RESEARCH ARTICLE

Gut Homing CD4+ and CD8+ T-Cell Frequencies in HIV Infected Individuals on Antiretroviral Treatment

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Abstract

The depletion of mucosal CD4+ T-cells occurs early in HIV infection and despite years on antiretroviral treatment (ART), this population never reconstitutes to pre-HIV infection levels. In an effort to understand the effect of ART initiation and different ART regimens on the reconstitution of mucosal T cells within the gut associated lymphoid tissue (GALT), we quantified the frequency of CD4+ and CD8+ T cells expressing the gut homing receptors CCR9 and β7 in peripheral blood (PB) of HIV infected individuals naive to ART and treated individuals on both short-term (less than a year) and long-term ART (more than 2 years). We found that the gut homing CD4+ T cells were depleted in ART-naive individuals and increased after ART initiation but levels were not comparable to HIV uninfected individuals. Gut homing CD4+ T cell activation decreased after ART initiation whilst gut homing CD8+ T cell activation remained elevated in ART experienced individuals, especially in those individuals taking protease inhibitors. Our findings provide new insights into the effects of ART initiation and ART regimens on the frequency and immune status of gut homing CD4+ and CD8+ T cells.

Introduction

During acute human immunodeficiency virus (HIV) infection, CD4+ T-cells within the gut associated lymphoid tissue (GALT) are depleted [1,2], preferentially the TH17 subset, which plays a key role in the homeostatic maintenance of the intestinal epithelial barrier [3]. The damage suffered by this latter leads to the translocation of bacterial products to the peripheral circulation [4]. Bacterial translocation is one of the main mechanisms responsible for the persistence of chronic immune activation and residual inflammation [5,6]. Indeed, chronic immune activation remains a hallmark of HIV disease progression [7,8] despite successful antiretroviral treatment (ART). Full reconstitution of both peripheral blood (PB) and mucosal CD4+ T-cells is a desirable goal upon ART initiation. However and despite several years on ART, mucosal CD4+ T-cell restoration is fairly modest [9,10]. The factors associated with poor mucosal CD4 T-cells reconstitution are still poorly understood. In 2012, Mavigner and

colleagues showed that gut homing CD4+ T-cells remained in circulation due to the lack of proper signaling by the CCR9 ligand, CCL25 [11]. Also, gut CD4+ T-cells, in particular the TH17 population, are susceptible to be infected and killed by HIV [12,13] as the integrin α4β7, necessary for gut homing trafficking, can be used by the virus to infect these cells, further adding to their depletion [14,15]. This in turn has been shown to contribute to the lack of epithelial barrier damage repair [3]. Studying gut homing T-cell frequencies (defined by the expression of α4+β7+ and CCR9+) in PB has been used by many as a useful surrogate to quantify immune reconstitution in the GALT in the absence of gut biopsies [11,16]. In the present study we report the frequency of gut homing CD4+ and CD8+ T-cells, gut homing CD4+ and CD8+ T-cell activation and gut homing TH17 frequencies (expression of the lineage marker CD161+) in PB of HIV-infected individuals naive to ART, ART experienced individuals on short- and long-term treatment, HIV controllers and compared them to HIV uninfected individuals.

Material and Methods

Cohort Characteristics

This study was evaluated and approved by the comité de ética en investigación y comité de ética of the Instituto Nacional de Enfermedades Respiratorias (INER) and conducted according to the principles expressed in the Declaration of Helsinki. All participants were adults (over 18 years old) and gave written informed consent. Our cohort was composed of Mexican Mestizo individuals. A total of 76 individuals were recruited for this study (Table 1) and included 5 HIV-uninfected individuals (who tested negative for HIV ELISA test), 6 HIV controllers, defined as HIV infected (HIV+) individuals with plasma Viral Load (pVL) levels <2,000 RNA copies/mL for at least 1 year in absence of ART, 18 HIV+ naive to ART and 47 HIV+ ART-experienced individuals. Our ART-experienced cohort was divided in two groups based on the length of treatment: short (less than a year on ART) and long term (more than 2 years on ART with undetectable pVL).

Plasma viral load and CD4 measurements

HIV viral load was determined by automated real time polymerase chain reaction (PCR) using the m2000 system (Abbott, Abbott Park, IL). CD4+ T-cell counts were obtained by flow

Table 1. Clinical characteristics. of the cohort.

Groups (n = 76)	Gender M/F	Age years	pVL HIV-1 RNA copies/mL	CD4 T Cells/mm^3	ART regimens	Time on ART
ART-naive (n = 18)	17/1	29 ± 5.9	129,814 ± 540,900	425 ±201	NA	NA
Short-term ART (n = 15)	15/0	31 ± 9.38	<40	302.04 ± 213	EFV/TDC/FTC	Less than 1 year
					EFV/TDC/FTC (n = 15)	
					LPV_r/TDC/FTC (n = 7)	
Long-term ART (n = 32)	29/3	40 ± 8.58	<40	514 ± 267.67	ATV_r/TDF/FTC (n = 8)	9 years ± 2.89
					AZT/3TC/LPV_r (n = 1)	
					TDF/FTC/LPV_r/AZT (n = 1)	
HIV Controllers (n = 6)	3/3	37 ± 15.43	75 ± 175	854 ± 304.93		NA
HIV SN (n = 5)	4/1	41 ±12.98	NA	1,050 ± 205.16		NA

Data is given as median ± standard deviation. Abbreviations: n = number of individuals per group, ART = antiretroviral treatment, M/F = male/female, NA = not applicable, pVL = plasma Viral Load, EFV = efavirenz, TDF = tenofovir, FTC = emtricitabine, LPV = lopinavir, r = ritonavir-boosted, ATV = atazanavir, AZT = zidovudine and 3TC = lamivudine.

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cytometry using the Trucount Kit in FACSCanto II instruments (BD Biosciences, San Jose, CA).

## Peripheral blood mononuclear cells (PBMCs) isolation

Thirty-six ml of PB were collected from each participant at study entry. PBMCs were isolated by density gradient centrifugation using lymphoprep (Axis-Shield, Oslo, Norway) according to the manufacturer´s instructions. Isolated PBMCs were frozen for batch analysis in 90% fetal calf-serum (Bio-west, California, USA) and 10% dimethyl sulfoxide (DMSO, Sigma-Aldrich, California, USA) and stored in liquid nitrogen until used.

Immunophenotyping of gut homing CD4+ and CD8+ T cells

A combination of monoclonal antibodies were used: anti-CD3 brilliant™ violet (BV) 570 (clone: UCHT1, diluted 1/100, Biolegend, California, USA), anti-CD4 phycoerythrin Cyanine5.5-conjugated (PE-Cy5.5) (clone: MHCD0418, diluted 0.35/100, Invitrogen, USA), anti-CD8 BV605 (clone: RPA-T8, diluted 1/100, Biolegend, California, USA), anti-CD45RO BV650 (clone: UCHL1, diluted 1/50, Biolegend, California, USA), anti-Integrin-β7 phycoerythrin (PE) (clone: FIB27, diluted 1/50, Biolegend, California, USA), anti-CCR9 PE (clone: 1125O9, diluted 3.5/50, BD, California, USA), anti-CD38 BV711 (clone: HIT2, diluted 1/100, Biolegend, California, USA), anti-HLADR BV785 (clone: UCHT1, diluted 1/50, Biolegend, California, USA) and anti-CD161 allophycocyanin (APC) (clone: HP-3G10, diluted 2.5/100, Biolegend, California, USA). Live/Dead Aqua dye (diluted 0.5/100, Invitrogen–Life Technologies, California, USA) was used to exclude dead cells. A dump gate was used to exclude unwanted populations and consisted of anti-CD14 (diluted 0.35/100), anti-CD19 (diluted 0.35/100), anti-CD56 (diluted 0.35/100), anti-CD11c (diluted 0.35/100) and anti-CD123 (diluted 0.45/100), all on BV510 channel (Biolegend, California, USA). As circulating gut homing T-cells compose less than 2% of total PB T-cells [17], we decided to detect both CCR9 and β7 markers in the same channel (PE) to enrich for this gut homing population. After thawing, total PBMCs were washed twice with phosphate-buffered saline (PBS) and counted manually. A total of 5 million PBMCs were stained extracellularly by incubating the cells with the antibody cocktail at laboratory temperature for 15 min. Cells were then washed twice with PBS and fixed in 300ul of 1% paraformaldehyde (Sigma-Aldrich, St. Louis, MO, USA). Samples were acquired on a LSRII cytometer (BD, Biosciences, San Jose California, USA) immediately after staining and fixing. FMO (fluorescence minus one) stained tubes were used as gating controls. Data were collected from single time points. The gating strategy is shown in Fig 1. Quality control was performed in each experiment using BD cytometer Setup & Tracking Beads (BD biosciences, San Jose California, USA). Also, a compensation matrix was calculated and applied individually to each experiment using BD Comp Beads Anti- Mouse Ig, k (BD biosciences, San Jose California, USA). Data was analyzed using FlowJo V10 software (Tree Star Inc.).

Statistical analysis

Correlations between T cell immunophenotyping and clinical data (Viral load, total CD4 or CD8 T-cell counts and time on ART) were evaluated using spearman's rank correlation coefficient. Clinical data for each individual was obtained on the same day PBMCs were isolated for T cell immunophenotyping. Comparison between groups was done using U-Mann Whitney test and Kruskal-Wallis test correcting for multiple corrections with Dunnett´s post-test. Corrected P values below 0.05 were considered significant. Statistical analyses were performed using GraphPad Prism version 5 (Statcon, La Jolla, CA, USA).
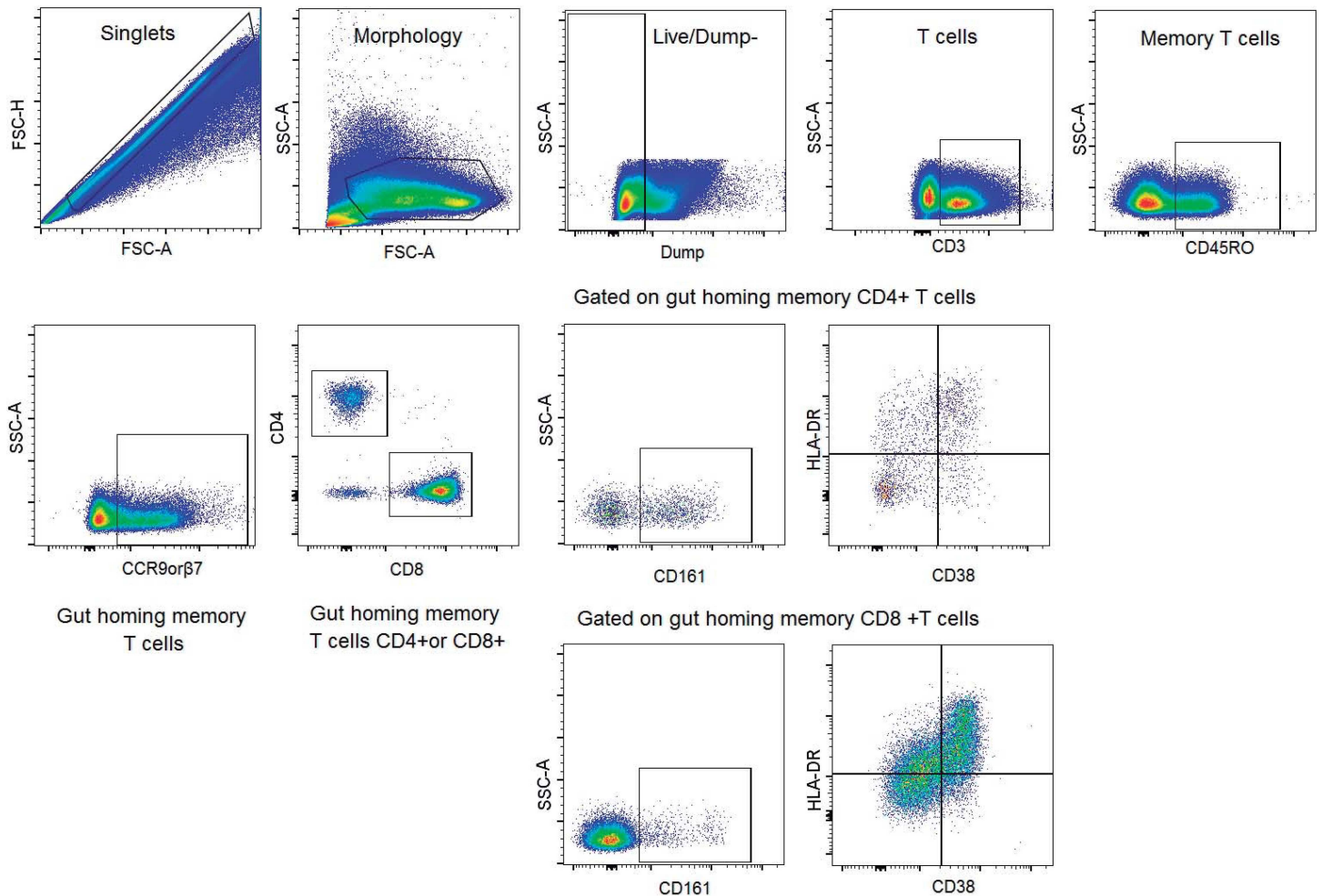
Fig 1. Gating strategy. The gating strategy used for all the samples was to first define singlet and morphology by using forward versus side scatter, followed by the exclusion of dead cells (aqua dye negative events) and the exclusion of CD14, CD19, CD56, CD11c and CD123 positive cells (dump gate). Live cells were gated on CD3+ and CD45RO+ cells. Next, CCR9 and/or β7+ cells were gated and defined as gut homing T-cells. Then CD4+ and CD8+ T cells were gated and finally the expression of CD161 on the gut homing CD4+ (top) and CD8+ (bottom) T-cells was gated. T cell activation was determined by the simultaneous expression of CD38 and HLADR on gut homing CD4+ and CD8+ T cells.

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Results

The frequency of PB gut homing CD4+ and CD8+ T-cells is altered in HIV infection

First, we wanted to determine the frequency of PB gut-homing CD4+ and CD8+ T-cells in our study groups. As shown in Fig 2A, ART-naive individuals had the lowest frequency of PB gut homing CD4+ T-cells compared to individuals on long term ART (p<0.0001), HIV controllers (p = 0.0355) and HIV SN individuals (p<0.0001). Individuals on short-term ART also had lower frequency of PB gut homing CD4+ T-cells compared to HIV SN individuals (p = 0.0036) but not to HIV controllers (p = 0.9615) and long-term ART experienced individuals (p = 0.0659). In contrast, as shown in Fig 2B, HIV+ individuals naive to ART and short-term ART experienced individuals had the highest frequencies of PB gut homing CD8+ T-cells compared to long-term ART individuals (p = 0.0002 and p = 0.0186 respectively), HIV controllers (p = 0.0125 only for ART naive individuals) and HIV SN individuals (p<0.0001 and
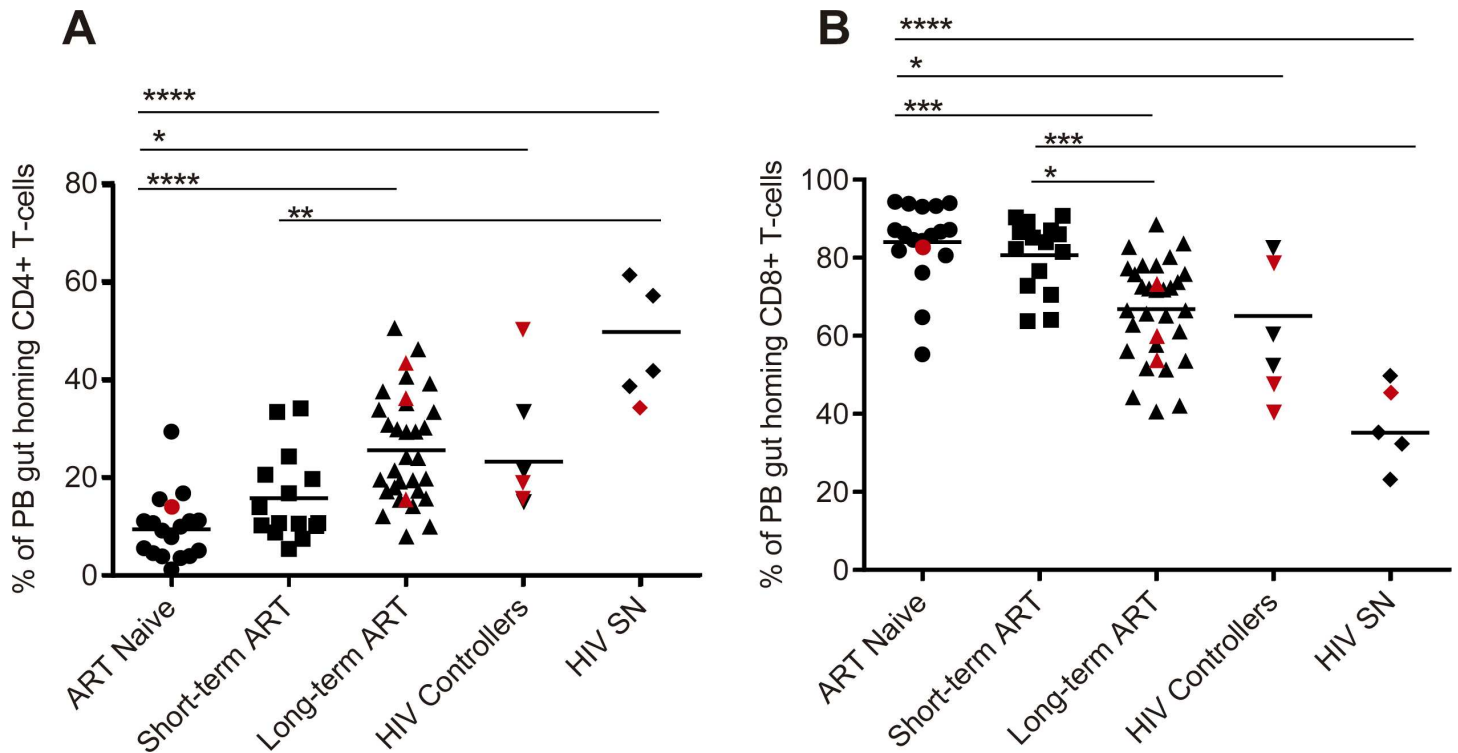
Fig 2. The frequency of PB gut homing CD4+ and CD8+ T-cells is altered in HIV infection. Frequencies (expressed as percentages) of peripheral blood (PB) memory gut homing (CD45RO+ CCR9+ β7+) CD4+ (A) and CD8+ (B) T-cells was determined by flow cytometry on PBMCs of HIV-infected individuals (HIV+), antiretroviral (ART) naïve (n = 18), ART experienced individuals on short-term (n = 15) and long-term treatment (n = 32), HIV controllers (n = 6) and HIV seronegative individuals (SN, n = 5). Scatter plots were used to represent the data. Horizontal lines indicate median values. Each symbol represents one individual. The red symbols represent the females in each group. Groups were compared using Kruskal-Wallis test correcting for multiple comparisons using the Dunnett´s post-test. Only significant corrected p values are shown ***p<0.0005, **p<0.005, *p<0.05. Gender was color-coded as follows: red dots, women and black dots, men.

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p = 0.0005 respectively). Frequencies of PB gut homing CD4+ and CD8+ T cells were similar between ART naive and short-term individuals (p>0.99 and p>0.99 respectively, Fig 2A and 2B). Long-term ART experienced individuals and HIV controllers also had similar frequencies of PB gut homing CD4+ and CD8+ T-cells (p>0.99 and p>0.99 respectively; Fig 2A and 2B). No correlations were found between the frequency of PB gut homing CD4+ or CD8+ T-cells and clinical data (total CD4 or CD8 T-cell counts, pVL or time on ART).

ART-naive and short-term ART experienced individuals have decreased frequency of CD161-expressing gut homing CD8+ T-cells

Because TH17 cells are essential for maintaining gut mucosa homeostasis, we next assessed the frequency of gut homing CD4+ expressing CD161 (marker of TH17 lineage [18]) in PB. Interestingly, we found no difference in the frequency of gut homing Th17 cells across our study groups (Kruskal-Wallis p = 0.1906, Fig 3A). We did however find a negative correlation between the frequency of PB gut homing CD4+ CD161+ T-cells and pVL in the ART naive group (r = -0.7089; p = 0.001, Fig 3B).

Recently, the expression of CD161+ on CD8+ T-cells has defined a new subset of TH17-like cells (T_C17) capable of expressing TH17 cytokines (IL-17) [19]. These T_C17 cells are enriched at sites of inflammation and have been postulated to play a role in disease pathogenesis [20,21]. Therefore, we assessed the frequency of CD161+ expressing gut homing CD8+ T cells
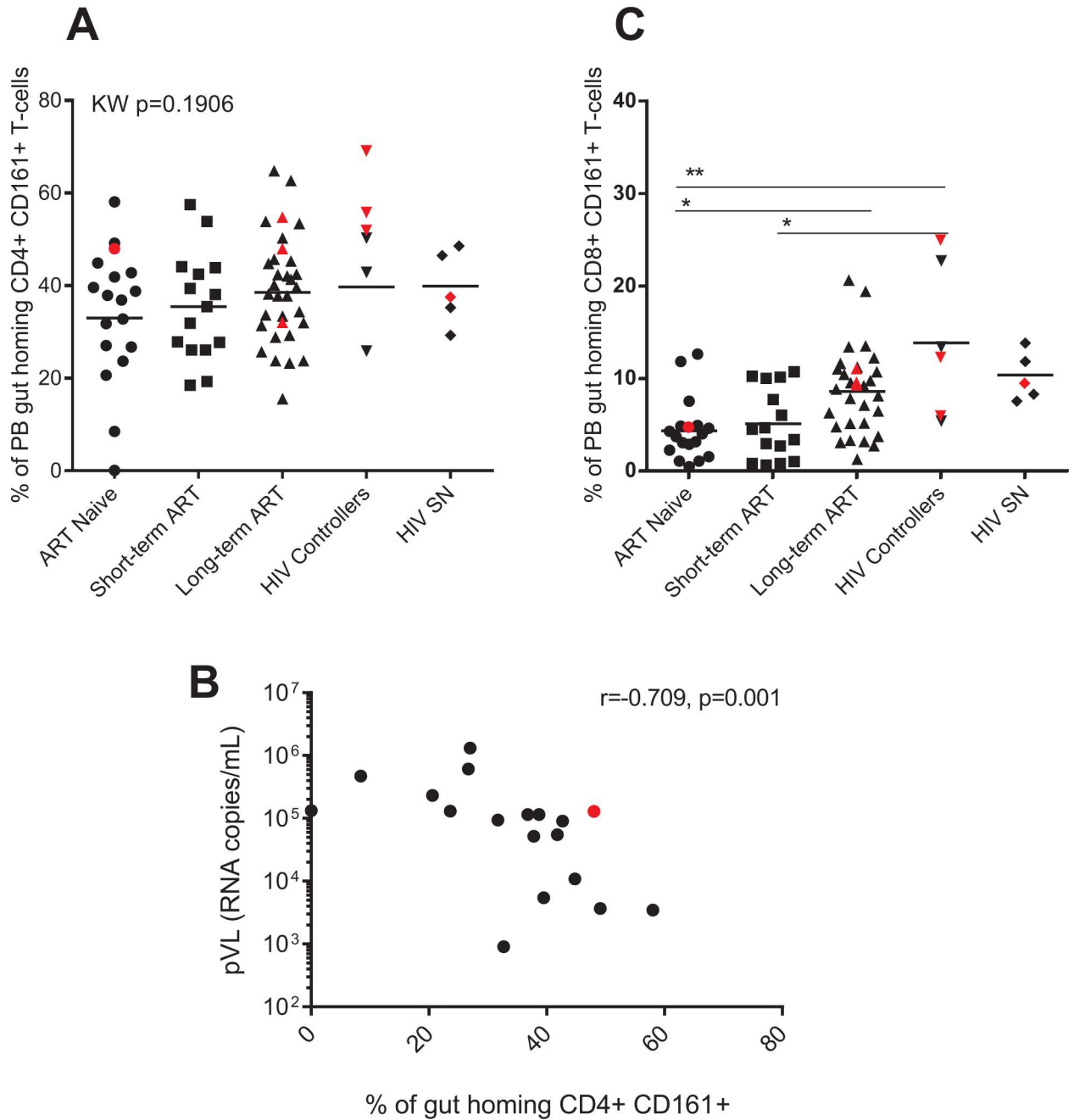
Fig 3. ART-naive and short-term ART experienced individuals have decreased frequency of CD161-expressing gut homing CD8+ T-cells. Frequencies (expressed as percentages) of peripheral blood (PB) memory gut homing (CD45RO+ CCR9+ B7+) CD4+ (A) and CD8+ (C) T-cells expressing CD161 were determined by flow cytometry on PBMCs of HIV-infected individuals (HIV+), antiretroviral (ART) naïve (n = 18), ART experienced individuals on short-term (n = 15) and long-term treatment (n = 32), HIV controllers (n = 6) and HIV seronegative individuals (SN, n = 5). Scatter plots were used to represent the data. Horizontal lines indicate median values. Each symbol represents one individual. The red symbols represent the females in each group. Groups were compared using Kruskal-Wallis test correcting for multiple comparisons using the Dunnett´s post-test. Only significant corrected p values are shown ***p<0.0005, **p<0.005, *p<0.05. Spearman's rank correlation between PB gut homing CD4+ CD161+ T-cells and plasma viral load (pVL) in the ART naive group (B). Gender was color-coded as follows: red dots, women and black dots, men.

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in our study groups. As shown in Fig 3C we found that ART naive individuals and short term treated individuals have comparable frequencies of gut homing CD8+ CD161+ T-cells (p>0.99). ART naive individuals showed decreased frequencies compared to long-term ART experienced individuals (p = 0.0115) and HIV controllers (p = 0.0071). The frequency of PB gut homing CD8+ CD161+ T-cells were comparable between long-term ART experienced individuals, HIV controllers and HIV SN individuals (p>0.05, Fig 3C).

PB gut homing CD4+ T activation decreases with ART, but PB gut homing CD8+ T-cell activation remains elevated in ART experienced individuals on protease inhibitors based therapy

We then examined the level of T cell activation (via the expression of the classical activation markers CD38+ and HLADR+) of gut homing CD4+ and CD8+ T-cells in PB. As shown in Fig 4A, gut homing CD4+ T cell activation was significantly higher in ART naive individuals compared to short-term ART experienced individuals (p = 0.0008). HIV+ individuals on short and long term ART and HIV controllers reach levels of CD4+ T-cell immune activation comparable to that of HIV SN individuals (p = 0.1897, p = 0.2707 and p = 0.6661 respectively; Fig 4A). However, when assessing gut homing CD8+ T cell activation, we found that ART naive individuals had significantly higher gut homing CD8 T cell activation compared to HIV+ on short-term ART (p = 0.0008, Fig 4B). Interestingly, gut homing CD8+ T cell activation remained elevated in long-term ART experienced individuals when compared to short term treated individuals (p = 0.0006, Fig 4B), indicating that short-term ART has a transient effect in diminishing gut homing CD8+ T cell activation. Because our long-term ART experienced individuals were on two distinct ART regimens based on Efavirenz (EFV) or protease inhibitors (PI, see Table 1), we subdivided the long-term ART experienced cohort into ART regimens to understand the effect of individual ART regimens on gut homing CD8+ T-cells T cell activation. We found that ART experienced individuals on PI had significantly higher gut homing CD8+ T cell activation compared to those on EFV (p = 0.0328) (Fig 4C). Additionally, we found a positive correlation between PB gut homing CD4+ T and CD8+ T cell activation and pVL in the ART naive group (r = 0.51, p = 0.02; Fig 4D and r = 0.65, p = 0.003; Fig 4E respectively), indicating that active viral replication is responsible to gut homing T cell activation in HIV infection.

Discussion

As far as we are aware, this is the first paper that describes the frequency of CD8+ T cells with gut homing properties in HIV infection. As previously shown, CD8+ T cells are essential for controlling viral replication [22,23]. We found an accumulation of PB gut homing CD8+ T cells in ART naive individuals as compared to HIV SN individuals. Whether this result can be explained by an attempt from the immune system to control HIV replication in the GALT by recruiting more CD8+ T cells with homing properties or a lack of proper signaling to the gut as described for CD4+ T cells [11] remains to be elucidated. Further research is needed to determine the role of gut homing CD8+ population in HIV infection. Interestingly, cytotoxic CD8+ T-cells with gut homing properties expressing the TH17 lineage marker CD161 have been recently described [19,20]. These CD161 +CD8+ T cells are polyfunctional [21] and have been shown to control viral infections [24]. They have also been shown to negatively correlated with CD8+ T cell immune activation in HIV infected individuals [25]. In our study, we found that PB gut homing CD161+ CD8+ frequency were depleted both ART naive and short-term ART experienced. Long-term ART experienced individuals and HIV controllers seemed to recover this population, as frequencies were comparable to HIV SN individuals. It is possible
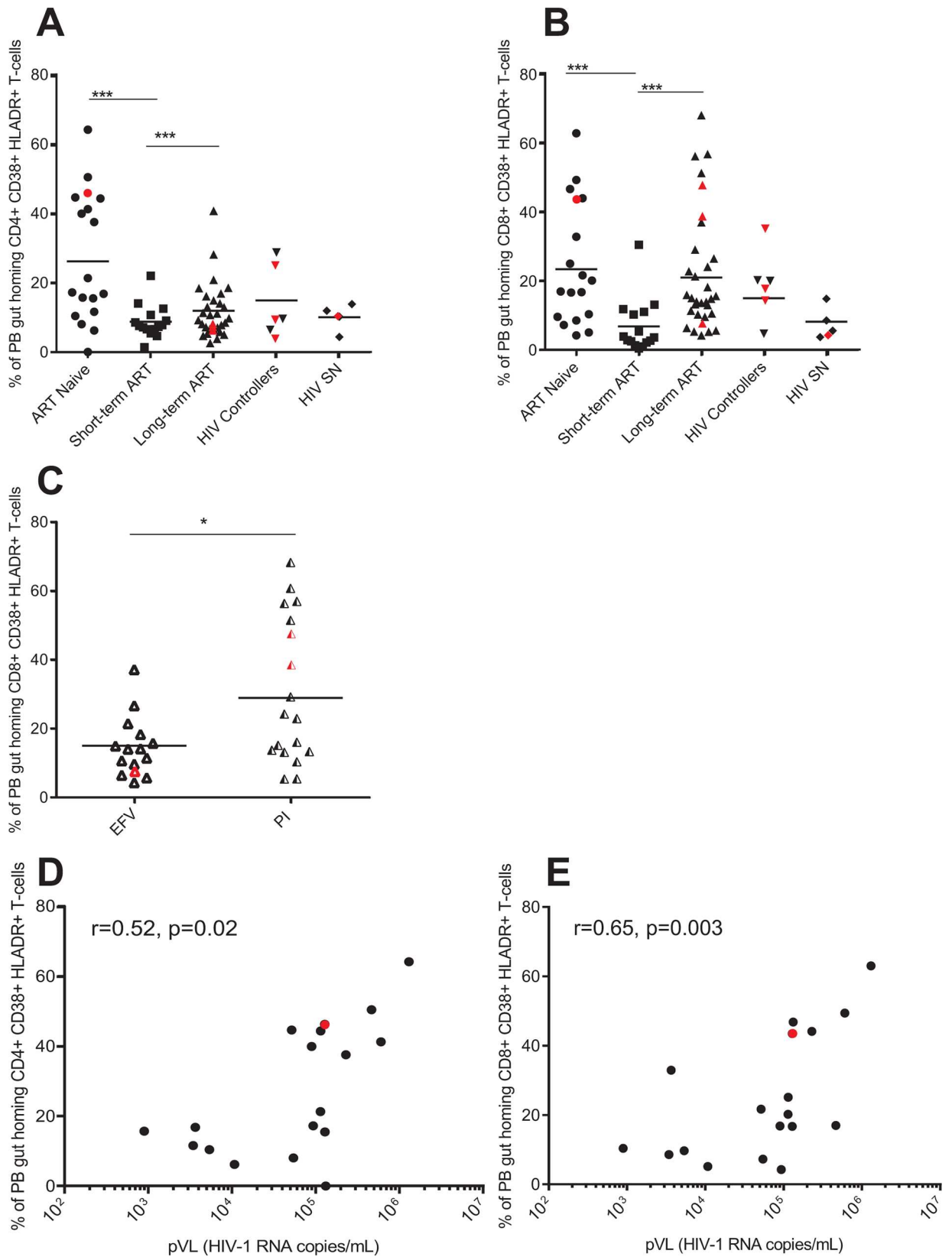
Fig 4. PB gut-homing CD8+ T activation remains elevated in long-term ART experienced individuals on protease inhibitors. Frequencies (expressed as percentages) of peripheral blood (PB) memory gut homing (CD45RO+ CCR9+ B7+) CD4+ (A) and CD8+ (B) T-cells expressing both CD38 and HLADR was determined by flow cytometry on PBMCs of HIV-infected individuals (HIV+), antiretroviral (ART) naïve (n = 18), ART experienced individuals on short-term (n = 15) and long-term treatment (n = 32), HIV controllers (n = 6) and HIV seronegative individuals (SN, n = 5). Frequencies of PB memory gut homing CD8+ T-cells expressing the activation markers CD38 and HLADR in efavirenz (EFV) or protease inhibitors (PI) based therapy is shown in (C). Scatter plots were used to represent the data. Horizontal lines indicate median values. Each symbol represents one individual. The red symbols represent the females in each group. Groups were compared using Kruskal-Wallis test correcting for multiple comparisons using the Dunnett´s post-test. Only significant corrected p values are shown ***p<0.0005, **p<0.005, *p<0.05. Spearman's rank correlation between PB memory gut homing CD4+ (D) and CD8+ (E) T cell activation and plasma viral load (pVL) in the ART naive group. Gender was color-coded as follows: red dots, women and black dots, men.

that this polyfunctional population could be associated with the functional immune responses that have been previously reported for HIV-controllers [26,27]. Tracking gut homing CD161+ CD8+ T-cells in PB could be used as a biomarker of effective immune responses reconstitution in the gut during HIV infection.

In this study, we showed that the frequency of gut homing CD4+ T-cells is significantly reduced in HIV infected individuals, in particular, in ART naive individuals. These results are in agreement with previous publications [28] but in disagreements with others [11]. The discrepancy in the results could be explained by differences in the clinical characteristics of the cohorts and/or the markers used to identify gut homing populations. It is well known that the TH17 subset arises from T-cells expressing the lineage marker CD161 and plays a fundamental role on the homeostatic maintenance of the GALT [18,29]. Contrary to what was expected, we found no differences in the frequencies of gut homing CD4+ CD161+ in our study groups. Whether this gut homing CD4+ CD161+ population found in PB is actually representative of Th17 frequencies in the GALT and whether they are responsible for maintaining gut Th17 population at levels comparable to an HIV uninfected individual remains to be elucidated. Also, we did find a negative correlation between the frequencies of gut homing CD4+ CD161+ with the pVL in the ART naïve group possibly indicating that active viral replication could be depleting this population.

We could not find previous reports that studied the effect of different ART regimens on gut homing CD4+ and CD8+ T cell activation. As expected, levels of CD4+ T immune activation decreased to levels comparable to HIV SN individuals. But when looking at gut homing CD8+ T cell activation, we found a transient decrease in PB gut homing CD8+ T cell activation upon ART initiation. Moreover, we found a positive correlation between the pVL with the frequency of activated gut homing CD4+ and CD8+ T-cells, as previously reported [28,30]. Interestingly, when looking at ART regimens separately, we found that ART experienced individuals on PI had significantly higher gut homing CD8+ T cell activation that those on EFV. Our data suggest that activated CD8+ T-cells are preferentially recruitment to the gut in those individuals on PI-based therapy, possibly as a response to more gut damage. Further research is needed to address the effect of different ART regimens on the reconstitution of the GALT.

We are aware that our study has several limitations. First the number of individuals in our HIV SN and short-term ART experienced group are small. Also, the results are based on a descriptive cross-sectional cohort study performed in peripheral blood. Further studies, including the use gut biopsies, are needed to confirm these results.

To conclude, our study sheds some lights into the effect of ART initiation and ART regimens on the frequency and activation status of gut homing CD4+ and CD8+ T-cells in HIV infection.

Author Contributions

Conceptualization: GRT SPC OB.

Data curation: SPC OB AMO.

Formal analysis: SPC.

Funding acquisition: GRT.

Investigation: OB NRB.

Methodology: GRT SPC OB.

Project administration: SPC AMO.

Resources: GRT AMO SPC.

Supervision: SPC.

Validation: OB NRB.

Visualization: OB SPC.

Writing – original draft: OB SPC.

Writing – review & editing: SPC GRT OB.

References

1. Brenchley JM, Schacker TW, Ruff LE, Price DA, Taylor JH, et al. (2004) CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. J Exp Med 200: 749–759. doi: 10.1084/jem.20040874 PMID: 15365096

2. Mehandru S, Poles MA, Tenner-Racz K, Horowitz A, Hurley A, et al. (2004) 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. doi: 10.1084/jem.20041196 PMID: 15365095

3. El Hed A, Khaitan A, Kozhaya L, Manel N, Daskalakis D, et al. (2010) Susceptibility of human Th17 cells to human immunodeficiency virus and their perturbation during infection. J Infect Dis 201: 843–854. doi: 10.1086/651021 PMID: 20144043

4. Nazli A, Chan O, Dobson-Belaire WN, Ouellet M, Tremblay MJ, et al. (2010) Exposure to HIV-1 directly impairs mucosal epithelial barrier integrity allowing microbial translocation. PLoS Pathog 6: e1000852. doi: 10.1371/journal.ppat.1000852 PMID: 20386714

5. Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, et al. (2006) Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med 12: 1365–1371. doi: 10.1038/nm1511 PMID: 17115046

6. Gordon SN, Cervasi B, Odorizzi P, Silverman R, Aberra F, et al. (2010) Disruption of intestinal CD4+ T cell homeostasis is a key marker of systemic CD4+ T cell activation in HIV-infected individuals. J Immunol 185: 5169–5179. doi: 10.4049/jimmunol.1001801 PMID: 20889546

7. Deeks SG, Kitchen CM, Liu L, Guo H, Gascon R, et al. (2004) Immune activation set point during early HIV infection predicts subsequent CD4+ T-cell changes independent of viral load. Blood 104: 942–947. doi: 10.1182/blood-2003-09-3333 PMID: 15117761

8. Giorgi JV, Hultin LE, McKeating JA, Johnson TD, Owens B, et al. (1999) Shorter survival in advanced human immunodeficiency virus type 1 infection is more closely associated with T lymphocyte activation than with plasma virus burden or virus chemokine coreceptor usage. J Infect Dis 179: 859–870. doi: 10.1086/314660 PMID: 10068581

9. Mehandru S, Poles MA, Tenner-Racz K, Jean-Pierre P, Manuelli V, et al. (2006) Lack of mucosal immune reconstitution during prolonged treatment of acute and early HIV-1 infection. PLoS Med 3: e484. doi: 10.1371/journal.pmed.0030484 PMID: 17147468

10. Guadalupe M, Reay E, Sankaran S, Prindiville T, Flamm J, et al. (2003) 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. doi: 10.1128/JVI.77.21.11708-11717.2003 PMID: 14557656

11. Mavigner M, Cazabat M, Dubois M, L'Faqihi FE, Requena M, et al. (2012) Altered CD4+ T cell homing to the gut impairs mucosal immune reconstitution in treated HIV-infected individuals. J Clin Invest 122: 62–69. doi: 10.1172/JCI59011 PMID: 22156200

12. Prendergast A, Prado JG, Kang YH, Chen F, Riddell LA, et al. (2010) HIV-1 infection is characterized by profound depletion of CD161+ Th17 cells and gradual decline in regulatory T cells. AIDS 24: 491–502. doi: 10.1097/QAD.0b013e3283344895 PMID: 20071976

13. Kader M, Wang X, Piatak M, Lifson J, Roederer M, et al. (2009) Alpha4(+)beta7(hi)CD4(+) memory T cells harbor most Th-17 cells and are preferentially infected during acute SIV infection. Mucosal Immunol 2: 439–449. doi: 10.1038/mi.2009.90 PMID: 19571800

14. Cicala C, Martinelli E, McNally JP, Goode DJ, Gopaul R, et al. (2009) The integrin alpha4beta7 forms a complex with cell-surface CD4 and defines a T-cell subset that is highly susceptible to infection by HIV-1. Proc Natl Acad Sci U S A 106: 20877–20882. doi: 10.1073/pnas.0911796106 PMID: 19933330

15. Arthos J, Cicala C, Martinelli E, Macleod K, Van Ryk D, et al. (2008) HIV-1 envelope protein binds to and signals through integrin alpha4beta7, the gut mucosal homing receptor for peripheral T cells. Nat Immunol 9: 301–309. doi: 10.1038/ni1566 PMID: 18264102

16. Wang X, Xu H, Gill AF, Pahar B, Kempf D, et al. (2009) Monitoring alpha4beta7 integrin expression on circulating CD4+ T cells as a surrogate marker for tracking intestinal CD4+ T-cell loss in SIV infection. Mucosal Immunol 2: 518–526. doi: 10.1038/mi.2009.104 PMID: 19710637

17. Schweighoffer T, Tanaka Y, Tidswell M, Erle DJ, Horgan KJ, et al. (1993) Selective expression of integrin alpha 4 beta 7 on a subset of human CD4+ memory T cells with Hallmarks of gut-trophism. J Immunol 151: 717–729. PMID: 7687621

18. Cosmi L, De Palma R, Santarlasci V, Maggi L, Capone M, et al. (2008) Human interleukin 17-producing cells originate from a CD161+CD4+ T cell precursor. J Exp Med 205: 1903–1916. doi: 10.1084/jem.20080397 PMID: 18663128

19. Kondo T, Takata H, Matsuki F, Takiguchi M (2009) Cutting edge: Phenotypic characterization and differentiation of human CD8+ T cells producing IL-17. J Immunol 182: 1794–1798. doi: 10.4049/jimmunol.0801347 PMID: 19201830

20. Billerbeck E, Kang YH, Walker L, Lockstone H, Grafmueller S, et al. (2010) Analysis of CD161 expression on human CD8+ T cells defines a distinct functional subset with tissue-homing properties. Proc Natl Acad Sci U S A 107: 3006–3011. doi: 10.1073/pnas.0914839107 PMID: 20133607

21. Fergusson JR, Huhn MH, Swadling L, Walker LJ, Kurioka A, et al. (2016) CD161(int)CD8+ T cells: a novel population of highly functional, memory CD8+ T cells enriched within the gut. Mucosal Immunol 9: 401–413. doi: 10.1038/mi.2015.69 PMID: 26220166

22. Migueles SA, Osborne CM, Royce C, Compton AA, Joshi RP, et al. (2008) Lytic granule loading of CD8 + T cells is required for HIV-infected cell elimination associated with immune control. Immunity 29: 1009–1021. doi: 10.1016/j.immuni.2008.10.010 PMID: 19062316

23. Killian MS, Johnson C, Teque F, Fujimura S, Levy JA (2011) Natural suppression of human immunodeficiency virus type 1 replication is mediated by transitional memory CD8+ T cells. J Virol 85: 1696–1705. doi: 10.1128/JVI.01120-10 PMID: 21147929

24. Hamada H, Garcia-Hernandez Mde L, Reome JB, Misra SK, Strutt TM, et al. (2009) Tc17, a unique subset of CD8 T cells that can protect against lethal influenza challenge. J Immunol 182: 3469–3481. doi: 10.4049/jimmunol.0801814 PMID: 19265125

25. Greathead L, Metcalf R, Gazzard B, Gotch F, Steel A, et al. (2014) CD8+/CD161++ mucosal-associated invariant T-cell levels in the colon are restored on long-term antiretroviral therapy and correlate with CD8+ T-cell immune activation. AIDS 28: 1690–1692. doi: 10.1097/QAD.0000000000000351 PMID: 24911351

26. Betts MR, Nason MC, West SM, De Rosa SC, Migueles SA, et al. (2006) HIV nonprogressors preferentially maintain highly functional HIV-specific CD8+ T cells. Blood 107: 4781–4789. doi: 10.1182/blood-2005-12-4818 PMID: 16467198

27. Ferre AL, Hunt PW, Critchfield JW, Young DH, Morris MM, et al. (2009) Mucosal immune responses to HIV-1 in elite controllers: a potential correlate of immune control. Blood 113: 3978–3989. doi: 10.1182/blood-2008-10-182709 PMID: 19109229

28. Peng Q, Wang H, Li X, Lu X, Liu L, et al. (2013) Imbalances of gut-homing CD4+ T-cell subsets in HIV-1-infected Chinese patients. J Acquir Immune Defic Syndr 64: 25–31. doi: 10.1097/QAI.0b013e318293a114 PMID: 23572009

29. Kleinschek MA, Boniface K, Sadekova S, Grein J, Murphy EE, et al. (2009) Circulating and gut-resident human Th17 cells express CD161 and promote intestinal inflammation. J Exp Med 206: 525–534. doi: 10.1084/jem.20081712 PMID: 19273624

30. Ciccone EJ, Greenwald JH, Lee PI, Biancotto A, Read SW, et al. (2011) CD4+ T cells, including Th17 and cycling subsets, are intact in the gut mucosa of HIV-1-infected long-term nonprogressors. J Virol 85: 5880–5888. doi: 10.1128/JVI.02643-10 PMID: 21471231