

$\alpha 4^{+} \beta 7^{\text{hi}} \text{CD4}^{+}$ memory T cells harbor most Th-17 cells and are preferentially infected during acute SIV infection

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Human immunodeficiency virus/simian immunodeficiency virus (HIV/SIV) infections are believed to infect minimally activated CD4^{+} T cells after viral entry. Not much is known about why SIV selectively targets these cells. Here we show that CD4^{+} T cells that express high levels of the $\alpha 4 \beta 7$ heterodimer are preferentially infected very early during the course of SIV infection. At days 2–4 post infection, $\alpha 4^{+} \beta 7^{\text{hi}} \text{CD4}^{+}$ T cells had $\sim 5 \times$ more SIV-gag DNA than $\beta 7^{-} \text{CD4}^{+}$ T cells. $\alpha 4^{+} \beta 7^{\text{hi}} \text{CD4}^{+}$ T cells displayed a predominantly central memory ($\text{CD45RA}^{-} \text{CD28}^{+} \text{CCR7}^{+}$) and a resting ($\text{CD25}^{-} \text{CD69}^{-} \text{HLA-DR}^{-} \text{Ki-67}^{-}$) phenotype. Although the expression of detectable CCR5 was variable on $\alpha 4^{+} \beta 7^{\text{hi}}$ and $\beta 7^{-} \text{CD4}^{+}$ T cells, both CCR5⁺ and CCR5⁻ subsets of $\alpha 4^{+} \beta 7^{\text{hi}}$ and $\beta 7^{-} \text{CD4}^{+}$ T cells were found to express sufficient levels of CCR5 mRNA, suggesting that both these subsets could be efficiently infected by SIV. In line with this, we found similar levels of SIV infection in $\beta 7^{-} \text{CD4}^{+} \text{CCR5}^{+}$ and $\beta 7^{-} \text{CD4}^{+} \text{CCR5}^{-}$ T cells. $\alpha 4 \beta 7^{\text{hi}} \text{CD4}^{+}$ T cells were found to harbor most T helper (Th)-17 cells that were significantly depleted during acute SIV infection. Taken together, our results show that resting memory $\alpha 4^{+} \beta 7^{\text{hi}} \text{CD4}^{+}$ T cells in the blood are preferentially infected and depleted during acute SIV infection, and the loss of these cells alters the balance between Th-17 and Th-1 responses, thereby contributing to disease pathogenesis.

INTRODUCTION

Acute human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) infections are characterized by a massive loss of memory CD4^{+} T cells,^{1–7} a process that is most pronounced in mucosal tissues, as these tissues are enriched in memory CD4^{+} T cells. As in non-natural hosts, severe depletion of CD4^{+} T cells has been reported in natural hosts, such as sooty mangabeys and African green monkeys, during acute SIV infection.^{8,9} Surprisingly, the kinetics of infection in memory CD4^{+} T cells that reach a peak ~ 10 days after SIV challenge appear to be independent of the route of infection.^{4,5}

The factors determining which target cells that are infected initially, and those responsible for the delay between initial infection and the explosive levels of viral replication observed 10 days later, remain unclear. Studies⁵ have shown that before day 7 post infection (pi) < 5 to 10% of CD4^{+} T cells in either the mucosal tissues or periphery are infected, whereas by day 10 pi, most memory CD4^{+} T cells are infected and carry viral DNA. Li

*et al.*⁴ showed that a few founder populations of CD4^{+} T cells in the gut-associated lymphoid tissue (GALT) are infected initially during SIV infection. Interestingly, these founder cells were minimally activated CD4^{+} T cells ($\text{CD25}^{-} \text{CD69}^{-} \text{Ki-67}^{-}$) and had a predominantly resting phenotype. It is not clear why SIV targets these CD4^{+} T cells during the early phase of viral infection.

Recent *in vitro* studies¹⁰ have shown that CD4^{+} T cells that express high levels of the $\alpha 4 \beta 7$ ($\alpha 4^{+} \beta 7^{\text{hi}}$) mucosal homing receptor can bind to HIV and SIVsmm with high affinity making these cells more susceptible to infection. Arthos *et al.*¹⁰ suggested that the expression of high levels of $\alpha 4 \beta 7$ on CD4^{+} T cells likely offers a selective advantage to the virus; hence, these cells may be the earliest cells targeted and infected by HIV. The loss of these mucosal homing T helper (Th) cells severely compromises the ability of the mucosal immune system. In line with this, recent studies have shown that mucosal Th-17 CD4^{+} T cells have a critical role in maintaining the integrity of the mucosal barrier.¹¹ Others have shown that alterations in the Th-17 and Th-1 responses contribute to disease progression.^{12–14}

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We hypothesized that if HIV selectively targeted the $\alpha 4^{+} \beta 7^{\text{hi}} \text{CD4}^{+}$ T cells during the early phase of infection, then these cells would be preferentially infected very early during the course of infection. We tested our hypothesis in the SIVmac251-infected rhesus macaque model by quantifying the cell-associated SIV DNA in $\alpha 4^{+} \beta 7^{\text{hi}} \text{CD4}^{+}$ T cells from peripheral blood during the early phase of SIV infection and compared them with SIV DNA levels in CD4^{+} T cells that either lacked ($\beta 7^{-}$) or expressed intermediate levels of $\alpha 4 \beta 7$ ($\alpha 4^{+} \beta 7^{\text{int}}$). Previous studies¹⁵ have shown that HIV-specific CD4^{+} T cells that were preferentially infected carried 2–5 \times more viral DNA than Epstein–Barr virus- or cytomegalovirus-specific CD4^{+} T cells. To determine whether the differentiated and activated nature of $\alpha 4^{+} \beta 7^{\text{hi}} \text{CD4}^{+}$ T cells contributes to their susceptibility to SIV infection, we evaluated the expression of phenotypic (CD28, CD45RA, and CCR7) and activation (CD25, CD69, HLA-DR, and Ki-67) markers on these cells. In addition, to evaluate whether preferential infection and depletion of $\alpha 4^{+} \beta 7^{\text{hi}} \text{CD4}^{+}$ T cells led to alterations in the balance between Th-17- and Th1-type responses, we evaluated the expression of interleukin (IL)-17 and interferon (IFN) γ in $\alpha 4^{+} \beta 7^{\text{hi}} \text{CD4}^{+}$ T cells and compared them with $\beta 7^{-} \text{CD4}^{+}$ T cells.

RESULTS

$\alpha 4^{+} \beta 7^{\text{hi}} \text{CD4}^{+}$ T cells are a minor population of peripheral CD4^{+} T cells

We used an antibody against the $\beta 7$ receptor to identify different subsets of CD4^{+} T cells. As $\beta 7$ can form a heterodimer with $\alpha 4$ (CD49d) or αE (CD103),¹⁶ we first evaluated the expression of these receptors on $\beta 7$ subsets in peripheral blood of healthy rhesus macaques. Our results showed that ~50% of peripheral blood CD4^{+} T cells expressed the $\beta 7$ receptor, whereas the rest of the 50% lacked the $\beta 7$ expression. Approximately 20% of CD4^{+} T cells that expressed the $\beta 7$ receptor did so at high levels and had a $\beta 7^{\text{hi}}$ phenotype, whereas ~30% of them expressed intermediate levels of $\beta 7$ receptor and had a $\beta 7^{\text{int}}$ phenotype (Figure 1a). More than 95% of $\beta 7^{\text{hi}}$ and ~65% of $\beta 7^{\text{int}}$ CD4^{+} T cells co-expressed the $\alpha 4$ receptor (Figure 1b), hereafter these are referred to as $\alpha 4^{+} \beta 7^{\text{hi}}$ and $\alpha 4^{+} \beta 7^{\text{int}}$ subsets. Studies have shown that cells expressing high levels of the $\alpha 4 \beta 7$ efficiently traffic to mucosal tissues.¹⁶

On the other hand, CD4^{+} T cells that lacked $\beta 7$ expression contained a mix of $\alpha 4^{+}$ (~75%) and $\alpha 4^{-}$ (~25%) cells (Figure 1c), and these subsets together are referred to as $\beta 7^{-} \text{CD4}^{+}$ T-cell subsets. $\alpha 4$ associates with $\beta 1$ to form the $\alpha 4 \beta 1$ heterodimer and binds to VCAM-1 (vascular cell adhesion molecule-1) on activated endothelial cells, which is involved in trafficking of T cells to peripheral lymphoid tissues.^{17–20} Interestingly, $\beta 7^{-} \text{CD4}^{+}$ T-cell subsets expressed $\alpha 4$ integrin at a significantly higher density than $\alpha 4^{+} \beta 7^{\text{hi}} \text{CD4}^{+}$ T-cell subsets (Figure 1e). Our results suggest that most of the $\beta 7^{-} \text{CD4}^{+}$ T-cell subsets likely home to peripheral lymphoid tissues.

All of the $\alpha 4^{+} \beta 7^{\text{hi}} \text{CD4}^{+}$ and $\beta 7^{-} \text{CD4}^{+}$ T-cell subsets expressed CD95, indicating that these were memory CD4^{+} T cells. On the other hand, $\alpha 4^{+} \beta 7^{\text{int}} \text{CD4}^{+}$ T cells lacked the CD95 expression, which is indicative of a naive phenotype. Previous

studies have shown that rhesus macaque T cells expressing CD95 were memory T cells, whereas T cells that lacked CD95 were naive T cells.²¹ As our analysis was confined to peripheral blood, it is possible that the pattern of $\alpha 4 \beta 7$ expression may vary from that seen in GALT.

$\alpha 4^{+} \beta 7^{\text{hi}} \text{CD4}^{+}$ T cells express a central memory phenotype

Next, we evaluated the phenotype of $\alpha 4^{+} \beta 7^{\text{hi}} \text{CD4}^{+}$ T-cell subsets based on the co-expression of $\alpha 4$ and $\beta 7$ integrins. Memory CD4^{+} T cells that express the $\alpha 4 \beta 7$ heterodimer appear on the diagonal, as the antibodies to $\alpha 4$ and $\beta 7$ integrins bind to the same heterodimerized molecule, and co-label with the Act-1 clone that recognizes an heterodimerized epitope of $\alpha 4$ and $\beta 7$ integrins²² (Supplementary Figure S1a online).

Three major subsets of memory CD4^{+} T cells could be delineated based on the differential expression of these two integrin molecules, namely $\alpha 4^{+} \beta 7^{\text{hi}}$ subset, and two subsets of $\beta 7^{-} \text{CD4}^{+}$ T cells, namely $\alpha 4^{+} \beta 7^{-}$ and $\alpha 4^{-} \beta 7^{-}$ subsets (Figure 2a, b). Similar proportions of the three subsets are found within human memory CD4^{+} T cells (M. Roederer, personal communication; Supplementary Figure S1e online).

Majority of the $\alpha 4^{+} \beta 7^{\text{hi}} \text{CD4}^{+}$ T-cell subsets expressed a central memory phenotype ($\text{CD28}^{+} \text{CCR7}^{+} \text{CD45RA}^{-}$), whereas most of the effector memory CD4^{+} T cells ($\text{CD28}^{-} \text{CCR7}^{-} \text{CD45RA}^{+}$) are found within the $\alpha 4^{+} \beta 7^{-}$ subsets (Figure 2c, d).

Interestingly, most (>95%) of the $\alpha 4^{+} \beta 7^{\text{hi}} \text{CD4}^{+}$ T-cell subsets expressed detectable levels of CCR5 on their surface (Figure 2e), whereas only a few (<20%) of the $\beta 7^{-} \text{CD4}^{+}$ T-cell subsets expressed detectable levels of CCR5. To determine whether these subsets expressed variable levels of CCR5 mRNA, we sorted CCR5^{+} and CCR5^{-} subsets of $\alpha 4^{+} \beta 7^{\text{hi}}$ and $\beta 7^{-} \text{CD4}^{+}$ T cells from uninfected animals (Supplementary Figure S1b online) and quantified the levels of CCR5 mRNA in these subsets using a relative quantitative PCR (qPCR) assay (Figure 2f). Our results show that although a majority of the $\beta 7^{-} \text{CD4}^{+}$ T-cell subsets expressed little CCR5 on their surface, they had significant levels of CCR5 mRNA that did not differ from that of the other memory CD4^{+} T-cell subsets.

$\alpha 4^{+} \beta 7^{\text{hi}} \text{CD4}^{+}$ T cells are preferentially infected during acute SIV infection

To determine whether the differential expression of $\alpha 4$ and $\beta 7$ integrins on CD4^{+} T-cell subsets made them variably susceptible to infection and subsequent loss, we evaluated the dynamics of $\alpha 4^{+} \beta 7^{\text{hi}} \text{CD4}^{+}$ T-cell subsets in peripheral blood and compared them with $\alpha 4^{+} \beta 7^{\text{int}}$ and $\beta 7^{-} \text{CD4}^{+}$ T-cell subsets.

We observed a significant loss of both $\alpha 4^{+} \beta 7^{\text{hi}}$ and $\beta 7^{-} \text{CD4}^{+}$ T-cell subsets in peripheral blood at day 63 pi compared with day 10 pi (Figure 3a, b), whereas there was no major difference in either the frequency or absolute numbers of $\alpha 4^{+} \beta 7^{\text{int}} \text{CD4}^{+}$ T-cell subsets or CD8^{+} T-cell subsets (Figure 3a–d). The loss of both $\alpha 4^{+} \beta 7^{\text{hi}}$ and $\beta 7^{-} \text{CD4}^{+}$ T-cell subsets was not unexpected, as previous studies⁵ have reported a significant loss of memory CD4^{+} T cells in peripheral blood during acute SIV infection; both $\alpha 4^{+} \beta 7^{\text{hi}}$ and $\beta 7^{-} \text{CD4}^{+}$ T-cell subsets have a memory phenotype. However, we observed a significantly

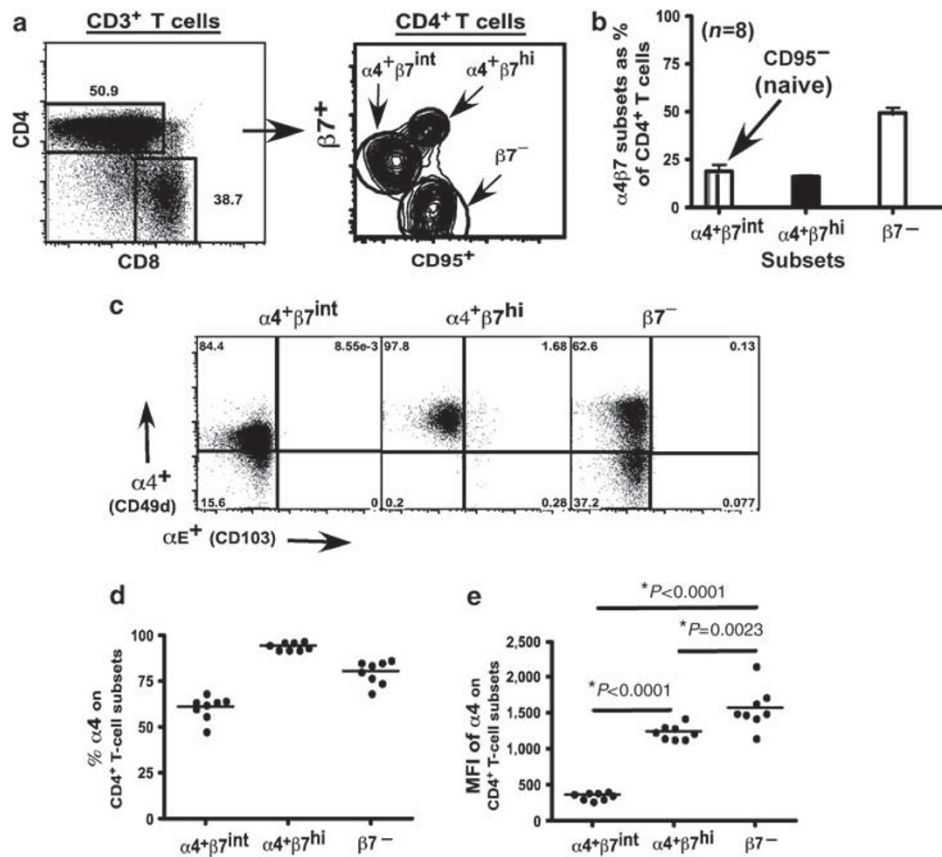


Figure 1 Phenotypic analysis of CD3⁺CD4⁺ T cells in the peripheral blood from healthy rhesus macaques (*n*=8). (a) Representative contour plots and (b) relative proportions of CD95⁺β7^{hi} ($\alpha 4^+ \beta 7^{\text{hi}}$), CD95⁻β7^{int} ($\alpha 4^+ \beta 7^{\text{int}}$), and CD95⁺β7⁻ ($\beta 7^-$) subsets of CD4⁺ T cells. (c) Expression of $\alpha 4$ and αE integrins on $\alpha 4^+ \beta 7^{\text{hi}}$, $\alpha 4^+ \beta 7^{\text{int}}$, and $\beta 7^-$ CD4⁺ T-cell subsets. (d) Relative proportions and (e) mean fluorescence intensity (MFI) of $\alpha 4$ expression on $\alpha 4^+ \beta 7^{\text{hi}}$, $\alpha 4^+ \beta 7^{\text{int}}$, and $\beta 7^-$ CD4⁺ T-cell subsets. MFI was determined using FlowJo 8.6 software after gating on each subset.

higher loss of $\alpha 4^+ \beta 7^{\text{hi}}$ CD4⁺ T-cell subsets by day 63 pi compared with the $\beta 7^-$ CD4⁺ T-cell subsets (Figure 3e), suggesting that $\alpha 4^+ \beta 7^{\text{hi}}$ CD4⁺ T-cell subsets were preferentially depleted during the course of acute SIV infection.

To determine whether the preferential loss of $\alpha 4^+ \beta 7^{\text{hi}}$ CD4⁺ T-cell subsets was due to a higher level of viral infection in these subsets, we measured the level of SIV-gag DNA in $\alpha 4^+ \beta 7^{\text{hi}}$ CD4⁺ T-cell subsets and compared them with $\alpha 4^+ \beta 7^{\text{int}}$ and $\beta 7^-$ CD4⁺ T-cell subsets. $\alpha 4^+ \beta 7^{\text{hi}}$, $\alpha 4^+ \beta 7^{\text{int}}$, and $\beta 7^-$ CD4⁺ T-cell subsets, discriminated based on the expression of $\beta 7$ and CD95²¹ (Supplementary Figure S1c online), were sorted from peripheral blood and used in a qPCR assay to measure SIV-gag DNA. We have shown earlier that this assay was highly sensitive for measuring viral infection in CD4⁺ T cells.⁵ Measuring the level of SIV DNA in target cells allows us to quantify both productively and non-productively infected cells; hence, it is a more sensitive assay for quantifying viral infection in cells than RNA-based assays that measure only productively infected cells. Previous studies have shown that most of the memory CD4⁺ T cells carried viral DNA during acute SIV infection, whereas only about 7–20% of them were productively infected.^{4,5}

We found ~5× more SIV-gag DNA in $\alpha 4^+ \beta 7^{\text{hi}}$ CD4⁺ T-cell subsets at days 2–4 pi compared with $\beta 7^-$ CD4⁺ T-cell subsets,

suggesting that $\alpha 4^+ \beta 7^{\text{hi}}$ CD4⁺ T-cell subsets were preferentially infected very early during the course of infection (Figure 4b, c). By day 10 pi, there was a significant increase in the number of SIV-gag DNA copies in both subsets compared with days 2–4 pi. However, as at days 2–4 pi, $\alpha 4^+ \beta 7^{\text{hi}}$ CD4⁺ T-cell subsets harbored ~3× more SIV-gag DNA than $\beta 7^-$ CD4⁺ T-cell subsets (Figure 4b, c).

By day 63 pi, however, the number of SIV-gag copies decreased significantly in both $\alpha 4^+ \beta 7^{\text{hi}}$ and $\beta 7^-$ CD4⁺ T-cell subsets (Figure 4b), indicating that most of the cells that were infected at day 10 pi were destroyed by day 63 pi. Interestingly, unlike days 2–4 and 10 pi, both $\alpha 4^+ \beta 7^{\text{hi}}$ and $\beta 7^-$ CD4⁺ T-cell subsets at day 63 pi had similar levels of SIV-gag DNA (Figure 4c).

Few $\alpha 4^+ \beta 7^{\text{hi}}$ CD4⁺ T-cell subsets expressed HLA-DR (Figure 4d) that did not change after infection. Previous reports²³ have shown that there was no significant change in the expression of HLA-DR on memory CD4⁺ T cells during the early phase of acute SIV infection. Similar to HLA-DR, few $\alpha 4^+ \beta 7^{\text{hi}}$ CD4⁺ T-cell subsets expressed CD25 (<0.5%), CD69 (<0.5%), or Ki-67 (~3%) before infection that did not change significantly at day 4 pi (Figure 4d–g). Previous studies have shown that there was little or no change in the expression of HLA-DR or Ki-67 on memory CD4⁺ T cells at days 4, 7, and 10 pi compared

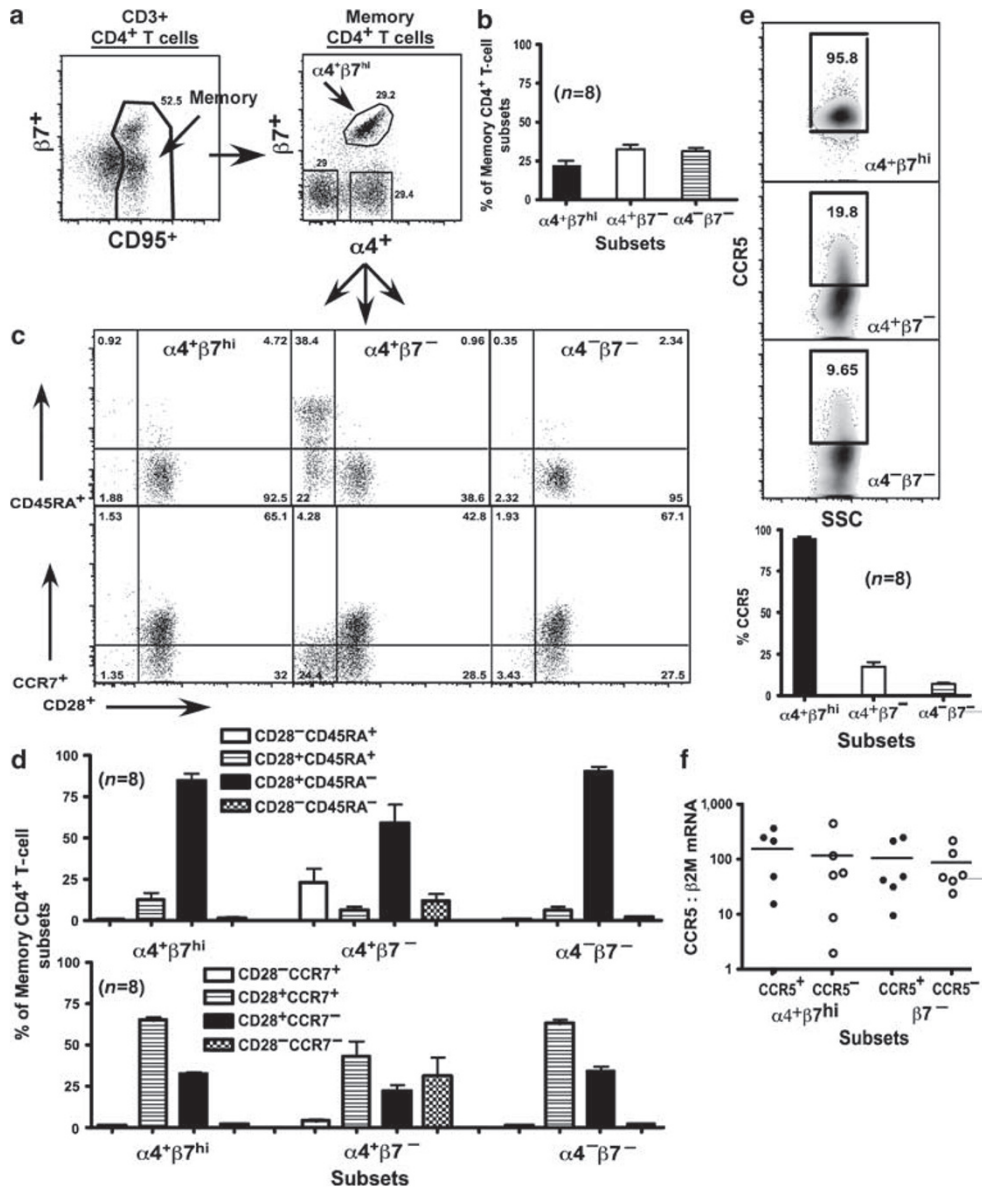


Figure 2 $\alpha 4^{+}\beta 7^{hi}$ CD4⁺ T-cell subsets show a predominantly central memory phenotype ($n=8$). Co-staining for $\alpha 4$ and $\beta 7$ identifies memory CD4⁺ T cells that express the $\alpha 4\beta 7$ heterodimer along a diagonal. (a) Representative dot plots and (b) relative proportions of $\alpha 4^{+}\beta 7^{hi}$, $\alpha 4^{+}\beta 7^{-}$, and $\alpha 4^{-}\beta 7^{-}$ subsets of CD4⁺ T cells. (c) Representative dot plots and (d) relative proportions of $\alpha 4^{+}\beta 7^{hi}$, $\alpha 4^{+}\beta 7^{-}$ and $\alpha 4^{-}\beta 7^{-}$ subsets of CD4⁺ T cells expressing CD45RA, CD28, and CCR7. (e) Representative dot plots and relative proportions of $\alpha 4^{+}\beta 7^{hi}$, $\alpha 4^{+}\beta 7^{-}$, and $\alpha 4^{-}\beta 7^{-}$ subsets of CD4⁺ T cells expressing CCR5. Most $\alpha 4^{+}\beta 7^{hi}$ subsets express detectable levels of surface CCR5. (f) Relative expression of CCR5 mRNA in CCR5⁺ and CCR5⁻ subsets of $\alpha 4^{+}\beta 7^{hi}$ and $\beta 7^{-}$ CD4⁺ T cells ($n=6$).

with pre-infection values,²³ whereas others have shown that very few peripheral blood CD4⁺ T cells express CD25, CD69, or Ki-67 during the acute phase of SIV infection.²⁴

Unlike $\alpha 4^{+}\beta 7^{hi}$ CD4⁺ T-cell subsets, higher proportions of $\beta 7^{-}$ CD4⁺ T-cell subsets expressed HLA-DR before infection, yet these cells were not preferentially infected. Similarly, $\beta 7^{-}$ CD4⁺ T-cell subsets were found to express significantly higher levels of Ki-67 at day 4 pi compared with uninfected animals. Taken together, these findings indicate that preferential infection during the early phase of SIV infection is confined to $\alpha 4^{+}\beta 7^{hi}$ CD4⁺ T-cell subsets that display a predominantly resting phenotype. This is in line with previous reports by

Li *et al.*,⁴ who showed that SIV primarily infects resting memory CD4⁺ T cells in the GALT that express a CD25⁻CD69⁻Ki-67⁻ phenotype. It is important to point out that our results only represent snapshots of the changes occurring *in vivo*. As such it is difficult to rule out if $\alpha 4^{+}\beta 7^{hi}$ CD4⁺ T-cell subsets were transiently activated at the time of infection.

To confirm whether SIV preferentially targeted CD4⁺ T-cell subsets that expressed the $\alpha 4\beta 7$ heterodimer during the early phase of SIV infection, we evaluated the levels of SIV-gag DNA in $\alpha 4^{+}\beta 7^{hi}$, $\alpha 4^{+}\beta 7^{-}$, and $\alpha 4^{-}\beta 7^{-}$ subsets of memory CD4⁺ T cells (Figure 5a) using peripheral blood samples (days 2–3 pi) that were obtained from an unrelated study.

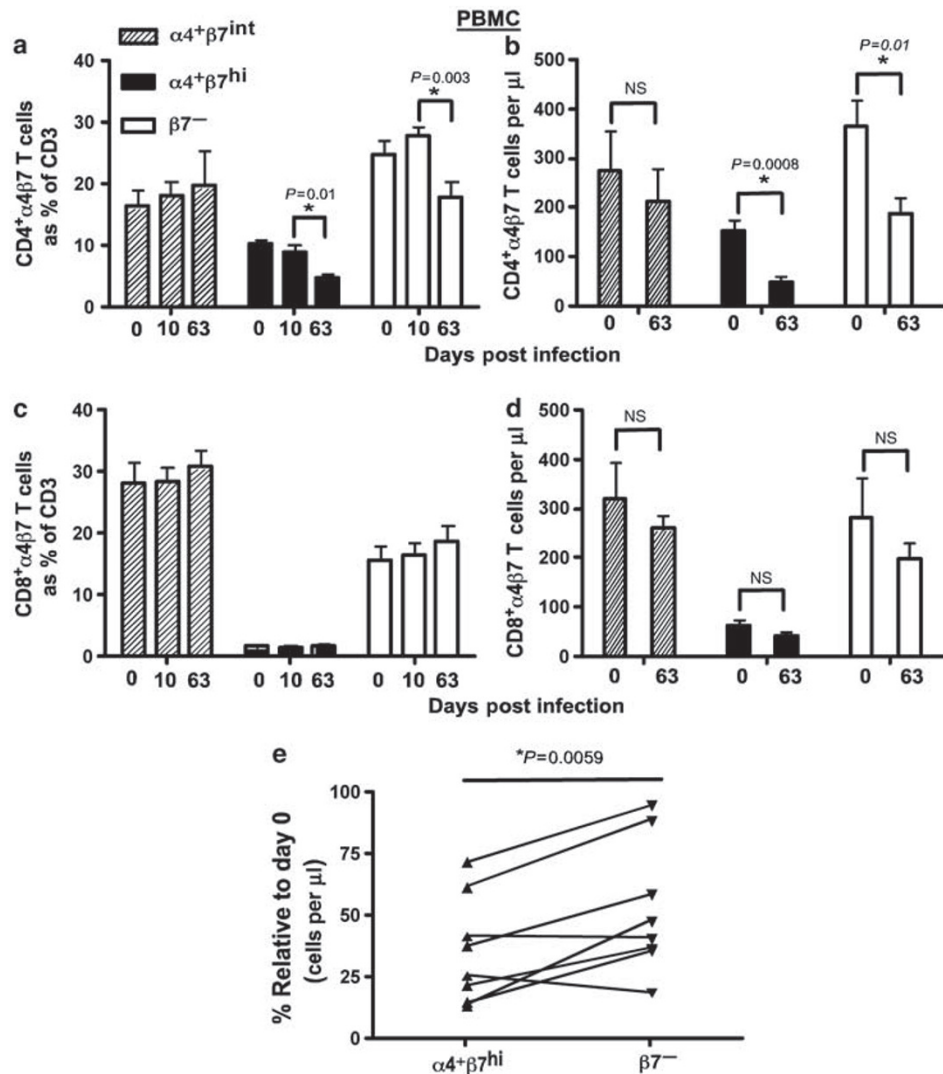


Figure 3 Dynamics of $\alpha 4\beta 7$ subsets of $CD4^+$ T cells in peripheral blood during acute simian immunodeficiency virus (SIV) infection. Significant loss of $\alpha 4\beta 7^{hi}$ and $\beta 7^-$ $CD4^+$ T cells is observed during acute SIV infection. Both frequency and absolute numbers are shown for (a, b) $CD4^+$ and (c, d) $CD8^+$ T cells ($n=8$). (e) Higher numbers of $\alpha 4\beta 7^{hi}CD4^+$ T cells were destroyed by day 63 pi (post infection) compared with $\beta 7^-$ $CD4^+$ T cells. The percentage (%) change in absolute numbers of $\alpha 4\beta 7^{hi}$ and $\beta 7^-$ $CD4^+$ T cells was calculated using each animal's day 63 values relative to its day 0 values with day 0 values as 100%.

Our results showed that during the first few days after infection, the virus is largely confined to memory $CD4^+$ T cells that express the $\alpha 4\beta 7$ heterodimer; $\alpha 4\beta 7^{hi}CD4^+$ T-cell subsets had significantly higher levels of SIV-gag DNA than either $\alpha 4\beta 7^-$ or $\alpha 4\beta 7^-CD4^+$ T-cell subsets (Figure 5a).

Taken together, these results support the *in vitro* findings reported by Arthos *et al.*,¹⁰ and show that SIVmac251 selectively target $CD4^+$ T cells that express high levels of the $\alpha 4\beta 7$ receptor.

Both $\beta 7^-CD4^+CCR5^+$ and $\beta 7^-CD4^+CCR5^-$ T-cell subsets are equally infected during acute SIV infection

To determine whether detectable surface expression of CCR5 makes $CD4^+$ T cells subsets more susceptible to SIV infection, we evaluated the levels of SIV infection in $\beta 7^-CD4^+CCR5^+$ T-cell subsets and compared them with $\beta 7^-CD4^+CCR5^-$ T-cell subsets. Krzysiek *et al.*,²⁵ showed that $CCR5^+CD4^+$ Th cells with

non-lymphoid homing potential are preferentially depleted during HIV infection despite early treatment. Similarly, a number of studies have shown that $CCR5^+CD4^+$ Th cells were preferentially depleted during HIV and SIV infection, whereas others have shown that both $CCR5^+$ and $CCR5^-CD4^+$ T-cell subsets were infected during acute SIV infection.^{1,5,26,27}

As most (>95%) of the $\alpha 4\beta 7^{hi}CD4^+$ T-cell subsets express detectable levels of CCR5 on their surface, we did not have sufficient samples to sort enough $\alpha 4\beta 7^{hi}CD4^+CCR5^-$ T-cell subsets for the qPCR analysis. Hence, we restricted our analysis to $\beta 7^-CD4^+$ T-cell subsets. We sorted $\beta 7^-CD4^+CCR5^+$ and $\beta 7^-CD4^+CCR5^-$ T-cell subsets from peripheral blood of SIV-infected animals (days 7 and 14 pi) obtained from an unrelated study and quantified the levels of SIV-gag DNA by qPCR.

Our results (Figure 5b) show that both $\beta 7^-CD4^+CCR5^+$ and $\beta 7^-CD4^+CCR5^-$ T-cell subsets had similar levels of SIV-gag

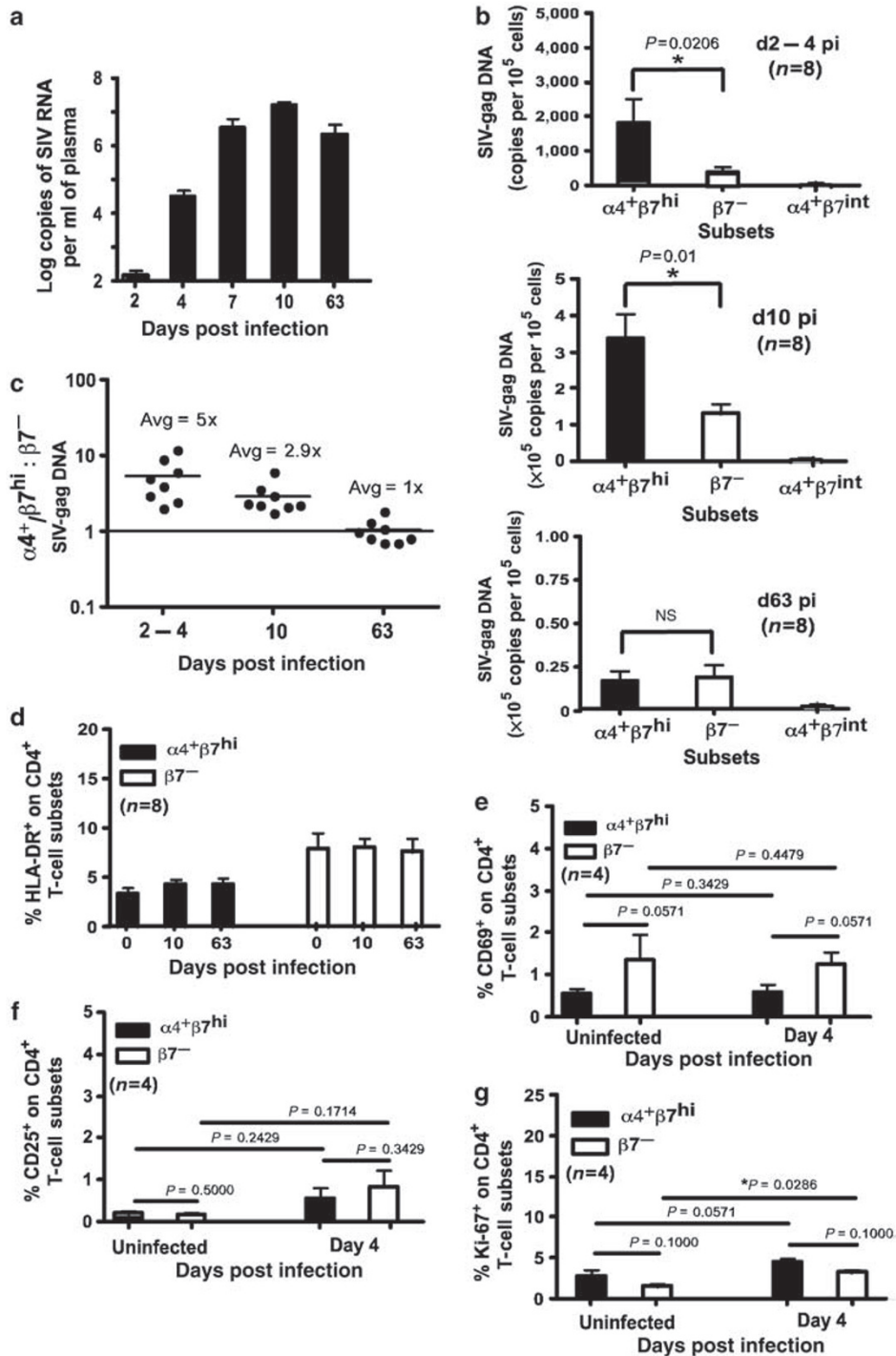


Figure 4 $\alpha 4^{+}\beta 7^{hi}$ CD4⁺ T cells are preferentially infected during acute simian immunodeficiency virus (SIV) infection. (a) Plasma viral loads. Limit of detection is <30 copies per ml of plasma. (b) Levels of SIV-gag DNA were determined using qPCR assay for SIV-gag in sorted subsets of $\alpha 4^{+}\beta 7^{hi}$, $\alpha 4^{+}\beta 7^{int}$, and $\beta 7^{-}$ CD4⁺ T cells. $\alpha 4^{+}\beta 7^{hi}$ CD4⁺ T cells are preferentially infected at days 2–4 and day 10 pi (post infection). (c) Ratio of SIV-gag DNA levels in $\alpha 4^{+}\beta 7^{hi}:\beta 7^{-}$ CD4⁺ T cells at days 2–4 pi, 10 pi, and 63 pi. The expression of (d) HLA-DR, (e) CD69, (f) CD25, and (g) Ki-67 on $\alpha 4^{+}\beta 7^{hi}$ and $\beta 7^{-}$ CD4⁺ T cells during the early phase of SIV infection.

DNA, which did not differ significantly, suggesting that preferential infection was not directly dependent on the surface level of CCR5 expression. As shown in **Figure 2f**, CCR5⁺ T-cell subsets express sufficient levels of CCR5 message that is likely sufficient

to make these cells susceptible to SIV infection. These results support previous studies,⁵ showing that there was no difference in the level of SIV infection in memory CD4⁺CCR5⁺ and memory CD4⁺CCR5[−] T-cell subsets at days 3, 7, 10, and 14 pi.

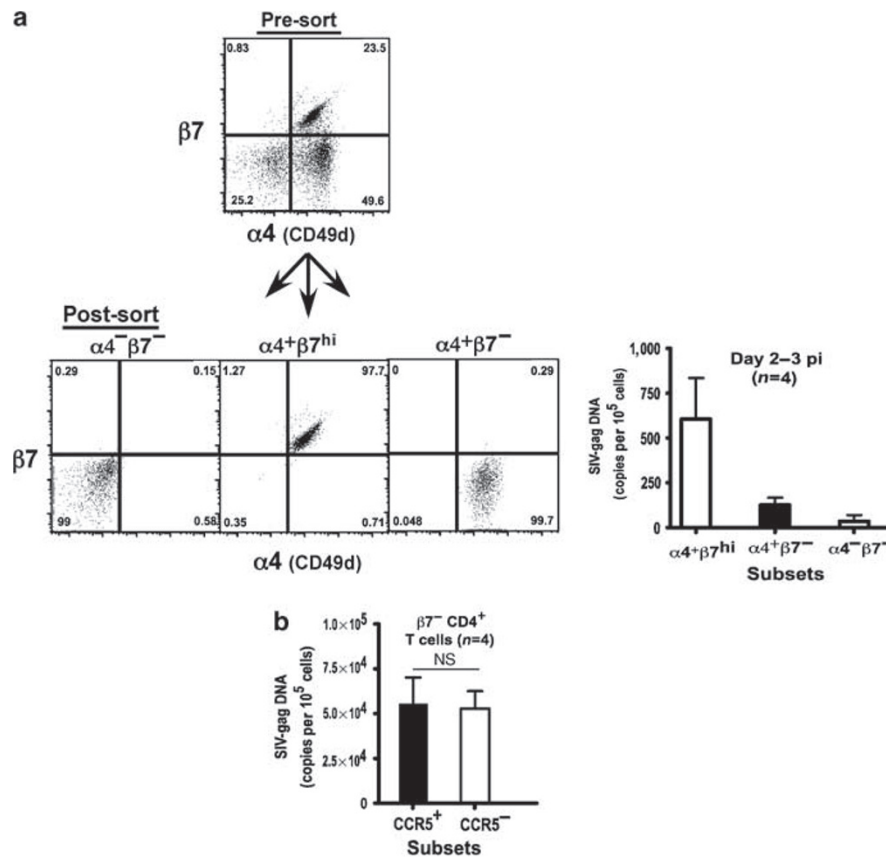


Figure 5 CD4⁺ T cells that express the $\alpha 4\beta 7$ heterodimer are preferentially infected *in vivo*. Memory CD4⁺ T cells were sorted based on the co-expression of $\alpha 4$ and $\beta 7$ integrins by flow cytometry, and used in the qPCR assay for simian immunodeficiency virus (SIV)-gag DNA. (a) Sorting strategy and purity of sorted subsets are shown along with the level of SIV infection in $\alpha 4^+ \beta 7^{hi}$, $\alpha 4^+ \beta 7^-$, and $\alpha 4^- \beta 7^-$ subsets at days 2–3 pi (post infection). $\alpha 4^+ \beta 7^{hi}$ subsets harbor significantly higher levels of SIV DNA at days 2–3 pi ($n=4$; $P=0.0244$) compared to $\alpha 4^+ \beta 7^-$ and $\alpha 4^- \beta 7^-$ subsets. Statistical analysis was performed using Kruskal–Wallis one way analysis of variance (ANOVA) (b) There is no significant difference in the level of SIV-gag DNA between $\beta 7^- CD4^+ CCR5^+$ and $\beta 7^- CD4^+ CCR5^-$ T-cell subsets of memory CD4⁺ T cells from the peripheral blood at days 7 and 14 pi (day 7: $n=2$, day 14: $n=2$). $\beta 7^-$ subsets were gated based on the lack of $\beta 7$ expression and CD95, followed by sorting of $CCR5^+$ and $CCR5^-$ subsets.

$\alpha 4^+ \beta 7^{hi} CD4^+$ T-cell subsets harbor most Th-17 cells and are lost during acute SIV infection

Previous studies have shown that mucosal CD4⁺ T cells are major producers of IL-17, and HIV infection was associated with alterations in the balance between Th17- and Th1-type responses.^{11–14} To determine whether the preferential loss of $\alpha 4^+ \beta 7^{hi} CD4^+$ T-cell subsets was accompanied by changes in the profile of Th17- vs. Th1-type responses, we evaluated the production of IL-17 and IFN γ by $\alpha 4^+ \beta 7^{hi} CD4^+$ T-cell subsets and compared them with $\beta 7^- CD4^+$ T-cell subsets.

Our results showed that $\alpha 4^+ \beta 7^{hi} CD4^+$ T-cell subsets harbored significantly higher frequencies of Th17-type cells, whereas $\beta 7^- CD4^+$ T-cell subsets harbored higher proportions of IFN γ -producing Th1-type cells (Figure 6a, b). Most of the Th-17 cells were found to be CD28⁺ memory CD4⁺ T cells (Supplementary Figure S1d online). The ratio of IFN γ :IL-17-producing cells was ~2 within the $\alpha 4\beta 7^{hi} CD4^+$ T-cell subsets (Figure 6c), whereas it was >10 within the $\beta 7^- CD4^+$ T-cell subsets, indicating that $\alpha 4\beta 7^{hi} CD4^+$ T-cell subsets were the primary source of Th-17 responses. SIV infection was found to significantly skew the ratio to a predominantly Th-1 phenotype,

as there was a significant depletion of Th-17 cells during early SIV infection.

DISCUSSION

Early SIV infection have been shown to target founder populations of CD4⁺ T cells.⁴ These cells are minimally activated, and have a central role in early viral replication and dissemination. Not much is known about why SIV specifically targets these founder populations of CD4⁺ T cells. Given the highly activated microenvironment in mucosal tissues, and the enrichment of target cells in these tissues, one would expect massive infection and replication to occur after viral entry. However, there appears to be a substantial delay of about 7–8 days before infection explodes out of the early founder population of cells to infect and destroy most of the memory CD4⁺ T-cell compartment.^{4,5} Arthos *et al.*¹⁰ using *in vitro* studies showed that HIV and SIVmm selectively targeted CD4⁺ T cells that expressed the $\alpha 4\beta 7$ heterodimer, suggesting that these cells could be earliest targets for viral infection *in vivo*. It is not known whether HIV preferentially targets memory CD4⁺ T cells that express the $\alpha 4\beta 7$ receptor *in vivo*.

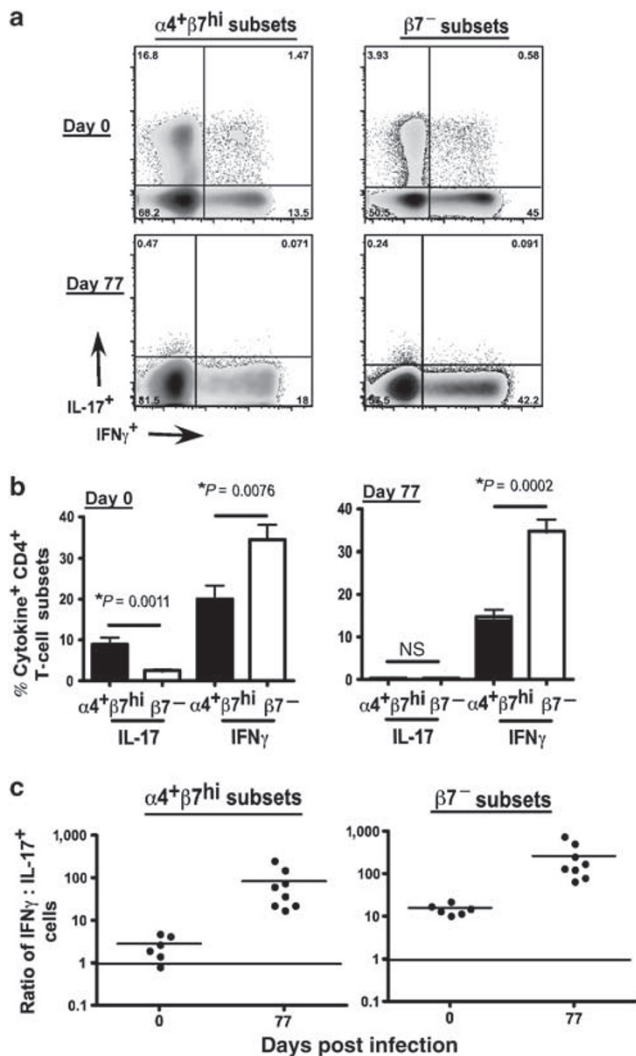


Figure 6 $\alpha 4 + \beta 7^{\text{hi}} \text{CD}4^+$ T cells harbor significantly higher frequencies of Th-17 cells that are lost after simian immunodeficiency virus (SIV) infection. $\alpha 4 + \beta 7^{\text{hi}} \text{CD}4^+$ T-cell subsets have a significantly higher capacity to produce interleukin (IL)-17 compared with $\beta 7^- \text{CD}4^+$ T-cell subsets. Expression of IL-17 and interferon (IFN) γ was determined by flow cytometry using peripheral blood mononuclear cells (PBMCs) from uninfected ($n=6$) and SIV-infected animals ($n=8$) after stimulation with phorbol myristate acetate (PMA)/ionomycin for 4 h. (a) Representative dotplots showing IL-17 and IFN γ production by $\alpha 4 + \beta 7^{\text{hi}}$ and $\beta 7^- \text{CD}4^+$ T-cell subsets at days 0 and 77 pi (post infection). (b) IL-17 and IFN γ production by $\alpha 4 + \beta 7^{\text{hi}}$ and $\beta 7^- \text{CD}4^+$ T-cell subsets at days 0 ($n=6$) and 77 pi ($n=8$). (c) The ratio of IFN γ :IL-17 producing $\alpha 4 + \beta 7^{\text{hi}}$ and $\beta 7^- \text{CD}4^+$ T-cell subsets at days 0 and 77 pi.

Using the SIVmac251 infection model, we show that memory $\text{CD}4^+$ T cells that expressed high levels of the $\alpha 4\beta 7$ heterodimer are preferentially infected during the early phase of viral infection; $\alpha 4 + \beta 7^{\text{hi}} \text{CD}4^+$ T cells harbored $\sim 5\times$ more SIV-gag DNA compared with $\beta 7^- \text{CD}4^+$ T cells. Surprisingly, $\alpha 4 + \beta 7^{\text{hi}} \text{CD}4^+$ T cells were found to be predominantly resting T cells ($\text{CD}25^- \text{CD}69^- \text{HLA-DR}^- \text{Ki}67^-$) with a central memory ($\text{CD}28^+ \text{CD}45\text{RA}^- \text{CCR}7^+$) phenotype that did not significantly change during the early phase of viral infection. Li *et al.*⁴ reported that during the early phase of infection, SIV

infection in the GALT is confined to resting $\text{CD}4^+$ T cells that were $\text{CD}25^- \text{CD}69^- \text{Ki}67^-$.

Both $\text{CCR}5^+$ and $\text{CCR}5^-$ subsets of $\alpha 4 + \beta 7^{\text{hi}} \text{CD}4^+$ and $\beta 7^- \text{CD}4^+ \text{CCR}5^+$ T cells were found to have significant levels of CCR5 mRNA, suggesting that all the memory $\text{CD}4^+$ T cells subsets have sufficient CCR5 for infection by SIV. In line with this, both $\beta 7^- \text{CD}4^+ \text{CCR}5^+$ and $\beta 7^- \text{CD}4^+ \text{CCR}5^-$ T cells had similar levels of SIV infection. It is likely that once SIV binds to the cells, the high levels of CCR5 expression enables SIV to more efficiently infect $\alpha 4 + \beta 7^{\text{hi}} \text{CD}4^+$ T cells, especially during the early phase of infection, when viral loads are very low, as seen at days 2–4 pi.

By days 7–10 pi, there was a significant increase in the extent of infection in both $\alpha 4 + \beta 7^{\text{hi}}$ and $\beta 7^- \text{CD}4^+$ T cells, most likely as a consequence of the massive viral replication occurring during this period. However, $\alpha 4 + \beta 7^{\text{hi}} \text{CD}4^+$ T-cell subsets were found to harbor $\sim 3\times$ more SIV DNA than $\beta 7^- \text{CD}4^+$ T-cell subsets.

Interestingly, $\alpha 4 + \beta 7^{\text{hi}} \text{CD}4^+$ T cells were found to contain ~ 3.5 to 4 copies of SIV per cell at day 10 pi, suggesting that there were likely more than 1 virion per cell. The assay used to quantify SIV infection in cells is stringently standardized using a non-expressing SIV-infected cell line that carries only a single copy for SIV DNA.⁵ As such, we do not think that these high copy numbers are assay related. It is more likely that the expression of the $\alpha 4\beta 7$ receptor along with CD4 and high levels of CCR5 makes $\alpha 4 + \beta 7^{\text{hi}} \text{CD}4^+$ T cells more susceptible to super infection with SIV. Previous studies have reported multiply infected $\text{CD}4^+$ T cells in HIV-infected patients. Jung *et al.*²⁸ using fluorescent *in situ* hybridization assays showed that $\text{CD}4^+$ T cells in the spleen of HIV-infected patients carried 4–6 proviruses per infected cell. Future studies will attempt to address this question in more detail.

By day 63 pi, the extent of infection had equalized between the two subsets, suggesting that there was a significantly higher loss of infected $\alpha 4 + \beta 7^{\text{hi}} \text{CD}4^+$ T cells after peak viral infection. In line with this conclusion, a significantly greater number of $\alpha 4 + \beta 7^{\text{hi}} \text{CD}4^+$ T-cell subsets were lost between days 0 and 63 pi compared with the $\beta 7^- \text{CD}4^+$ T-cell subsets.

A number of factors likely contribute to the amplification of infection in $\beta 7^- \text{CD}4^+$ T-cell subsets. Acute SIV infection has been shown to be associated with acute immune activation,^{23,24} leading to the production of numerous pro-inflammatory mediators such as IL-15.²³ Similarly, acute HIV infection is associated with an increase in numerous pro-inflammatory cytokines, such as TNF α (tumor necrosis factor- α), IFN γ , IL-6, IL-8, MCP-1 (monocyte chemotactic protein-1), IFN α .²⁹ These factors likely have a role in making $\text{CD}4^+$ T cells more susceptible to infection. Eberly *et al.*²³ showed that there was a significant increase in the production of IL-15 between days 7 and 14 pi, which was found to correlate with infection in memory $\text{CD}4^+$ T cells during the early stages of infection. Others have shown that efficiency of infection was greatly influenced by the density of CD4, but not CCR5, expression on the surface of target cells.^{30–33}

It is also possible that during periods of high viremia, as seen during the ramp-up phase of acute viral infection, the threshold for efficient infection is lower than during the pre-ramp phase, when

viral loads are very low. Thus, although $\beta 7^{-}CD4^{+}$ T-cell subsets inherently expressed low levels of CCR5, it was likely sufficient for SIV to efficiently infect these cells between days 7 and 14 pi when plasma viral loads are as high as 7–8 logs per ml.

Previous studies^{4,5} have shown that < 5% of memory $CD4^{+}$ T cells were infected before day 7 pi, whereas by days 10–14 pi, there was a significant increase in infection of these cells. Although these memory $CD4^{+}$ T cells lacked detectable CCR5 on their surface, they were found to have 20× more CCR5 mRNA compared with naive $CD4^{+}$ T cells, which was sufficient for them to be infected by SIV. In line with these studies, we found significant levels of CCR5 mRNA in all the subsets of $\alpha 4^{+}\beta 7^{hi}$ and $\beta 7^{-}CD4^{+}$ T cells. Others have shown that, minimal expression of CCR5 was sufficient for infection of $CD4^{+}$ T cells.^{30,32,33} Eberly *et al.*²³ showed that acute SIV infection was associated with little or no upregulation of CCR5 expression on memory $CD4^{+}$ T cells during the first 10 days of infection, yet there was a significant increase in the level of SIV infection in these cells between days 7 and 10 pi. Taken together, these studies indicate that when viral loads are high, minimal levels of CCR5 is likely sufficient to support efficient infection of memory $CD4^{+}$ T-cell subsets.

Interestingly, $\alpha 4^{+}\beta 7^{hi}CD4^{+}$ T-cell subsets harbored a significantly higher potential to produce IL-17, suggesting that most of the Th-17 cells are found within these mucosal homing T-cell subsets. The preferential loss of these subsets during acute SIV infection likely cripples the ability of the immune system to maintain homeostasis in mucosal tissues. On the other hand, most of the IFN γ -producing Th-1 cells were found to be $\beta 7^{-}CD4^{+}$ T-cell subsets, and fewer of these cells were lost, suggesting a skewing of the Th cells response to a Th-1 phenotype. Recent studies¹¹ have shown that Th-17 responses have a central role in protecting the mucosal barrier function, and the loss of Th-17 cells was associated with systemic dissemination of *Salmonella* in SIV-infected rhesus macaques. Cecchinato *et al.*¹³ showed that altered balance between Th-17 and Th-1 cells in mucosal sites predicted AIDS progression in SIV-infected rhesus macaques. On the other hand, Favre *et al.*¹⁴ showed that pathogenic SIV infection was associated with altered balance between Th-17 and T-regulatory populations. Similarly, Brenchley *et al.*¹² showed that Th-17 cells were differentially depleted in pathogenic vs. non-pathogenic lentiviral infections. Macal *et al.*³⁴ showed that restoration of mucosal $CD4$ T cells was associated with enhanced Th-17 responses in HIV-infected patients. Taken together, these studies suggest that preferential infection and depletion of $\alpha 4^{+}\beta 7^{hi}CD4^{+}$ T-cell subsets significantly skews Th cell responses toward a Th-1 phenotype, thereby contributing to disease progression.

In conclusion, our results provide *in vivo* evidence that, during the early phase of SIV infection, SIVmac251 selectively targets $\alpha 4^{+}\beta 7^{hi}CD4^{+}$ T-cell subsets. These cells are predominantly central memory T cells with a resting phenotype and resemble the founder populations of $CD4^{+}$ T cells described by Li *et al.*⁴ The inherently high level of CCR5 expression likely allows SIV to more efficiently infect the $\alpha 4^{+}\beta 7^{hi}CD4^{+}$ T-cell subsets very early during the course of viral infection. Development of

therapeutic strategies targeted at preventing the early infection of memory $CD4^{+}$ T-cell subsets that express high levels of $\alpha 4\beta 7$ heterodimer may significantly aid in the early control of viral infection and contribute to the maintenance of homeostasis in mucosal tissues.

METHODS

Animals and infection. Mamu* A01^{neg} rhesus macaques (*Macaca mulatta*) of Indian origin ($n=8$) housed at Advanced Bioscience Laboratories (MD) were used in this study. The animals were housed in accordance with the American Association for Accreditation of Laboratory Animal Care guidelines and were sero-negative for SIV, simian retrovirus, and simian T-cell leukemia virus type-1. All the animals were infected with 100 animal-infectious doses of uncloned pathogenic SIVmac251 intravenously. Peripheral blood was collected at different time points (days 0, 2, 4, 10, and 63) after challenge, and complete blood count data were obtained at days 0 and 63 pi. No complete blood count data were collected at other time points; hence, absolute counts for various subsets could only be determined at days 0 and 63 pi. In addition, peripheral blood samples were also obtained from 14 SIVmac251-infected rhesus macaques at days 2–3 ($n=4$) and 7–14 pi ($n=10$), 77 ($n=8$), and from uninfected animals ($n=6-8$).

Tissue samples. Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation, as previously described.⁵ Plasma viral loads were determined by real-time PCR (ABI Prism 7700 Sequence Detection System, Applied Biosystems, Foster City, CA) using reverse-transcribed viral RNA as templates using methods, as previously described³⁵ (limit of detection < 30 copies per ml of plasma).

Antibodies and flow cytometry. All antibodies, except for CD103, used in this study were obtained from BD Biosciences (San Diego, CA), and titrated using rhesus macaque PBMC. For phenotypic analysis and sorting of $CD4^{+}$ T-cell subsets, cells were labeled simultaneously with the following combinations of antibodies: CD3-Cy7APC, CD8-Alexa-700, CD4-APC, CD95-FITC, CCR5-PE, HLA-DR-Texas Red-PE, and Integrin $\beta 7$ (FIB504 clone)-Cy5-PE. FIB504 clone has been shown to cross-react with rhesus macaques.^{10,21} $\beta 7$ Integrin forms a heterodimer with $\alpha 4$ (CD49d) or αE (CD103) integrins, and has a central role in homing of T cells to mucosal tissues.³⁶⁻³⁹ To delineate $\alpha 4^{+}\beta 7^{+}CD4^{+}$ T-cell subsets from $\alpha E\beta 7^{+}CD4^{+}$ T-cell subsets, cells were labeled with CD3-Cy7APC, CD8-Alexa-700, CD4-Qdot-605, CD95-APC, CD49d-PE, CD103-FITC (clone 2G5 from Beckman Coulter Fullerton, CA), and Integrin $\beta 7$ -Cy5-PE. Clone 2G5 has been shown to cross-react with rhesus macaque mucosal homing T cells, and expressed by intraepithelial lymphocytes.^{40,41} To evaluate the phenotype of $\alpha 4\beta 7$ subsets of $CD4^{+}$ T cells, the cells were labeled with a panel of CD3-Cy7APC, CD8-Qdot-605, CD4-APC, CD95-FITC, CD49d-PE and $\beta 7$ -Cy5-PE, CD45RA-TR-PE, CD28-Cy7-PE, and CCR7-Alexa-680. The expression of activation markers was determined by using a panel of CD3-Cy7APC, CD8-Alexa-700, CD4-TR-PE, CD95-FITC, CD49d-PE, $\beta 7$ -Cy5-PE, CD69, or CD25-APC.

To determine which $\alpha 4\beta 7$ subsets were preferentially infected, $\alpha 4\beta 7$ subsets of $CD4^{+}$ T cells (discriminated based on the expression of $\beta 7$ and CD95²¹) were sorted, and subjected to qPCR assay for measuring SIV-gag DNA, as described previously.^{5,15} To determine whether $CD4^{+}$ T cells that expressed the $\alpha 4\beta 7$ heterodimer were preferentially infected,

PBMCs were labeled with CD3-Cy7APC, CD8-Alexa-700, CD4-APC, CD95-FITC, CD49d-PE, and β 7-Cy5-PE. In addition, KI-67 expression was evaluated using a panel of CD3-Cy7APC, CD8-Alexa-700, CD4-APC, CD95-PE, and β 7-Cy5-PE. After surface labeling, the cells were fixed/permeabilized using the Cytofix/Perm Kit (BD Biosciences) and labeled with Ki-67-FITC.

To evaluate the ability of α 4 β 7 subsets of CD4⁺ T cells to produce IL-17 and IFN γ , PBMCs were stimulated with phorbol myristate acetate at 10 ng ml⁻¹ and ionomycin at 500 ng ml⁻¹ in the presence of Brefeldin-A (1 μ M) for 4 h. The cells were harvested and labeled with CD3-Cy7APC, CD8-Alexa-700, CD4-Qdot-605, CD95-APC, and β 7-Cy5-PE. After surface labeling, the cells were fixed/permeabilized using the Cytofix/Perm Kit and labeled with IL-17-PE (e-Biosciences, San Diego, CA) and IFN γ -FITC (BD Biosciences).

Labeled cells were fixed in 0.5% paraformaldehyde and analyzed using a modified Becton Dickinson Aria sorter.

qPCR assay. T-cell-associated viral DNA was measured by a qPCR assay for SIV *gag* using a Perkin-Elmer ABI 7500 instrument, as previously described^{5,15} using SIV *gag* primers and probe as described by Lifson *et al.*⁴² Cell numbers were quantified simultaneously using rhesus macaque albumin-specific primers and probe using previously described protocols.⁵ The assay was calibrated using a cell line that carried a single copy of proviral SIV DNA as shown previously.⁵ Sorted samples were lysed in Proteinase-K and used for PCR analysis. We have previously shown that this assay was highly sensitive and can be used to successfully measure SIV-DNA in single cells.⁵

CCR5 mRNA levels were determined using primer/probes as described previously,⁵ and normalized to β 2M levels in sorted subsets. Data are shown relative to α 4⁺ β 7^{int}CD4⁺ (naive) T cells.

Data analysis. Flow cytometric data were analyzed using FlowJo version 8.6 (Tree Star, Ashland, OR). Statistical analysis was carried out using GraphPad Prism Version 4.0 software (GraphPad Prism Software, San Diego, CA) using non-parametric tests. As the data did not exhibit normal distribution due to small sample size, comparisons between groups were carried out using Mann-Whitney *U*-test, and multiple comparisons between groups was carried out using the Kruskal-Wallis one way analysis of variance (ANOVA). *P* < 0.05 was considered significant.

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at <http://www.nature.com/mi>

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AUTHOR CONTRIBUTIONS

M.K. processed the samples, performed all the experiments, analyzed and helped in the interpretation of data, and preparation of the paper. X.W. and R.S.V. helped with samples and preparation of the paper. M.P. and J.L. helped with plasma viral load assays, and reviewing the paper. M.R. provided certain reagents and helped in reviewing the paper. J.J.M. designed, helped with analysis and interpretation of data, preparation of the paper, and supervised the study.

DISCLOSURE

The authors declared no conflict of interest.

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REFERENCES

- Brenchley, J.M. *et al.* CD4⁺ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. *J. Exp. Med.* **200**, 749–759 (2004).
- Guadalupe, M. *et al.* Severe CD4⁺ T-cell depletion in gut lymphoid tissue during primary human immunodeficiency virus type 1 infection and substantial delay in restoration following highly active antiretroviral therapy. *J. Virol.* **77**, 11708–11717 (2003).
- Kewenig, S. *et al.* Rapid mucosal CD4(+) T-cell depletion and enteropathy in simian immunodeficiency virus-infected rhesus macaques. *Gastroenterology* **116**, 1115–1123 (1999).
- Li, Q. *et al.* Peak SIV replication in resting memory CD4⁺ T cells depletes gut lamina propria CD4⁺ T cells. *Nature* **434**, 1148–1152 (2005).
- Mattapallil, J.J. *et al.* Massive infection and loss of memory CD4⁺ T cells in multiple tissues during acute SIV infection. *Nature* **434**, 1093–1097 (2005).
- Mehandru, S. *et al.* Primary HIV-1 infection is associated with preferential depletion of CD4⁺ T lymphocytes from effector sites in the gastrointestinal tract. *J. Exp. Med.* **200**, 761–770 (2004).
- Veazey, R.S. *et al.* Gastrointestinal tract as a major site of CD4⁺ T cell depletion and viral replication in SIV infection. *Science* **280**, 427–431 (1998).
- Gordon, S.N. *et al.* Severe depletion of mucosal CD4⁺ T cells in AIDS-free simian immunodeficiency virus-infected sooty mangabeys. *J. Immunol.* **179**, 3026–3034 (2007).
- Pandrea, I.V. *et al.* Acute loss of intestinal CD4⁺ T cells is not predictive of simian immunodeficiency virus virulence. *J. Immunol.* **179**, 3035–3046 (2007).
- Arthos, J. *et al.* HIV-1 envelope protein binds to and signals through integrin α 4 β 7, the gut mucosal homing receptor for peripheral T cells. *Nat. Immunol.* **9**, 301–309 (2008).
- Raffatelli, M. *et al.* Simian immunodeficiency virus-induced mucosal interleukin-17 deficiency promotes *Salmonella* dissemination from the gut. *Nat. Med.* (2008).
- Brenchley, J.M. *et al.* Differential Th17 CD4 T-cell depletion in pathogenic and nonpathogenic lentiviral infections. *Blood* **112**, 2826–2835 (2008).
- Cecchinato, V. *et al.* Altered balance between Th17 and Th1 cells at mucosal sites predicts AIDS progression in simian immunodeficiency virus-infected macaques. *Mucosal Immunol.* **1**, 279–288 (2008).
- Favre, D. *et al.* Critical loss of the balance between Th17 and T regulatory cell populations in pathogenic SIV infection. *PLoS Pathog.* **5**, e1000295 (2009).
- Douek, D.C. *et al.* HIV preferentially infects HIV-specific CD4⁺ T cells. *Nature* **417**, 95–98 (2002).
- Butcher, E.C. & Picker, L.J. Lymphocyte homing and homeostasis. *Science* **272**, 60–66 (1996).
- Chan, B.M., Elices, M.J., Murphy, E. & Hemler, M.E. Adhesion to vascular cell adhesion molecule 1 and fibronectin. Comparison of α 4 β 7 (VLA-4) and α 4 β 7 on the human B cell line JY. *J. Biol. Chem.* **267**, 8366–8370 (1992).
- Hemler, M.E., Huang, C. & Schwarz, L. The VLA protein family. Characterization of five distinct cell surface heterodimers each with a common 130,000 molecular weight beta subunit. *J. Biol. Chem.* **262**, 3300–3309 (1987).

19. Rose, D.M., Han, J. & Ginsberg, M.H. Alpha4 integrins and the immune response. *Immunol. Rev.* **186**, 118–124 (2002).
20. Takada, Y., Elices, M.J., Crouse, C. & Hemler, M.E. The primary structure of the alpha 4 subunit of VLA-4: homology to other integrins and a possible cell-cell adhesion function. *EMBO J.* **8**, 1361–1368 (1989).
21. Pitcher, C.J. *et al.* Development and homeostasis of T cell memory in rhesus macaque. *J. Immunol.* **168**, 29–43 (2002).
22. Lazarovits, A.I. *et al.* Lymphocyte activation antigens. I. A monoclonal antibody, anti-Act I, defines a new late lymphocyte activation antigen. *J. Immunol.* **133**, 1857–1862 (1984).
23. Eberly, M.D. *et al.* Increased IL-15 production is associated with higher susceptibility of memory CD4 T cells to simian immunodeficiency virus during acute infection. *J. Immunol.* **182**, 1439–1448 (2009).
24. Kaur, A., Hale, C.L., Ramanujan, S., Jain, R.K. & Johnson, R.P. Differential dynamics of CD4(+) and CD8(+) T-lymphocyte proliferation and activation in acute simian immunodeficiency virus infection. *J. Virol.* **74**, 8413–8424 (2000).
25. Krzysiek, R. *et al.* Preferential and persistent depletion of CCR5+ T-helper lymphocytes with nonlymphoid homing potential despite early treatment of primary HIV infection. *Blood* **98**, 3169–3171 (2001).
26. Veazey, R.S. *et al.* Dynamics of CCR5 expression by CD4(+) T cells in lymphoid tissues during simian immunodeficiency virus infection. *J. Virol.* **74**, 11001–11007 (2000).
27. Veazey, R.S., Marx, P.A. & Lackner, A.A. Vaginal CD4+ T cells express high levels of CCR5 and are rapidly depleted in simian immunodeficiency virus infection. *J. Infect. Dis.* **187**, 769–776 (2003).
28. Jung, A. *et al.* Multiply infected spleen cells in HIV patients. *Nature* **418**, 144 (2002).
29. Stacey, A.R. *et al.* Induction of a striking systemic cytokine cascade prior to peak viraemia in acute human immunodeficiency virus type 1 infection, in contrast to more modest and delayed responses in acute hepatitis B and C virus infections. *J. Virol.* **83**, 3719–3733 (2009).
30. Kozak, S.L., Kuhmann, S.E., Platt, E.J. & Kabat, D. Roles of CD4 and coreceptors in binding, endocytosis, and proteolysis of gp120 envelope glycoproteins derived from human immunodeficiency virus type 1. *J. Biol. Chem.* **274**, 23499–23507 (1999).
31. Kozak, S.L. *et al.* CD4, CXCR-4, and CCR-5 dependencies for infections by primary patient and laboratory-adapted isolates of human immunodeficiency virus type 1. *J. Virol.* **71**, 873–882 (1997).
32. Pesenti, E. *et al.* Role of CD4 and CCR5 levels in the susceptibility of primary macrophages to infection by CCR5-dependent HIV type 1 isolates. *AIDS Res. Hum. Retroviruses* **15**, 983–987 (1999).
33. Platt, E.J., Wehrly, K., Kuhmann, S.E., Chesebro, B. & Kabat, D. Effects of CCR5 and CD4 cell surface concentrations on infections by macrophagetropic isolates of human immunodeficiency virus type 1. *J. Virol.* **72**, 2855–2864 (1998).
34. Macal, M. *et al.* Effective CD4+ T-cell restoration in gut-associated lymphoid tissue of HIV-infected patients is associated with enhanced Th17 cells and polyfunctional HIV-specific T-cell responses. *Mucosal Immunol.* **1**, 475–488 (2008).
35. Cline, A.N., Bess, J.W., Piatak, M. Jr & Lifson, J.D. Highly sensitive SIV plasma viral load assay: practical considerations, realistic performance expectations, and application to reverse engineering of vaccines for AIDS. *J. Med. Primatol.* **34**, 303–312 (2005).
36. Andrew, D.P., Rott, L.S., Kilshaw, P.J. & Butcher, E.C. Distribution of alpha 4 beta 7 and alpha E beta 7 integrins on thymocytes, intestinal epithelial lymphocytes and peripheral lymphocytes. *Eur. J. Immunol.* **26**, 897–905 (1996).
37. Berlin, C. *et al.* Alpha 4 beta 7 integrin mediates lymphocyte binding to the mucosal vascular addressin MAdCAM-1. *Cell* **74**, 185–195 (1993).
38. Briskin, M. *et al.* Human mucosal addressin cell adhesion molecule-1 is preferentially expressed in intestinal tract and associated lymphoid tissue. *Am. J. Pathol.* **151**, 97–110 (1997).
39. Campbell, D.J. & Butcher, E.C. Rapid acquisition of tissue-specific homing phenotypes by CD4(+) T cells activated in cutaneous or mucosal lymphoid tissues. *J. Exp. Med.* **195**, 135–141 (2002).
40. Mattapallil, J.J., Letvin, N.L. & Roederer, M. T-cell dynamics during acute SIV infection. *AIDS* **18**, 13–23 (2004).
41. Veazey, R.S. *et al.* Characterization of gut-associated lymphoid tissue (GALT) of normal rhesus macaques. *Clin. Immunol. Immunopathol.* **82**, 230–242 (1997).
42. Lifson, J.D. *et al.* Role of CD8(+) lymphocytes in control of simian immunodeficiency virus infection and resistance to rechallenge after transient early antiretroviral treatment. *J. Virol.* **75**, 10187–10199 (2001).