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The frequency of $\alpha_4\beta_7^{\text{high}}$ memory CD4⁺ T cells correlates with susceptibility to rectal SIV infection

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Abstract

Background—Integrin $\alpha_4\beta_7$ ($\alpha_4\beta_7$) mediates the homing of CD4⁺ T cells to gut-associated lymphoid tissues (GALT), which constitute a highly favorable environment for HIV expansion and dissemination. HIV and SIV envelope proteins bind to and signal through $\alpha_4\beta_7$ and during acute infection SIV preferentially infects $\alpha_4\beta_7^{\text{high}}$ CD4⁺ T cells. We postulated that the availability of these cells at the time of challenge could influence mucosal SIV transmission and acute viral load (VL).

Methods—We challenged 17 rhesus macaques with 3000 TCID₅₀ of SIV_{mac239} rectally and followed the subsets of $\alpha_4\beta_7^+$ T and dendritic cells (DCs) by flow cytometry in blood and tissues, before and after challenge.

Results—We found that the frequency of memory CD4⁺ T cells that expressed high levels of $\alpha_4\beta_7^{\text{high}}$ ($\alpha_4\beta_7^{\text{high}}$ memory CD4⁺ T cells) in blood before challenge correlated strongly with susceptibility to infection and acute VL. Notably, not only at the time of challenge, but also their frequency 3 weeks before challenge correlated with infection. This association extended to the rectal tissue as we observed a strong direct correlation between the frequency of $\alpha_4\beta_7^{\text{high}}$ memory CD4⁺ T cells in blood and rectum before and after challenge. The frequency of $\alpha_4\beta_7^+$ myeloid DCs and $\alpha_4\beta_7^{\text{high}}$ CD80⁺ DCs also correlated with infection and acute VL, while blood CCR5⁺ and CD69⁺ CD4⁺ T cells could not be associated with infection.

Conclusions—Our results suggest that animals with higher frequency of $\alpha_4\beta_7^{\text{high}}$ CD4⁺ T cells in circulation and in rectal tissue could be more susceptible to SIV rectal transmission.

Keywords

HIV; SIV; mucosa transmission; integrin alpha-4 beta-7; susceptibility; gut

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Introduction

Mucosal HIV transmission is considered a rare event [1]. The site of exposure (rectal or vaginal), the viral load (VL) of the infected partner and the presence of other sexually transmitted infections (STIs) constitute variables with major impact on the likelihood of transmission [2, 3]. However, there may be additional unidentified viral and host-related characteristics that sway the chances of productive infection.

In order to proliferate and disseminate after exposure, HIV needs to evade numerous barriers in the mucosa and find its way to draining lymph nodes (LNs) and gut inductive sites [4]. The availability of HIV targets cells at the site of exposure is influenced by several factors (e.g. STIs) that impact the quality and quantity of cell subsets with differential ability to spread infection (CCR5⁺ and $\alpha 4 \beta 7^{\text{high}}$ CD4⁺ T cells) [5–8].

$\alpha 4 \beta 7^{\text{high}}$ CD4⁺ T cells constitute a major target of HIV/SIV infection and are preferentially infected during the acute stages [5, 6, 8]. It was reported that at day 2–4 post-infection $\alpha 4 \beta 7^{\text{high}}$ CD4⁺ T cells have 5 times more SIV-gag DNA than $\alpha 4 \beta 7^{-}$ CD4⁺ T cells [6] and their frequency in blood correlates with the loss of CD4⁺ T cells in the gut [9]. Of note, blocking $\alpha 4 \beta 7$ by injecting a monoclonal antibody (mAb) against $\alpha 4 \beta 7$ prior to intravenous SIV challenge reduces peak VL, decreasing pro-viral DNA in PBMCs, jejunal and colorectal tissues [10].

Once viral replication is underway at the site of exposure, the next critical stage in mucosal transmission is the ability of HIV infected cells to rapidly reach the mucosal draining LNs [4]. Notably, $\alpha 4 \beta 7$ is responsible for trafficking and retention of lymphocytes in the gut-associated lymphoid tissue (GALT), Peyer's patches (PP), mesenteric LNs (MLNs) and gut lamina propria (LP) [11]. These are among the first sites reached by HIV and where the virus replicates at a very high rate. Moreover, $\alpha 4 \beta 7$ -expressing leukocytes play an essential role in mucosal immunity and are also important in the immune response of the female genital tract (FGT) [12, 13].

Thus, the presence of $\alpha 4 \beta 7^{\text{high}}$ CD4⁺ T cells at the site of exposure can impact (i) the availability of good substrate and, therefore, the initial rate of viral replication (ii) the ability of infected cells to rapidly reach the mucosal draining LNs and (iii) the local immune response.

Additional evidence of the importance of $\alpha 4 \beta 7^{\text{high}}$ CD4⁺ T cells in HIV transmission and pathogenesis is reflected in the ability of HIV

gp120 to bind $\alpha 4 \beta 7$. Recent findings suggest that the ability of HIV to interact with $\alpha 4 \beta 7$ might be one of the viral features that help HIV to survive the mucosal barriers [5, 6, 9, 10, 14, 15].

The different lines of evidence noted above point to a role for $\alpha 4 \beta 7^{\text{high}}$ CD4⁺ T cells during the earliest stages of mucosal transmission. We postulated a link between the availability of these cells at the time of challenge and susceptibility to rectal SIV infection. Herein, we show that the frequency of $\alpha 4 \beta 7^{\text{high}}$ memory CD4⁺ T cells in blood before challenge correlates with the rate of acquisition of SIV_{mac239} infection. Moreover, we observed a strong direct correlation between the frequency of $\alpha 4 \beta 7^{\text{high}}$ CD4⁺ T cells in blood and rectal tissue. This indicates that the frequency of $\alpha 4 \beta 7^{\text{high}}$ CD4⁺ T cells in the rectal mucosa at the time of challenge correlated with susceptibility to infection. Additionally, the frequency of $\alpha 4 \beta 7^{+}$ CD11c⁺ myeloid DCs (mDCs) and $\alpha 4 \beta 7^{+}$ CD80⁺ DCs correlated with infectious status and acute VL, while blood CCR5⁺ CD4⁺ T cells did not. Taken together our results

suggest that the availability of $\alpha 7^{\text{high}}$ CD4⁺ T cells plays a critical role at a very early stage of SIV transmission.

Methods

Macaque treatments and challenge

Adult male Indian rhesus macaques (*Macaca mulatta*, RM) were housed in compliance with the regulations under the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, at Tulane National Primate Research Center (TNPRC; Covington, LA). Monkeys were anesthetized with ketamine-HCl (10mg/kg) or tiletamine/zolazepam (6mg/kg) prior to all procedures. Preemptive and post procedural analgesia (buprenorphine 0.01mg/kg) was required for procedures causing more than momentary pain or distress. 17 animals were challenged rectally with 3000TCID₅₀ of SIV_{mac239} in 1ml of PBS. Virus was propagated in PBMCs and titrated 174xCEM cells. The SIV chronically infected RMs (n=10, >1 year post-infection) used for the correlation of $\alpha 7^{\text{high}}$ CD4⁺ T cells between tissues (Fig 1B) were healthy with CD4 count ranging from 364 to 785 cells/ μ l.

Determination of SIV infection

Plasma VLs were measured by quantitative RT-PCR [16–18]. Infection was confirmed by SIV-PCR on MLNs and iliac LNs at day 10 and 14. 1 animal was negative to SIV-PCR in plasma on day 10 and VL at day 14 could not be tested. This animal was positive by SIV-PCR in MLNs and iliac LNs both on day 10 and day 14 and was considered infected. The animal is included in the graphs that show POS vs NEG animals, but not in the graphs that correlate VLs with the frequency of $\alpha 7^{\text{high}}$ or CCR5⁺ CD4⁺ T cells and $\alpha 7^{+}$ DCs. For 1 animal the data of the T cells panel at day 0 were not available, therefore this animal is not represented in the graphs that show correlations with the frequency of the various T subsets at day 0, but included in the correlations at earlier time-points.

Cell isolation and flow cytometry

PBMCs were isolated using Ficoll-Hypaque density gradient centrifugation. Axillary, inguinal, iliac and MLNs, were cut in small pieces and passed through a 40 μ m cell strainer. Rectal biopsies were incubated 1h in R10 with 1mg/ml hyaluronidase, 0.5mg/ml Collagenase II (Sigma-Aldrich) 1mg/ml DNaseI (Roche). The cell suspension was passed through a 40 μ m nylon cell strainer. Cells were stained with the LIVE/DEAD Aqua dye (Invitrogen), anti-CD4-QDotTM 605 (Non-human primate repository, Beth Israel Medical Center, Boston, MA), anti-HLA-DR-QDotTM 605 (Invitrogen) and anti-dimeric $\alpha 7$ (NIH AIDS Research and Reference program) conjugated using PE-Conjugation Kit (AbD-Serotec). Also included in the T cells panel anti-: CD8-APC-Cy7, CD95-FITC, CD3-V450, CCR5-PeCy7, CD69-Alexa700, CD28-APC; DCs anti-: CD3-14-20(Lin)-V450, CD80-APC-H7, CD11c-Alexa700, CD11c-APC, CD123-PCPCy5.5; BDBioscience). >200,000 events were acquired in the lymphocyte live-cells gate using the BD LSRII Flow Cytometer.

Statistics

Mann-Whitney non-parametric test was used to compare variables between groups (SIV-POS versus NEG). Linear regression analysis was performed to determine the correlation between cell subsets in blood and other tissues and VLs. A two-tailed p= <0.05 was considered significant. The analysis was performed using Prism5a (GraphPad Software, Inc).

Results

The frequency of $\alpha_4\beta_7^{\text{high}}$ memory CD4⁺ T in blood correlates with their frequency in the rectal mucosa

Upon rectal challenge, the mucosa is the first site where the virus encounters susceptible target cells. Thus, its attributes are likely to have a strong impact on susceptibility to SIV infection. The $\alpha_4\beta_7^{\text{high}}$ T cells home to the gut and the gut inductive sites and are reportedly a major target population in acute infection [5, 6, 19]. We postulated that there could be an association between the frequency of $\alpha_4\beta_7^{\text{high}}$ CD4⁺ T cells in the colorectal area and in blood. If so, measuring the frequency of $\alpha_4\beta_7^{\text{high}}$ CD4⁺ T cells in blood could provide a surrogate for their presence at the site of challenge.

Since the majority of $\alpha_4\beta_7^{\text{high}}$ CD4⁺ T cells are CD95⁺ T cells (Supplemental Fig 1 and [6, 20]), we measured the frequency of $\alpha_4\beta_7^{\text{high}}$ within the CD95⁺ CD4⁺ T cells subset. This gating strategy allowed us to measure $\alpha_4\beta_7^{\text{high}}$ cells independently of memory T cells (Supplemental Fig 1). This gating strategy was used for blood, rectum and LNs.

Indeed, the frequency of $\alpha_4\beta_7^{\text{high}}$ CD4⁺ T cells in blood and colorectal tissues of 17 animals 8 weeks before challenge strongly correlated (Fig 1A, left). In contrast, the frequency of $\alpha_4\beta_7^{\text{high}}$ memory CD4⁺ T cells in axillary (AX) and inguinal (ING) LNs biopsies 6 weeks before infection did not correlate with their frequency in blood on the same day (Fig 1A, center and right). Since it was not possible to sample MLNs and iliac LNs from naïve animals, we measured the frequency of this population in blood, rectal tissues and AX, ING, MLNs and iliac LNs of 10 healthy chronically infected animals (> than 1 year post-infection) at the time of their euthanization. We found that also in chronically infected animals the frequency of $\alpha_4\beta_7^{\text{high}}$ memory CD4⁺ T cells significantly correlated between blood and rectum (Fig 1B, left). Moreover, there was a significant correlation between blood and iliac LNs (Fig 1B, center), but not with MLNs (Fig 1B, right), AX or ING LNs (not shown).

Higher frequency of $\alpha_4\beta_7^{\text{high}}$ CD4⁺ memory T cells in blood associates with infection

We next asked whether the frequency of $\alpha_4\beta_7^{\text{high}}$ memory CD4⁺ T cells in peripheral blood was related to susceptibility to infection through the rectal route. 10 naïve rhesus macaques were challenged rectally with 3000 TCID₅₀ of SIV_{mac239} (Group 1) and 10 months later an additional 7 animals were challenged rectally with 3000 TCID₅₀ of the same stock of SIV_{mac239} (Group 2). As determined by quantitative SIV-RNA PCR on plasma (VL) and SIV-DNA nested PCR on iliac LNs, 12 out of 17 animals became infected.

We found that the animals that became infected (POS) had a significantly higher frequency of $\alpha_4\beta_7^{\text{high}}$ memory CD4⁺ T cells in blood 21 days before challenge (Fig 2, left) and at the time of challenge (Fig 2, right) than the ones that remained uninfected (NEG). This was most significant if we separated the animals in positive (POS) and negative (NEG) by Plasma VLs at day 7 p.c. (Supplemental Fig 2).

The acute VLs confirmed a positive association between the rate of viral replication and the frequency of $\alpha_4\beta_7^{\text{high}}$ CD4⁺ T cells at the time of exposure. In fact, there was a strong direct correlation between the acute VLs and the frequency of this population in blood at the time of challenge (Fig 3A. VLs at day 7 post-challenge, left; VLs at day 10 post-challenge, right). Interestingly, the frequency of these cells in the infected animals declined at a rate similar to the rate of increase in VL (Fig 3B, left). This was in agreement with data from other groups [6]. In contrast, there was little variation in the animals that remained uninfected (Fig 3B, right).

Notably, the strong direct correlation between blood and rectal $\alpha 7^{\text{high}}$ CD4⁺ T cells (Fig 1A) suggests that the animals with high levels of $\alpha 7^{\text{high}}$ CD4⁺ T cells in blood also had high levels in their rectal tissue. This indicates that the frequency of $\alpha 7^{\text{high}}$ memory CD4⁺ T cells in the rectal mucosa correlated with susceptibility to SIV infection.

It has been proposed that binding to $\alpha 7$ might constitute an advantage for the virus during transmission (increased transmission fitness, [15]). Indeed, an interaction between the of SIV_{mac239} and $\alpha 7$ could help explain the correlation between infection and the frequency of $\alpha 7^{\text{high}}$ CD4⁺ T cells at the time of challenge. However, the strength of the gp120- $\alpha 7$ binding varies among gp120s from different viral isolates [15] and this prompted us to examine the binding of the gp120 of SIV_{mac239} to macaque $\alpha 7$. The relative affinity of SIV_{mac239} gp120 for $\alpha 7$ is in the range of HIV gp120s previously tested (Supplemental Fig 3 and [5, 15, 19]). If a role for this interaction in SIV/HIV transmission is confirmed, it is possible that the association of $\alpha 7$ with susceptibility to infection is lost when the challenging virus has low or no affinity for $\alpha 7$.

The frequency of CCR5⁺ T cells does not associate with infection

It has been reported that memory CD4⁺ T cells that express very high levels of $\alpha 7$ are highly susceptible to HIV/SIV infection [5, 9]. Thus, we investigated if the expression of other HIV/SIV receptors on highly susceptible cell subsets associated with rectal SIV infection. We found no association between higher frequency of CCR5⁺ or memory CCR5⁺ (frequency of CD95⁺ that are CCR5⁺) CD4⁺ T cells and infection status (Fig 4A). Neither did we find a correlation with acute VL (Fig 4B).

In the gut tissue the majority of $\alpha 7^{\text{high}}$ CD4⁺ T cells expresses CCR5 [5]. However, in blood, only a fraction of $\alpha 7^{\text{high}}$ CD4⁺ T cells expresses CCR5 (mean 13.8% range 13.2 – 18.1% at the time of challenge) and the majority of CCR5⁺ CD4⁺ T cells did not express $\alpha 7$ (gating strategy in Supplemental Fig. 4). We found no correlation between the frequency of CCR5⁺ memory CD4⁺ T cells in blood and their frequency in the rectal tissue in the macaques 8 weeks before infection (Fig 4C). Finally, it has been shown that when measured by flow cytometry the expression of CCR5 may be underestimated and that RT-qPCR measurement of the CCR5 mRNA may be a more sensitive method [21, 22]. These observations indicate that we cannot rule out a correlation between the frequency of CCR5⁺ CD4⁺ T cells in the rectal tissue and the susceptibility to SIV infection.

It is also possible that high expression of $\alpha 7$ on lymphocytes is a characteristic of the activation state of the immune system, which favors virus survival and spread early after challenge. However, we found no association between infection status (or VL) and the frequencies of CD69⁺ CD4⁺ T cells or $\alpha 7$ + CD8⁺ T cells in blood at the time of challenge (not shown).

Higher frequency of $\alpha 4\beta 7$ + DCs in blood associates with infection

DCs are among the first cell targets that HIV encounters and likely play a key role in driving viral expansion after transmission [23–26]. Thus, we investigated if the expression of $\alpha 7$ on DCs at the time of challenge could be associated with infection and/or VL. We found that the majority of $\alpha 7$ + Lin[−] HLA-DR⁺ DCs in blood of rhesus macaques are CD11c[−] (mean 77.4%; range: 61.9%–87.6%), while the CD11c⁺ $\alpha 7$ + myeloid DCs (mDCs) express low/intermediate level of $\alpha 7$ (Supplemental Fig 5). Interestingly, all the Lin[−] HLA-DR⁺ DCs that express intermediate/high levels $\alpha 7$ are CD80+ and there is a small population of DCs that expresses high levels of $\alpha 7$ and CD80 ($\alpha 7^{\text{high}}$ CD80⁺ DCs, Supplemental Fig 5).

Indeed, we found that the frequency of $\alpha 7$ + Lin[−] HLA-DR⁺ DCs, $\alpha 7$ + mDCs and $\alpha 7^{\text{high}}$ CD80⁺ DCs at the time of challenge was higher in the animals that were positive

(POS) for SIV-RNA PCR on plasma at day 7 post-infection than those that were negative (NEG) (Fig 5A; frequency of γ^{high} CD80⁺ DCs was available only for Group 1). Instead, when the animals were divided in positive and negative by VLs at day 10 or 14 (therefore the final results of infected vs uninfected as in Fig 2 right and 4A) only the frequency of γ^{high} mDCs was significantly different. Higher frequency of γ^{high} mDCs and γ^{high} CD80⁺ DCs in blood at the time of challenge also correlated with acute VL (Fig 5B, center and right), while total γ^{high} Lin⁻ HLA-DR⁺ DCs did not (Fig 5B, left).

In contrast, the frequency of CD1c⁺ DCs and CD123^{high} pDCs, which express low levels of γ (Supplemental Fig 5), were not associated with infection or VL (not shown).

Discussion

Our results show that susceptibility to rectal SIV infection could be associated with the frequency of γ^{high} memory CD4⁺ T cells. After a single rectal exposure to a high-dose of SIV_{mac239}, the 12 (out of 17) macaques that became infected had a significantly higher frequency of γ^{high} CD4⁺ T cells before challenge than did the animals that remained uninfected. Notably, the same significant difference was found when we compared the frequency of this cell subset in blood 3 weeks before challenge, suggesting that each macaque had a relatively stable frequency of blood γ^{high} memory CD4⁺ T cells. In contrast, this correlation was not present with CCR5⁺ memory CD4⁺ T cells. However, when we tried to associate the susceptibility to SIV with the frequencies of blood or rectal γ^{high} CD4⁺ T cells (or blood/rectal CCR5⁺ CD4⁺ T cells) 8 weeks before infection, we found no correlation (although there was a tendency for a higher frequency of blood γ^{high} CD4⁺ T cells for animals who become infected; not shown). This may be explained by the several factors that can impact the frequency of these cells during the 8 weeks before challenge.

Moreover, we found a strong direct correlation between the frequency of γ^{high} CD4⁺ T cells before challenge and acute VL. Importantly, higher acute VL is associated with CD4⁺ T cell decline and disease progression [27]. Therefore, although it is unclear if γ^{high} CD4⁺ T cells are the main source of virus in plasma during the acute phase [8], our findings suggest a link between availability of γ^{high} CD4⁺ T cells at the time of challenge and the magnitude of viral replication during acute infection. Of note, previous reports described the frequency of γ^{high} cells within the CD4⁺ T cells population [6, 9, 19]. However, having shown that the vast majority of γ^{high} CD4⁺ T cells are CD95⁺ (Supplemental Fig. 1 and [6, 20]), we followed the γ^{high} CD4⁺ T cells within the CD95⁺ cells, excluding the possibility that a higher frequency of γ^{high} CD4⁺ T cells is a consequence of higher frequency of all memory cells.

The correlation between susceptibility to rectal SIV and γ^{high} CD4⁺ T cells can be explained, at least in part, by the role played by γ in directing lymphocytes to gut inductive sites [28] and by the high susceptibility of γ^{high} CD4⁺ T cells [5, 6, 9]. We propose that a higher frequency of γ^{high} CD4⁺ T cells at the site of exposure increases the probability that SIV infected cells reach MLNs and PPs, where they encounter a large number of highly susceptible HIV cell targets. Therefore the γ^{high} CD4⁺ T cells could affect (i) the rate of viral replication at the site of exposure and (ii) the rate with which the virus accesses mucosal draining LNs and gut-inductive sites. Both these factors constitute two major determinants of transmission and impact the chances of this rare event to occur.

By facilitating the rapid delivery of virus to inductive sites, infection of γ^{high} CD4⁺ T cells may not only increase the likelihood of establishing infection, but may also influence the identity of the viral isolate that initiates infection. In fact, productive mucosal SIV/HIV

infection, in most instances, reflects an expansion from a single viral isolate [29, 30]. It has been reported that the removal of transmission-linked PNGs in HIV-gp120s can increase $\alpha 7$ -affinity [15, 30–32] and it was proposed that, in regard to transmission fitness, a strong $\alpha 7$ -reactivity might constitute an advantage during mucosal exposure. We found that the relative affinity of SIV_{mac239} gp120 for $\alpha 7$ fell into in the range of other medium-reactive HIV-gp120s, however, gp120s from other SIVs might react with different strengths and this could influence the correlation between $\alpha 7^{\text{high}}$ CD4⁺ T cells and infection.

We report that in uninfected animals the frequency of $\alpha 7^{\text{high}}$ CD4⁺ T cells was quite stable over time. However, STIs such as HSV-2, Chlamydia and HPV modulate the frequency of $\alpha 7$ expressing T cells in the FGT and systemically [12, 13, 20, 33], influencing in this way susceptibility to HIV mucosal infection. Moreover other host-related factors, e.g. hormonal environment, presence of other pathogens and/or autoimmunity conditions could impact the frequency of this cell subset and influence immune responses, in a way that also influences infection and could confound the correlation we are describing.

Of interest, not only to the HIV field, is the finding that the frequency of $\alpha 7^{\text{high}}$ CD4⁺ memory T cells circulating in blood correlates with their frequency in the rectal mucosa and, perhaps, its draining LNs. This strong correlation suggests that at the time of challenge the animals that exhibited the highest frequency in blood also had the highest frequency in the rectal mucosa. Nevertheless, correlation does not prove causation and the link between the frequency of $\alpha 7^{\text{high}}$ CD4⁺ memory T cells and transmission might be indirect. More studies are needed to clarify the role of these cells in transmission.

We also found a correlation between the frequency of $\alpha 7$ ⁺ DCs in blood at the time of challenge and VL. It has been shown that $\alpha 7$ ⁺ pDCs are modulated by SIV infection and accumulate in the gut mucosa [34]. However, we did not find a correlation between $\alpha 7$ ⁺ pDCs in blood and infection status. DCs at mucosal surfaces possess very different characteristics than circulating DCs, therefore it might be inappropriate to draw a parallel between circulating DCs and the DCs present at the mucosal site of challenge [35, 36]. Nonetheless, like the $\alpha 7^{\text{high}}$ T cells, $\alpha 7$ ⁺ DCs traffic to the mucosal inductive sites. Here the DCs acquire the ability to induce $\alpha 7$ on CD4⁺ T cells, which could be a way to influence the dynamics of viral replication during the acute phase of infection.

In conclusion, our results suggest that $\alpha 7^{\text{high}}$ CD4⁺ T cells and possibly $\alpha 7$ ⁺ DCs play an important role during transmission by the mucosal route and may constitute a determinant of susceptibility to infection. To the extent that these results can be extrapolated to mucosal transmission of HIV in humans, they present a novel and straightforward mean of identifying individuals at higher risk of rectal transmission within and across different groups of people.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. The frequency of $4 \gamma^{\text{high}}$ CD4⁺ T cells in blood correlates with the frequency in rectum and iliac LNs

(A) The frequencies of $4 \gamma^{\text{high}}$ cells within CD95⁺ CD4⁺ CD3⁺ T cells in blood of the animals (each dot corresponds to one animal) before challenge are plotted against their frequencies in rectum (all animals, 8 weeks before-challenge), axillary and inguinal LNs (6 weeks before challenge; Group 1 only, Group 2 data not available) (B) The frequencies of $4 \gamma^{\text{high}}$ cells within CD95⁺ CD4⁺ CD3⁺ T cells in blood of chronic infected animals (> 1 year from infection) are plotted against their frequencies in rectum at the same time point (each dot corresponds to one animal). For (A) and (B) linear regression p values (significance $p < 0.05$) and r square coefficients are shown.

Figure 2. Higher frequency of $\alpha_4\beta_7^{\text{high}}$ CD4⁺ T cells in blood associates with infection

The frequency of blood $\alpha_4\beta_7^{\text{high}}$ within CD95⁺ CD4⁺ CD3⁺ T cells 21 days before challenge (A) and at time of challenge (B) of animals that resulted positive (POS) or negative (NEG) by SIV-PCR on plasma, iliac LNs and MLNs. Data on the frequency of $\alpha_4\beta_7^{\text{high}}$ cells at the time of challenge is not available for 1 animal. Error bars represent means $\pm$ SEM. The shown p values (significance $p < 0.05$) were calculated with the non-parametric Mann-Whitney test.

Figure 3. VLs correlate with the frequencies of $\alpha_4\beta_7^{\text{high}}$ T cells at time of challenge
 (A) The number of SIV-RNA copies in plasma of the infected animals at 7 (left) and 10 (right) post-infection is plotted against the frequency of $\alpha_4\beta_7^{\text{high}}$ cells within $\text{CD95}^+ \text{CD4}^+ \text{CD3}^+$ T cells in blood at the time of challenge. Linear regression p values (significance $p < 0.05$) and r square coefficients are shown. (B) The frequency of blood $\alpha_4\beta_7^{\text{high}}$ cells within $\text{CD95}^+ \text{CD4}^+ \text{CD3}^+$ T cells (filled circles) and the corresponding numbers of SIV-RNA copies in plasma (open squares) are shown over time for 1 representative infected animal (left) and 1 representative uninfected animal (right).

Figure 4. The frequency of CCR5⁺ T cells does not associate with infection

(A) The frequencies of CCR5⁺ within the CD95⁺ CD4⁺ CD3⁺ T cells at time of challenge are shown from animals that were positive (POS) or negative (NEG) by SIV-PCR on plasma, iliac LNs and MLNs. Error bars represent means $\pm$ SEM. The p values (significance $p < 0.05$) were calculated with the non-parametric Mann-Whitney test. (B) The plasma VLs day 10 post-infection is plotted against the frequency of CCR5⁺ within the CD95⁺ CD4⁺ CD3⁺ T cells in blood at the time of challenge. The linear regression p values (significance $p < 0.05$) and r square coefficient is shown. (C) The frequencies of CCR5⁺ within CD95⁺ CD4⁺ CD3⁺ T cells in blood of the animals (each dot corresponds to one animal) 8 weeks before challenge are plotted against their frequencies in rectum (all animals, same time point). Linear regression p value (significance $p < 0.05$) and r square coefficient is shown.

Figure 5. The frequency of $\alpha_4\beta_7^+$ DCs in blood at time of challenge correlates with infection
 (A) The frequency of blood $\alpha_4\beta_7^+$ Lin⁻ HLA-DR⁺ DCs (left), $\alpha_4\beta_7^+$ CD11c⁺ Lin⁻ HLA-DR⁺ myeloid DCs (center) and $\alpha_4\beta_7^{\text{high}}$ CD80⁺ Lin⁻ HLA-DR⁺ DCs (right) at the time of challenge of animals that resulted positive (POS) or negative (NEG) by SIV-PCR on plasma at day 7 post-challenge. Error bars represent means $\pm$ SEM. The shown p values (significance $p<0.05$) were calculated with the non-parametric Mann-Whitney test. (B) The number of SIV-RNA copies in plasma of each animal at different time points is plotted against the frequency of blood $\alpha_4\beta_7^+$ Lin⁻ HLA-DR⁺ DCs (left) and $\alpha_4\beta_7^+$ CD11c⁺ Lin⁻ HLA-DR⁺ DCs (center) and $\alpha_4\beta_7^{\text{high}}$ CD80⁺ Lin⁻ HLA-DR⁺ DCs (right) at the time of challenge.