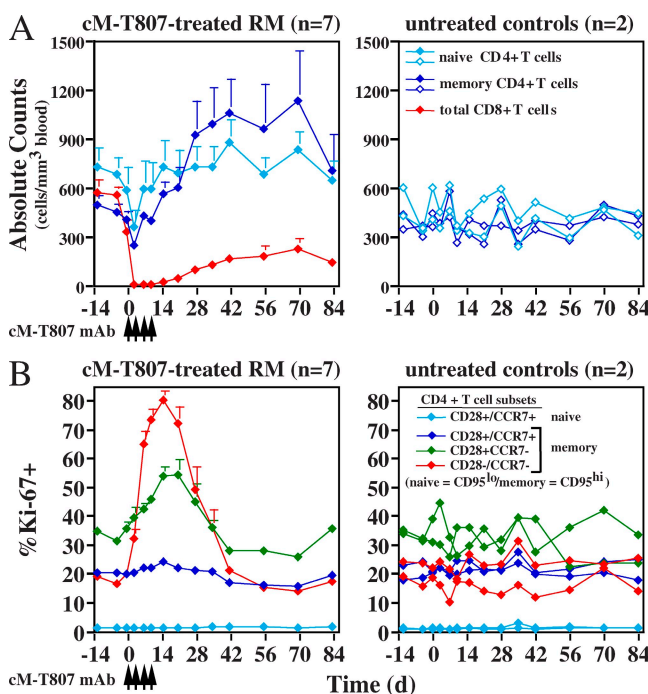


Figure 2. $CD8^+$ lymphocyte depletion of SIV^- RMs induces $CD4^+$ memory T cell proliferation and expansion. (A and B) The effect of cM-T807-mediated $CD8^+$ lymphocyte depletion on $CD4^+$ T cell subset dynamics was evaluated in seven SIV^- RMs. Two cohoused untreated SIV^- RMs served as negative controls. (A) The fraction of naive versus memory $CD4^+$ T cells in blood was determined by flow cytometric evaluation of CD28, CD95, and CCR7 expression patterns (Walker et al., 2004; Picker et al., 2006), and was used to calculate absolute numbers of these cells, with the mean \pm SEM shown at each time point relative to cM-T807 treatment (the results for the two control RMs are shown individually). Absolute numbers of total $CD8^+$ T cells in blood are also shown for the cM-T807-treated RMs. In the period from 28 to 70 d after initial treatment, absolute $CD4^+$ memory cell counts were 2.1 ± 0.3 -fold higher than at baseline, whereas the absolute $CD4^+ T_N$ cell counts increased by only 1.1 ± 0.02 -fold. The two untreated RMs showed no net increase in either subset. (B) The proliferative fractions of $CD4^+ T_N$ cells and the $CD4^+$ memory T cell subsets defined by CD28 and CCR7 expression were evaluated by determination of the percentage of each subset expressing Ki-67. The difference in mean change \pm SEM in percent Ki-67 from baseline to posttreatment peak was 61.9 ± 3.2 , 20.3 ± 2.4 , and $3.9 \pm 1.09\%$ for $CD28^+$, CCR7⁺ memory $CD4^+$ T cells, $CD28^+$, CCR7⁺ T_{TrM} , and $CD28^+$, CCR7⁺ T_{CM} cells, respectively ($P < 0.0001$, and $P = 0.0001$ and 0.012 , respectively, by the paired Student's *t* test). The two untreated control RMs showed only random fluctuation of percent Ki-67 over the same time period.

$CD8^+$ lymphocyte depletion during acute SIV infection is associated with the activation and rapid consumption of optimal SIV "target" T cells

These data indicate that $CD8^+$ lymphocyte depletion with mAb cM-T807 results in the robust activation, proliferation, and expansion of $CD4^+$, CCR5⁺ memory T cells, precisely those cells targeted by CCR5-tropic SIV and capable of the highest viral production (Picker et al., 2004; Zhang et al., 2004). As suggested above, it is conceivable that such target cell enhancement during the upswing of viral replication in acute SIV infection contributes to or even accounts for the accelerated viral dynamics and pathogenesis associated with $CD8^+$ lymphocyte depletion during acute infection. In keeping with this, we observed that $CD8^+$ lymphocyte depletion induces massive proliferation of $CD4^+ T_{TrM}$ and, particularly, $CD4^+ T_{EM}$ cells 7 d after SIV infection, dramatically higher than what is observed in control SIV infections at this time point (Fig. 4). This effect is quantified for seven cM-T807-treated versus eight untreated control RMs with acute SIV-mac239 infection in Fig. 5. The induction of proliferation in the $CD4^+ T_{TrM}$ and T_{EM} cell populations in cM-T807-treated, SIV-infected RMs was similar to that observed in uninfected RMs (Fig. 3), but in striking contrast to the uninfected RMs, these populations did not increase in size but instead dramatically collapsed, to a much greater extent than control SIV infections (Fig. 5 B). $CD4^+ T_{TrM}$ and T_{EM} cells were therefore activated and then destroyed in the setting of cM-T807 treatment during acute SIV infection, likely by direct infection. In addition, these $CD4^+ T_{TrM}$ and T_{EM} cells were not partially regenerated by enhanced production, as they typically are in control infections. We have previously shown that such new $CD4^+ T_{TrM}$ and T_{EM} cell production (and AIDS-free survival) is dependent on inducing and maintaining increased

proliferation and differentiation of CD4⁺ T_{CM} cells, the substrate for CD4⁺ T_{TrM} and T_{EM} cell production (Picker et al., 2004; Okoye et al., 2007). This response, which characteristically initiates on approximately PID 17, is significantly delayed in the CD8⁺ lymphocyte-depleted RMs, and CD4⁺ T_{CM} cell populations in these RMs stabilize at significantly lower levels than in controls (Fig. 5 B). This compromise of CD4⁺ T_{CM} cell homeostasis and the associated failure of CD4⁺ T_{TrM} and T_{EM} cell production is similar to that observed in spontaneous rapid progressors (Picker et al., 2004), and almost certainly underlies (or significantly contributes to) the rapid clinical progression observed in these RMs.

IL-15 mediates the CD4⁺ T_{EM} cell proliferative response associated with CD8⁺ lymphocyte depletion, but IL-15 blockade does not abrogate accelerated viral replication and disease progression of CD8⁺ lymphocyte depletion in acute SIV infection

The pattern of selective CD4⁺ T_{TrM} and T_{EM} cell proliferation induced by CD8⁺ lymphocyte depletion is highly reminiscent of the effects of *in vivo* administration of IL-15 in RMs (Picker et al., 2006). In addition, IL-15 can promote simultaneous proliferation and effector memory differentiation among highly purified CD4⁺ T_{CM} cells (unpublished data), consistent with an ability to recruit and expand T_{TrM} and T_{EM} cells from recirculating T_{CM} cell precursors. We therefore

hypothesized that the ability of mAb cM-T807 to abruptly deplete CD8⁺ T cells and NK cells from blood and lymphoid tissues would either decrease consumption or increase production of IL-15, potentially exposing CD4⁺ T cells in these sites to high levels of this regulatory cytokine. To investigate this possibility, we treated two cohorts of CD8⁺ lymphocyte-depleted, acutely SIV-infected RMs with either a neutralizing anti-IL-15 mAb or an isotype-matched control. As shown in Fig. 6 A, CD8⁺ lymphocyte depletion was associated with a marked spike in plasma IL-15 levels in the control but not in the anti-IL-15-treated RMs. Moreover, the CD4⁺ T_{EM} cell proliferative response to CD8⁺ lymphocyte depletion was completely abrogated by the anti-IL-15 mAb, although the smaller CD4⁺ T_{TrM} cell proliferative response was not (Fig. 6 B). Most significantly, despite complete inhibition of the CD4⁺ T_{EM} cell proliferative response, the anti-IL-15 treatment did not alter the other pathophysiologic characteristics of CD8⁺ lymphocyte-depleted acute infection, including (a) the rapid consumption of CD4⁺ T_{EM} cells, (b) the delayed CD4⁺ T_{CM} cell proliferative response, (c) the lower plateau levels of CD4⁺ T_{CM} cells, (d) the failure to lower postpeak viral replication, and (e) accelerated disease progression (Fig. 6, B and C). The extent of derangement of these parameters was somewhat reduced in both the anti-IL-15- and control-treated cohorts compared with the CD8⁺ lymphocyte-depleted RMs presented in Fig. 5 (most likely because of the shorter duration

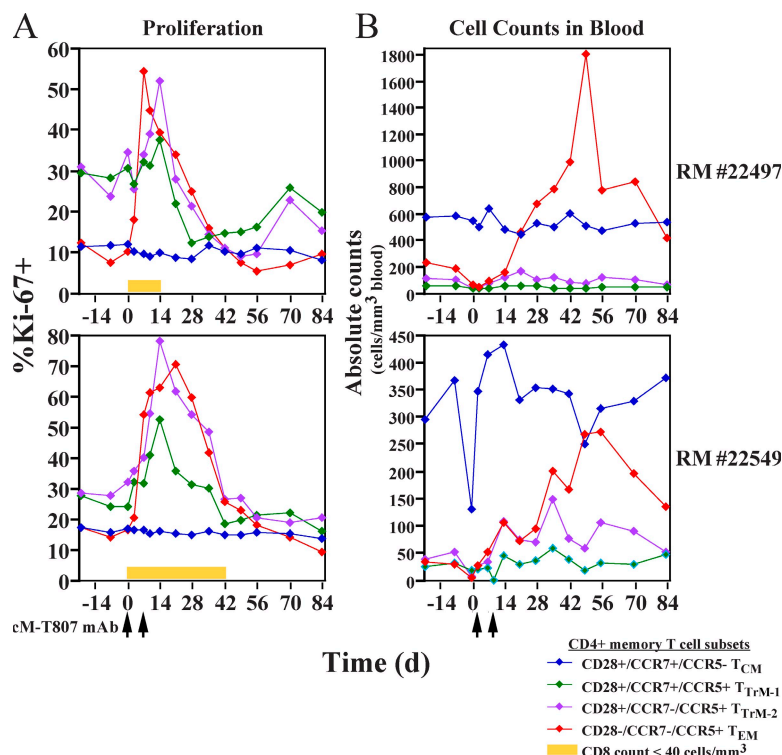


Figure 3. The effect of CD8⁺ lymphocyte depletion on CD4⁺ memory T cell proliferation and expansion is restricted to CCR5-expressing cells and increases with progressive effector memory differentiation. (A and B) Two additional SIV⁻ RMs were CD8⁺ lymphocyte-depleted with 50 mg/kg of mAb cM-T807 at days 0 and 7, and followed for changes in (A) the proliferative fraction and (B) absolute number of circulating CD4⁺ memory T cell subsets defined by CD28, CCR7, and CCR5 expression (T_{CM} → T_{TrM-1} → T_{TrM-2} → T_{EM}, from least to most "effector" differentiated).

of CD8⁺ lymphocyte depletion in this vs. the previous experiment: 17–21 vs. 35 d; Veazey et al., 2008), but the observation that the anti-IL-15- and control-treated cohorts did not show significant differences in these parameters indicates that the IL-15-induced CD4⁺ T_{EM} cell proliferative response is not a primary mediator of the accelerated pathogenesis of CD8⁺ lymphocyte depletion.

Administration of recombinant IL-15 during acute SIV infection recapitulates the CD4⁺ T_{EM} and T_{RM} cell proliferation of CD8⁺ lymphocyte depletion but not the immediate postpeak loss of virologic control

Anti-IL-15 did not block the CD4⁺ T_{EM} cell proliferative response to CD8⁺ lymphocyte depletion, leaving open the possibility that this response, though of smaller magnitude than the CD4⁺ T_{EM} cell proliferative response, plays a role in supporting the high level, postpeak viral replication characteristic of CD8⁺ lymphocyte depletion. To address this issue, we treated five acutely SIV-infected RMs with 50 µg/kg of recombinant IL-15 on PID 0, 3, and 7 (Picker et al., 2006), and compared cell dynamic and virologic parameters of these RMs with those of untreated, acutely infected controls.

As shown in Fig. 7, exogenous IL-15 induced CD4⁺ T_{EM} and T_{RM} cell proliferative responses with kinetics similar to CD8⁺ lymphocyte depletion. The magnitude of peak CD4⁺ T_{EM} cell proliferation was slightly reduced compared with that observed with CD8⁺ lymphocyte depletion, but the magnitude of the CD4⁺ T_{RM} cell proliferative response was equivalent. However, the virologic course of these IL-15-treated SIV infections did not recapitulate the CD8⁺ lymphocyte depletion pattern, most notably by the ability of these RMs to initially control viral replication rates immediately after the PID 10 peak. Indeed, through day 42, the mean plasma viral loads of these RMs overlapped those of untreated controls (although it is notable that a slight “blip” in viral replication was noted at PID 17 in the IL-15-treated group).

IL-15 administration in acute SIV infection has been associated with accelerated progression (Mueller et al., 2008), and strikingly, mean plasma viral loads began to diverge from those of the control group at PID 56 and rose to levels associated with rapid disease progression by PID 84 (Fig. 7 A). These data suggest that the accelerated progression of IL-15-treated RMs is not related to target cell activation and/or expansion (which was not apparent immediately before or at

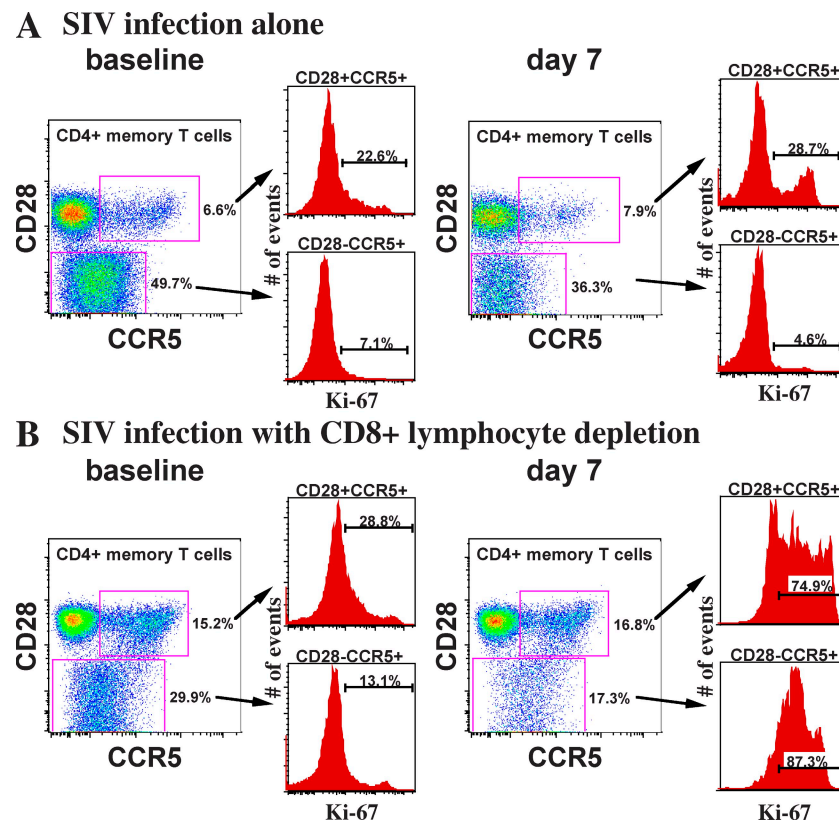


Figure 4. CD8⁺ lymphocyte depletion during acute SIV infection is associated with a marked increase in the proliferating fraction of circulating, CD4⁺, CCR5⁺ “SIV targets” during the rising phase of viral replication. (A and B) Representative flow cytometric profiles of CD4⁺ memory subset proliferation at baseline and at 7 d after i.v. inoculation with SIVmac239 in (A) an untreated control RM and (B) an RM treated with 10, 5, 5, and 5 mg/kg of mAb cM-T807 at days 0, 3, 7, and 10, respectively. The bivariate dot plots in the figure reflect events that were gated on CD3⁺, CD4⁺, small lymphocytes first, followed by gating on the CD28 versus CD95 profiles to include the overall memory population (Picker et al., 2006). The Ki-67 histograms were gated as shown in the dot plots (pink boxes).

the time of the viral load rise) but rather to a loss of immunological control. Indeed, it is noteworthy that CD8⁺ T_{EM} and T_{TrM} cells, like their CD4⁺ counterparts, were highly responsive to IL-15 and manifested a profound burst of proliferation at PID 7, followed by an abrupt decline (Fig. 7 C). Moreover, whereas control infections typically manifest and maintain an increase in proliferation by CD8⁺ (as well as CD4⁺) T_{EM} and T_{TrM} cells starting at PID 14–17, almost certainly including SIV-specific effectors, these responses were diminished and/or poorly sustained in IL-15-treated RMs. These results suggest that premature and/or excessive exposure of

the developing SIV-specific CD8⁺ (and perhaps CD4⁺) T cell response to IL-15 might compromise SIV-specific memory T cell generation and stability, and thereby account for the subsequent loss of virologic control in these IL-15-treated, acutely SIV-infected RMs (see Discussion).

DISCUSSION

The ability of HIV and SIV to target the major (CD4⁺) lineage of memory/effector T cells and to replicate to the highest level in activated members of this lineage sets up a dilemma for the adaptive immune system: a strong response to infection

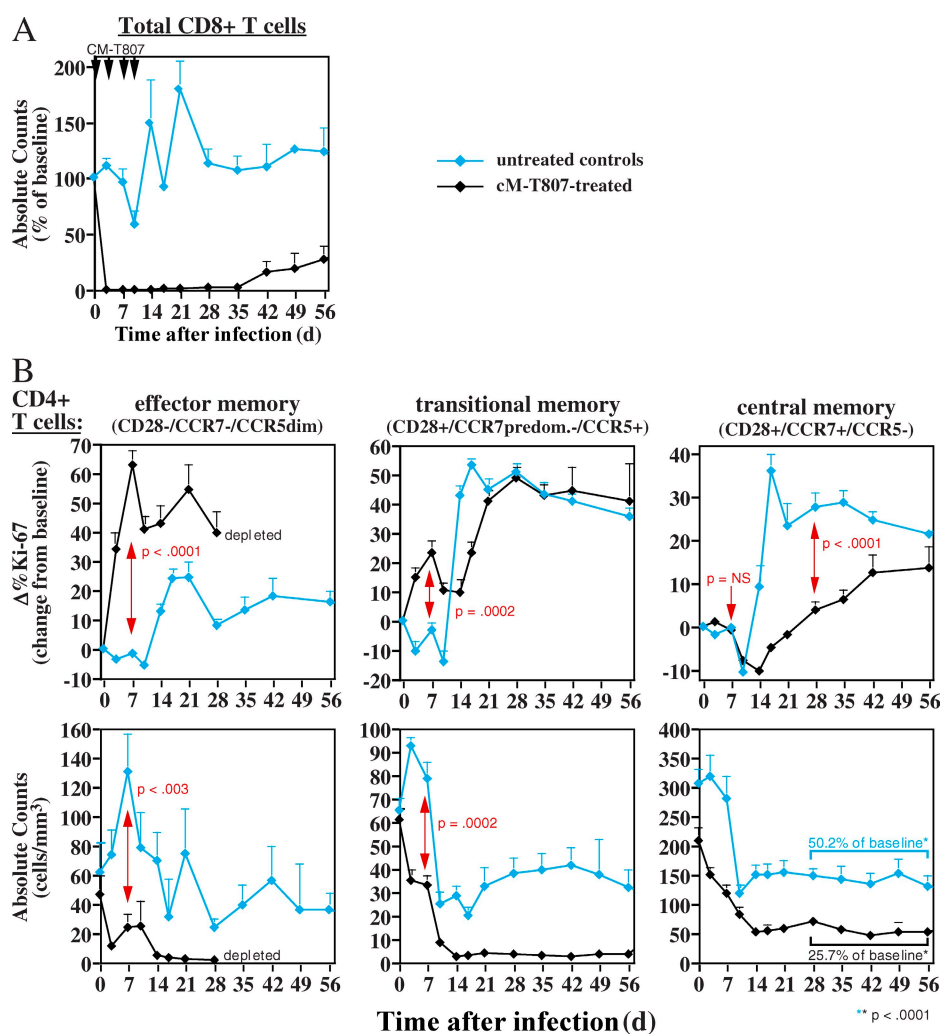


Figure 5. The induction of massive proliferation among CD4⁺, CCR5⁺ viral targets accelerates their depletion in acute SIV infection and is associated with an inadequate CD4⁺ T_{CM} cell homeostatic response. (A and B) Comparison of (A) total CD8⁺ T cell counts and (B) CD4⁺ memory T cell subset dynamics in the peripheral blood of seven CD8⁺ lymphocyte-depleted, SIVmac239-infected RMs (10, 5, 5, and 5 mg/kg of mAb cM-T807 at days 0, 3, 7, and 10, respectively; black) versus eight control SIVmac239 infections (blue). The viral load profiles of the CD8⁺ lymphocyte-depleted group are shown in Fig. 1. The control group used in this experiment is a subset of the normal progressing SIVmac239 infections shown in Fig. 1. This subgroup had a mean \pm SEM log-transformed peak (day 10) and plateau phase (days 42–87) viral load of 7.5 ± 0.2 and 5.9 ± 0.3 copies/ml, respectively. The data shown reflect the means \pm SEM of the designated parameters, presented as either change from preinfection baseline (CD8⁺ T cell counts and proliferative fraction) or the actual measured value (absolute CD4⁺ memory T cell subset counts). Analysis of the CD4⁺ T_{EM} cell subset was discontinued at day 28, as this population was completely depleted in the majority of CD8⁺ lymphocyte-depleted RMs by day 35, preventing further analysis. The red arrows indicate analysis of the significance of the difference in the designated values by the unpaired Student's *t* test, with the *p*-values shown. Student's *t* test analysis of differences in plateau-phase CD4⁺ T_{CM} cell numbers (bottom right) was made after normalization of the data to "change from baseline," with the *p*-value shown.

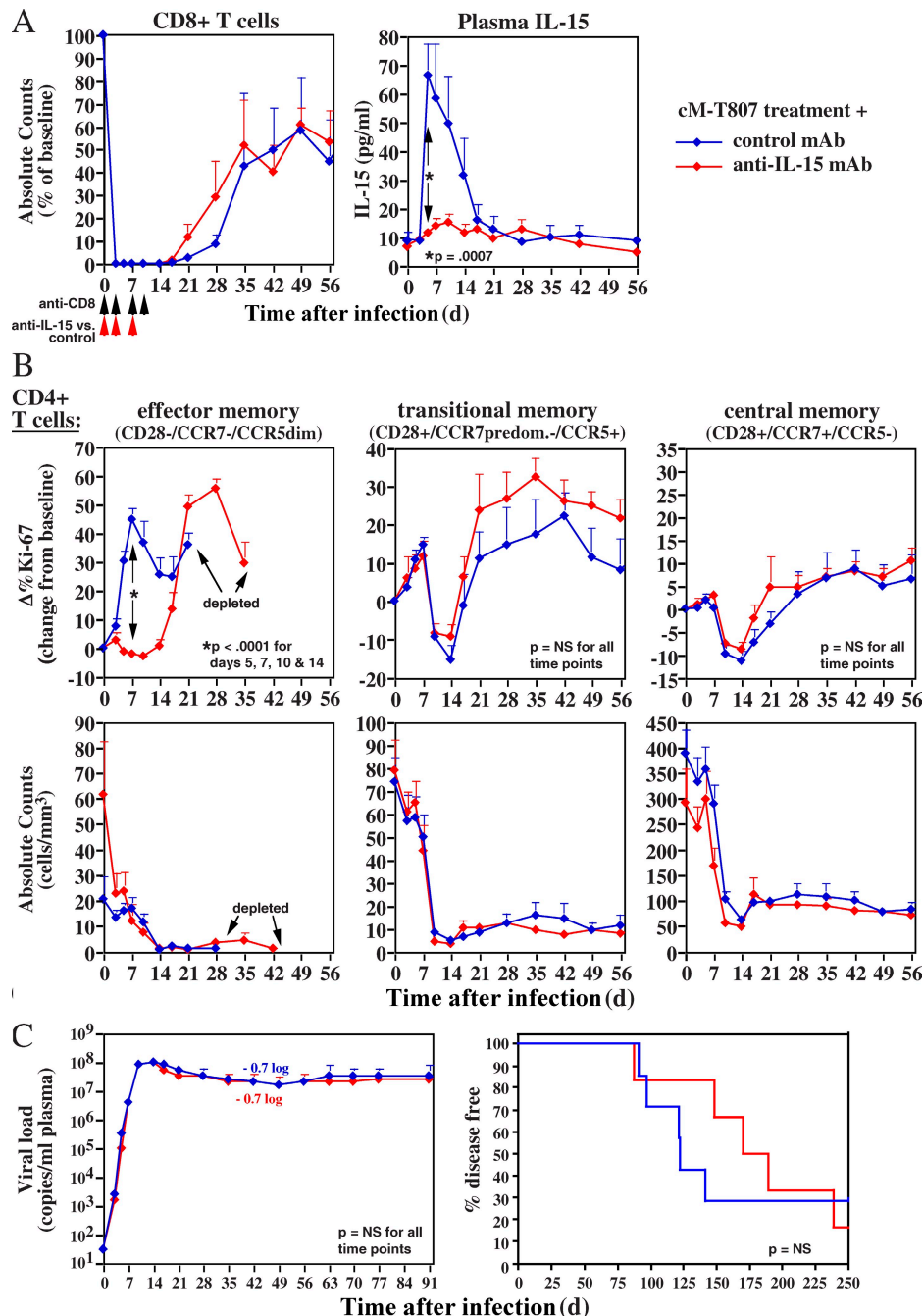


Figure 6. A neutralizing anti-IL-15 mAb specifically blocks the CD4⁺ T_{EM} cell proliferative response associated with CD8⁺ lymphocyte depletion during acute SIV infection but does not block accelerated viral replication rates or prevent rapid progression in these depleted RMs. 16 RMs that were SIVmac239 infected and CD8⁺ lymphocyte depleted with mAb cM-T807, as described in Fig. 5, were divided into two groups of 8 RMs each, with one group additionally receiving the neutralizing anti-IL-15 M111 mAb and the other group receiving an isotype-matched control (both given at 5 mg/kg i.v. on days 0, 3, and 7). One RM in the M111-treated group had inadequate CD8⁺ lymphocyte depletion and was not further considered, leaving a total of seven mAb M111-treated and eight control-treated RMs for analysis. (A) The means \pm SEM of the change in CD8⁺ T cell counts from baseline and of the concentration of plasma IL-15 of the anti-IL-15- (left) and control-treated (right) RMs are shown. The significance of the difference in IL-15 levels in day 5 plasma in these two groups was determined by the unpaired Student's *t* test, with the *p*-value shown. (B) The means \pm SEM of the change from baseline in percent Ki-67 within the designated CD4⁺ memory T cell subsets (top) and of the absolute cell counts of these subsets in blood (bottom) are shown for the anti-IL-15- (red) and control-treated (blue) groups. Analysis of the CD4⁺ T_{EM} cell subset was discontinued at days 21 and 35 for the control- and M111-treated groups, respectively, as this population was completely depleted in the majority of RMs in these groups at these times, preventing further analysis. The significance of the differences in Δ Ki-67 between the anti-IL-15- and control-treated groups was determined by the unpaired Student's *t* test, with the *p*-values shown. (C, left) The means \pm SEM of the log-transformed plasma viral loads of the anti-IL-15- (red) and control-treated (blue) groups are shown, with the significance of

might bring to bear potent antiviral effector responses, but if it includes a CD4⁺ T cell response, it will also, inevitably, increase both the number of potential target cells and the level of virus production per infected target cell, potentially enhancing viral replication and accelerating pathogenesis (Douek et al., 2002; Staprans et al., 2004; Haase, 2005; Grossman et al., 2006). This theoretical conundrum has been recognized for many years, but the actual, relative contribution of these forces to the outcome of acute infection has never been clarified. The ability of adaptive immunity, in particular the CD8⁺ T cell effector response, to modulate HIV/SIV replication set points and subsequent pathogenesis is convincingly documented by evidence of class I MHC allele-associated control of infection, either spontaneously or after vaccination (Goulder and Watkins, 2008), but the extent to which cellular immune responses contribute to the partial (but crucial) plateau-phase control of typical infection (those in the individuals without clearly protective alleles) is less clear. The massive destruction of target cells in acute SIV infection, coincident with the fall of plasma viral loads from peak levels, and the subsequent switch of viral targeting from resting to activated/proliferating targets (Picker et al., 2004; Zhang et al., 2004; Haase, 2005; Li et al., 2005; Mattapallil et al., 2005) suggests that target cell dynamics may regulate acute-phase viral replication. However, evidence showing that differences in CD4⁺ target cell numbers, rates of target cell expansion, and/or activation can independently determine viral replication set points and the pathogenic outcome of acute infection is quite limited, consisting largely of observations that during acute SIV infection, simultaneous coinfection with other pathogens or immune manipulations increasing CD4⁺ T cell activation and proliferation are associated with increased viral replication rates and more rapid disease progression (Folks et al., 1997; Zhou et al., 1999; Sequer et al., 2002; Garber et al., 2004; Staprans et al., 2004; Cecchinato et al., 2008). Cause and effect have not been shown in these studies, but in several instances, a significant correlation has been observed between the number of activated CD4⁺ T cells and outcome (Garber et al., 2004; Staprans et al., 2004).

In this study, we have used the experimental model of CD8⁺ lymphocyte depletion in acutely SIV-infected RMs to directly determine the relative contribution of these two potentially conflicting consequences of immune responsiveness—target cell augmentation versus antiviral effector responses—to viral dynamics and pathogenesis. Previous work has established that efficient CD8⁺ lymphocyte depletion (defined as depletion lasting ≥ 4 wk) with the CD8 α -specific cM-T807 mAb universally abrogates virologic control in acute SIV infection

and precipitates rapid disease progression (Schmitz et al., 1999; Veazey et al., 2008). Although it has been long recognized that this manipulation eliminates adaptive (and likely innate) effector responses to the virus mediated by CD8 α -expressing cells (Schmitz et al., 1999; Choi et al., 2008; Kim et al., 2008; Veazey et al., 2008), we further demonstrate that this CD8⁺ lymphocyte depletion instigates a robust proliferative response by CD4⁺ T_{EM} and, to a lesser extent, CD4⁺ T_{TM} cell populations, which both express CCR5 and are known primary targets of CCR5-tropic SIV (Picker, 2006; Grossman and Picker, 2008). In uninfected RMs, this CD8⁺ lymphocyte depletion-associated proliferative response leads to prolonged expansion of CD4⁺ T_{EM} cells, but in the setting of acute SIV infection, such expansion is not observed, and in fact, both circulating CD4⁺ T_{EM} and T_{TM} cell populations precipitously collapse in the early plateau phase of infection. These observations suggest that activation of CD4⁺ T_{EM} and T_{TM} cells by CD8⁺ lymphocyte depletion facilitate their infection by SIV, which in combination with delayed regeneration from CD4⁺ T_{CM} cell precursors leads to their accelerated loss. As activated T cells produce more virus than resting T cells (Zhang et al., 2004), the profound CD4⁺ T_{EM} and T_{TM} cell activation noted in our studies might be expected to increase and/or prolong high level acute-phase viral replication, perhaps directly precipitating immune failure as well as facilitating selection of viruses capable of efficiently targeting macrophages (which are thought to maintain viral replication after memory CD4⁺ T cell depletion; unpublished data; Igarashi et al., 2001).

These potential mechanisms raise the possibility that the rapid disease progression associated with CD8⁺ lymphocyte depletion in primary SIV infection may arise, alone or in significant part, from enhancing the number of target cells, their susceptibility to virus, and the virus yield per infected cell rather than loss of immune effector function. In this case, inhibition of the CD8⁺ lymphocyte depletion-associated, CD4⁺ target cell activation might be expected to actually lower post-peak viral replication rates and ameliorate progression, even in the continued absence of CD8⁺ lymphocytes. This, however, was not the case. We were able to demonstrate that the major component of CD8⁺ lymphocyte depletion-associated target cell activation, that of CD4⁺ T_{EM} cells, was IL-15 dependent and completely inhibited by a neutralizing anti-IL-15 mAb. This abrogation of the CD4⁺ T_{EM} cell proliferative response did not, however, alter the accelerated viral replication and pathogenesis typical of CD8⁺ lymphocyte depletion. CD4⁺ T_{TM} cells are responsive to IL-2 and IL-7 as well (unpublished data; Picker et al., 2006), and the CD8⁺ lymphocyte depletion-associated proliferation of these cells was not significantly

differences between groups assessed by the unpaired Student's *t* test. (right) Kaplan-Meier plot of disease-free survival of the two groups, with the statistical differences between groups determined by the log-rank test. Note that two animals, one each for the anti-IL-15 and control groups, were lost to follow up at day 87 (when disease free), and were censored from this analysis at this time point. Of note, it was retrospectively determined that 1 and 2 of the mAb M111-treated and 1 and 4 of the control mAb-treated RMs in this experiment expressed the potentially protective *Mamu* B*08 and B*17 alleles, respectively, but in keeping with the documented ability of CD8⁺ lymphocyte depletion to abrogate CD8⁺ T cell-mediated antiviral function (Kim et al., 2008), none of these RMs controlled SIV replication and no consistent effect of these alleles on survival was identified.

inhibited by anti-IL-15. However, administration of recombinant IL-15 to CD8⁺ lymphocyte-intact RMs in acute infection did induce a CD4⁺ T_{TrM} cell proliferative response of

similar magnitude and kinetics as CD8⁺ lymphocyte depletion (as well as a CD4⁺ T_{EM} cell proliferative response), but in striking contrast to CD8⁺ lymphocyte depletion, did not

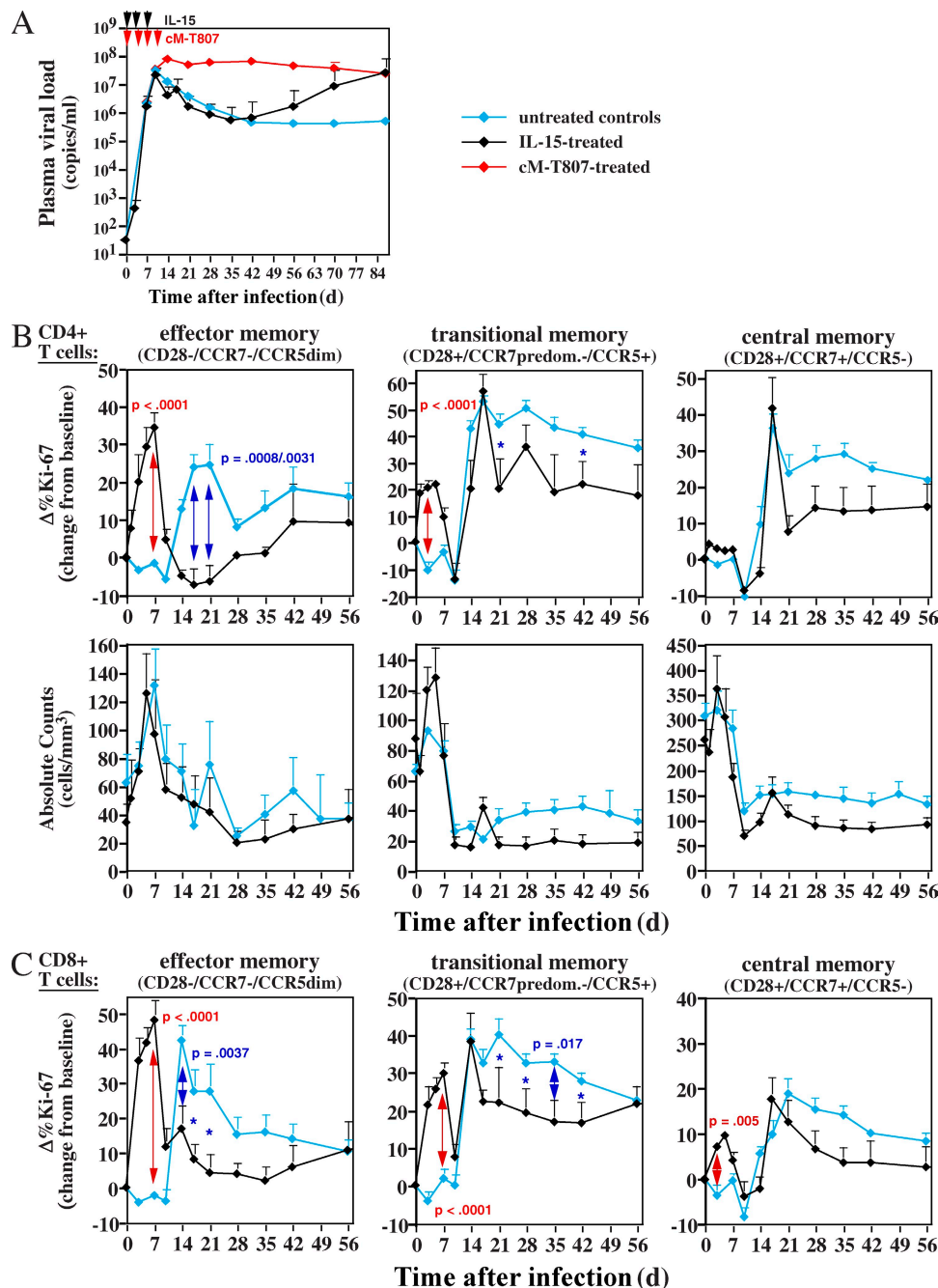


Figure 7. Recombinant IL-15 induces extensive proliferation among CD4⁺, CCR5⁺ viral targets during acute SIV infection but does not recapitulate the failure to diminish postpeak viral replication manifested by CD8⁺ lymphocyte-depleted RMs. (A) The plasma viral load patterns, and (B) both CD4⁺ memory T cell subset dynamics and (C) CD8⁺ memory T cell subset proliferation in the peripheral blood of five RMs that were SIVmac239 infected and treated with 50 μg/kg rhIL-15 on PID 0, 3, and 7 (but are CD8⁺ lymphocyte intact) in comparison to the same untreated SIV-infected controls (A–C) and CD8⁺ lymphocyte-depleted, SIV-infected RMs (A) shown in Fig. 5. None of the IL-15-treated RMs expressed class I MHC alleles (*Mamu* B*08 and B*17) associated with spontaneous SIV control (Goulder and Watkins, 2008). The data shown reflect the means ± SEM of the log-transformed plasma viral loads and the means ± SEM of the designated cellular parameters, presented as either change from preinfection baseline (proliferative fraction) or the actual measured value (viral loads, absolute CD4 memory T cell subset counts). Analysis of the significance of the difference in the designated values was done by the unpaired Student's *t* test (*, *P* > 0.03 and < 0.08, borderline significance/trends).

result in failure to control postpeak viral loads. At best, IL-15 treatment induced a slight postpeak blip in viral replication (which, interestingly, was also observed in a previous report of IL-15 administration in acute SIV infection; Mueller et al., 2008). Whether this blip was caused by CD4⁺ target T cell activation/expansion is uncertain, but even if it were entirely attributable to this mechanism, the effect would clearly be quite small. Thus, when the major CD4⁺ T_{EM} cell proliferative response is abrogated in the absence of CD8⁺ cells, or when CD4⁺ T_{EM} and T_{TM} cell proliferative responses are induced in the presence of CD8⁺ cells, there is no major change in early postpeak viral replication (uncontrolled in the former situation, controlled in the latter).

Collectively, these data strongly argue against a major role for CD4⁺ target cell activation and expansion in the initial establishment of postpeak viral load set points in acute SIV infection, and conversely, strongly imply that functional activities of CD8⁺ lymphocytes play the determining role. The adaptive, SIV-specific, CD8⁺ T cell response is the most obvious candidate for such antiviral activity, and if this is the case, it would imply that the delivery of SIV-specific CD8⁺ T cell effectors to sites of viral production at days 10–14 after infection is, though not maximal (Reynolds et al., 2005), sufficient to affect viral replication at these sites. However, the possibility that this initial control is mediated by innate immune function of CD8⁺ lymphocytes must also be considered. With respect to this possibility, CD8⁺ NK cells are quite efficiently depleted by cM-T807 treatment (unpublished data) and are, therefore, a potential contributor to postpeak viral load control. The observation that CD16⁺ lymphocyte depletion did not materially alter the virologic and clinical course of acute SIV infection argues against a major NK cell contribution to this control but does not rule it out, because not all RM NK cells express CD16 and the depletion reported with the CD16 mAb was not complete (Choi et al., 2008). However, NK cells are not the only CD8⁺ cells capable of innate responses: conventional memory CD8⁺ T cells can also mediate such innate-like responses (Lertmemongkolkhai et al., 2001; Kambayashi et al., 2003), and the overall memory CD8⁺ T cell population is obviously widely dispersed, side-by-side with memory CD4⁺ viral targets, at essentially all potential sites of viral replication throughout infection. It is therefore possible that a generalized (nonantigen-specific) release of β chemokines or other HIV/SIV inhibitory factors (DeVico and Gallo, 2004), induced by innate stimulation, hinders viral spread sufficiently to lower postpeak viral loads.

Our demonstration that the robust CD4⁺, CCR5⁺ memory T cell proliferation induced by CD8⁺ lymphocyte depletion has little effect on subsequent viral dynamics contradicts the prevailing hypothesis that coinfection or other immune manipulations leading to CD4⁺ T cell activation and proliferation mediate increased pathogenesis by expanding CD4⁺ target cell populations. In this regard, the effect of IL-15 treatment is illustrative. Although IL-15 administration in acute infection accelerates SIV

pathogenesis, its effect on viral replication is relatively late (after PID 50), long after its stimulation of CD4⁺ memory T cell proliferation (the current study; Mueller et al., 2008). This pattern strongly suggests the initial establishment and then loss of immune control, possibly caused by dysregulated development of the SIV-specific T cell and/or antibody response. Consistent with this, we demonstrated a significant diminution of CD4⁺ and CD8⁺ T_{EM} cell proliferation after the viral load peak in IL-15-treated versus control RMs in this study (Fig. 7), and Mueller et al. (2008) report that IL-15 administration significantly changes the character of cellular and humoral immune responses to SIV in the plateau phase of infection. As IL-15 potentially promotes expansion, differentiation, and effector site migration of effector lymphocyte populations (Picker et al., 2006), as well as inducing negative regulators of itself and other common γ -chain cytokines (Ramsborg and Papoutsakis, 2007), it is quite plausible that premature or excessive IL-15 activity would compromise the extent, quality, and longevity of a developing pathogen-specific immune response. In this regard, it is noteworthy that IL-15 administration specifically abrogated the protective effects of a therapeutic SIV vaccine in SIV-infected, virally suppressed RMs (Hryniewicz et al., 2007). Whether such immune dysregulation is the basis for the accelerated SIV disease associated with acute-phase coinfection with other agents and other acute-phase immune modulations remains to be determined, but given the insensitivity of SIV replication and disease progression to target cell proliferation/activation status in this paper, this possibility clearly merits further investigation.

The issue of the impact of acute-phase CD4⁺ memory activation on viral replication and pathogenesis has crucial implications for HIV/AIDS vaccine development. With few exceptions, prophylactic HIV/AIDS vaccines generate specific CD4⁺ memory T cell responses that are primed for anamnestic expansion and CCR5⁺ T_{EM} and T_{TM} cell production after pathogenic viral challenge. A central tenet of the HIV/AIDS T cell vaccine concept is that the T cell effector activity generated by such responses will outweigh the impact of providing more or better viral targets, but there is concern for, and some evidence of, such vaccination-induced responses making subsequent infection more, rather than less, pathogenic (Staprans et al., 2004). Although it remains possible that the IL-15-mediated homeostatic activation studied in this paper differs from the antigen-driven CD4⁺ T cell expansions that would occur in primary HIV/SIV infections of subjects with vaccine-generated HIV/SIV-specific CD4⁺ T cell memory, our data provide some reassurance on this issue, suggesting that CD4⁺ target cell activation and expansion has less effect on the outcome of systemic infection than feared. It should be noted that we investigated systemic infection via i.v. challenge; our data do not speak to the issue of whether the number or activation status of CD4⁺ T cell targets in mucosal sites affect the development of HIV/SIV infection after mucosal exposure (Li et al., 2009).

The major implications of these studies are that the size and activation status of CD4⁺ target cell populations do not appear to be primary limiting factors in the replication of highly pathogenic, CCR5-tropic SIV in acute infection, and that increasing target cell number, infection susceptibility, and/or progeny virus productivity during the rising phase of acute-phase SIV replication does not, by itself, significantly accelerate the tempo of SIV infection and pathogenesis. Rather, CD8⁺ lymphocyte function, innate and/or adaptive, appears to play the dominant role in setting this tempo, and the increased pathogenicity observed in concomitant infection and other settings of generalized immune stimulation is most likely secondary to dysregulation of this function. Target cell availability is even less likely to be limiting in human HIV infection, as the tempo of viral replication and target cell destruction in typical HIV infection is somewhat slower than that seen in SIV infection of RMs (Mellors et al., 1996; Brenchley et al., 2004; Mehandru et al., 2004; Mattapallil et al., 2005; Okoye et al., 2007). Finally, these studies emphasize the dynamic, reactive nature of the in vivo immune system, in particular highlighting homeostatic responses to subset perturbations. We describe a major homeostatic response to CD8⁺ lymphocyte depletion in primates and define its dependence on IL-15. This is likely not the only homeostatic response to CD8⁺ lymphocyte depletion, and it is certain that perturbation of other subsets have analogous reactions. This complexity does not undermine the value of exploring such experimental perturbations, but it does speak to the need to identify such reactive responses and define their effects.

MATERIALS AND METHODS

Animals and viruses. A total of 62 purpose-bred male RMs (*Macaca mulatta*) were used in this study, all of Indian genetic background and free of Cercopithecine herpesvirus 1, D type simian retrovirus, simian T-lymphotrophic virus type 1, and SIV infection at study initiation. Of this total, 51 were infected with SIVmac239 (Kestler et al., 1990) and 11 were used as uninfected controls; 32 were CD8⁺ lymphocyte depleted with mAb cM-T807 (23 concomitantly infected with SIVmac239 and 9 uninfected; cM-T807 was provided by the National Institutes of Health [NIH] Nonhuman Primate Reagent Resource Program, Boston, MA; Schmitz et al., 1999; Veazey et al., 2008); and 5 were treated with rhIL-15 (all SIV infected). mAb cM-T807 was administered in multiple doses, typically 10 mg/kg (subcutaneous injection) at day 0, followed by three i.v. doses of 5 mg/kg at days 3, 7, and 10. An alternative (and functionally equivalent) regimen, used in two uninfected RMs (Fig. 3), was 50 mg/kg i.v. at days 0 and 7. The anti-IL-15 mAb M111 (American Type Culture Collection; Barzegar et al., 1998) and isotype-matched control mAb 1B7.11 (anti-trinitrophenyl [TNP]) were administered by i.v. injection at a dose of 5 mg/kg on days 0, 3, and 7 (4 h after cM-T807 injection). rhIL-15 was prepared as previously described (Villinger et al., 2004) and administered subcutaneously at 50 µg/kg at days 0, 3, and 7. SIVmac239 infections were initiated with i.v. injection of 5-ng equivalents of SIV p27 ($2.7\text{--}3 \times 10^4$ infectious centers), as previously described (Picker et al., 2004). Plasma SIV RNA was assessed using a real-time RT-PCR assay (Cline et al., 2005) with a threshold sensitivity of 30 SIV gag RNA copy equivalents per milliliter of plasma. All RMs were housed at the Oregon National Primate Research Center according to standards of the Animal Care and Use Committee and the NIH Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Research, 1996). Disease onset (AIDS) was defined by the presence of

clinical and/or laboratory evidence of opportunistic infection, a wasting syndrome unresponsive to therapy, and/or non-Hodgkin lymphoma (McClure et al., 1989). RMs that developed disease states that were not manageable were euthanized according to the recommendations of the AVMA Panel on Euthanasia, American Veterinary Medical Association (2001).

Flow cytometric analysis. Whole blood was obtained and stained for flow cytometric analysis as previously described (Walker et al., 2004). Polychromatic (8–12 parameter) flow cytometric analysis was performed on an LSR II instrument (BD) using Pacific blue, AmCyan, FITC, PE, PE–Texas red, PE–Cy7, PerCP–Cy5.5, allophycocyanin (APC), APC–Cy7, and Alexa Fluor 700 as the available fluorescent parameters. Instrument set up and data acquisition procedures were performed as previously described (Walker et al., 2004). List mode multiparameter data files were analyzed using FlowJo software (version 6.3.1; Tree Star, Inc.). Criteria for delineating naive and memory T cell subsets and for setting + versus – markers for Ki-67 expression have been previously described in detail (Picker et al., 2006).

mAbs. mAbs L200 (CD4; AmCyan and APC–Cy7), SP34-2 (CD3; Alexa Fluor 700), SK1 (CD8α; APC–Cy7, PerCP–Cy5.5, and PE–Cy7), CD28.2 (CD28; PE and PerCP–Cy5.5), B56 (Ki-67; FITC and PE), DX2 (CD95; PE, APC, and PE–Cy7), and 3A9 (CCR5; PE and APC) were obtained from BD. mAbs 2ST8.5h7 (CD8β; PE and PE–Cy7), CD28.2 (PE–Texas red), and streptavidin–PE–Cy7 were obtained from Beckman Coulter. mAb DK25 (CD8α; PE and APC), which is non-cross-reactive with cM-T807, was obtained from Dako. FN-18 (CD3) was produced and purified in house and conjugated to Pacific blue or Alexa Fluor 700 using a conjugation kit from Invitrogen. mAb 150503 (anti-CCR7) was purchased as purified immunoglobulin from R&D Systems, conjugated to biotin using a biotinylation kit (Thermo Fisher Scientific), and visualized with streptavidin–Pacific blue (Invitrogen). Production and purification of anti-IL-15 and isotype control antibodies was performed using cell lines producing the m111 (anti-IL-15) and 1B7.11 (anti-TNP–isotype control) antibodies obtained from the American Type Culture Collection. Scale-up growth of the hybridomas was performed in bioreactors (CL1000; BD). Antibodies were captured from growth medium using cation exchange chromatography and further purified using recombinant protein A columns. The purified antibodies were dialyzed into PBS and frozen at –80°C before use.

Determination of plasma IL-15 levels. Multiplex kits for measuring cytokines and chemokines on the Luminex platform were obtained from Millipore. The kits were used according to the manufacturer's instructions. Frozen plasma samples collected from RMs were thawed rapidly and clarified by centrifugation at 10,000 g for 5 min. 25-µl aliquots were used per well in a 96-well filter plate. Incubations were performed with agitation on a plate shaker overnight at 4°C. After the final wash, beads in the 96-well microtiter plate were resuspended in 125 µl of Luminex sheath fluid and loaded into the Luminex instrument. An acquisition gate was set between 8,000 and 15,000 for the doublet discriminator, and at least 100 events per region were acquired. The IL-15 concentration in the plasma samples were determined by the standard curves generated with recombinant rhesus IL-15 using a weighted 5-parameter logistic method.

Statistical analysis. Statistical analysis was conducted with the programs Statview (Abacus Concepts) and SAS version 9.1 (SAS Institute Inc.). Comparisons between different groups of animals (treated vs. control) were made using unpaired Student's *t* tests. The significance of differences between pre- and post-treatment values was assessed with paired Student's *t* tests. The significance of differences in disease-free survival was determined with the Kaplan-Meier analysis and log-rank testing. In all analyses, a two-sided significance level (α) of 0.05 was used, with correction made for multiple comparisons by the Bonferroni method.

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