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Early and long-lasting alteration of effector CD45RA(-)Foxp3(high) regulatory T-cell homeostasis during HIV infection

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Abstract

Regulatory T cells (Treg) quantification in HIV infection remains ill defined due to the lack of reliable specific markers to identify human Treg and the diversity of clinical stages of HIV infection. Employing a recently described Treg identification strategy based on CD45RA and FOXP3 expression, we performed here an extensive quantification of total, naïve (CD45RA+ Foxp3low) and effector (CD45RA- Foxp3hi) Treg in different contexts of HIV infection: primary HIV infection, long term viremic patients, HAART treated aviremic patients and HIV controllers. We showed that, whereas total Treg percentages were mildly affected by HIV infection, Treg absolute numbers were significantly reduced in all groups studied. We demonstrated that whereas naïve Treg numbers were essentially preserved, effector Treg were consistently affected during HIV infection. Finally we demonstrated that effector but not total or naïve Treg numbers negatively correlated with the magnitude of HIV specific CD8 T cell responses.

MESH Keywords Adult; Aged; Antigens, CD45; metabolism; Antigens, CD8; metabolism; Antiretroviral Therapy, Highly Active; Case-Control Studies; Cell Proliferation; Flow Cytometry; Forkhead Transcription Factors; metabolism; France; HIV Infections; drug therapy; immunology; virology; HIV-1; drug effects; immunology; Homeostasis; Humans; Middle Aged; T-Lymphocytes, Regulatory; immunology; metabolism; Viral Load

Author Keywords HIV; AIDS; Treg; FOXP3

INTRODUCTION

HIV infection is characterized by massive CD4 depletion, inefficient HIV specific immune responses and chronic immune hyper activation. Multiple mechanisms lead to dysfunctions of both HIV specific and non specific immune responses. In that respect, alterations of regulatory T cells (Treg) numbers and/or functions have been considered. Treg have been shown to exert suppressive activity during infectious immune responses[1]. However, the overall influence of Treg during HIV infection remains ill defined: Treg induced suppression may prove deleterious to the development of efficient HIV specific immune responses or beneficial by preventing inappropriate immune activation [2]. Suppressive activity of Treg on HIV specific responses has been repeatedly described[3–5]. However, very few consensual data are available concerning Treg functions and numbers during HIV infection. A strong suppressive activity of Treg has been initially associated to decreased viral load[3] but most studies point to a pathogenic role of Treg on HIV infection[4,5]. Recently, CD8 T cells restricted by protective HLA alleles have been characterized by their capacity to evade Treg suppression, thus associating delayed disease progression and low Treg suppression[6]. It has also been demonstrated that Treg proliferate during HIV infection[7] and are directly targeted by HIV infection[8–10]. However, Treg quantification in HIV infection remains controversial in part due to the lack of reliable specific marker to identify human Treg. CD25 and to a lesser extent Foxp3 expression are transiently expressed by activated T cells, a bias especially deleterious in the context of chronic immune activation. These uncertainties in Treg characterization led to conflicting data on the quantification of regulatory T cells[11]. Combination of CD127 low expression and CD25 or Foxp3 expression is currently used to enhance Treg characterization presumably by excluding effector non Treg cells[12,13]. However, the purity of regulatory T cells reached using such strategy remains under debate[14]. This strategy also excludes a fraction of Treg which may include activated Treg as demonstrated in mice[15]. Importantly, heterogeneity in Treg quantification appears to differ depending on clinical stage of HIV infection. Whereas Treg percentages are shown to be consistently increased in HIV infected patients with low CD4 count[16–22], various results emerged depending on the viral, immunological and clinical status of HIV infected patients. During primary HIV infection, decreased Treg percentages have been described[23,24] although results differ depending on the staining strategy[24]. Treg percentages in HIV controllers were alternately described as unchanged[25–26] or reduced[27].

The aim of this work was to readdress Treg quantification during HIV infection by integrating the diversity of HIV infections and novel Treg identification strategy. We distinguished different groups of HIV infected patients based on viral levels, time of infection and/or clinical profile. Primary HIV infected viremic patients, untreated viremic patients, ART treated aviremic patients and HIV controllers were considered separately. Treg quantification was performed using CD45RA/RO and Foxp3 combination as proposed by Miyara et al[28]. Although CD45RA expression has been previously studied among Foxp3 expressing cells[21], the interest of Miyara's
strategy lied on the functional delineation of effector non regulatory T cells (CD45RA+Foxp3low), naïve (CD45RA*Foxp3low) and effector Tregs (CD45RA+Foxp3hi) among Foxp3 expressing cells. Such strategy provides a unique tool to distinguish effector non regulatory T cells from Treg and allows evaluating functional Treg activity by defining ‘naïve’ and ‘effector’ regulatory T cells distribution. We quantified Treg using both classical (CD127lowCD25+Foxp3+) and novel (CD45RA and Foxp3 combination) identification strategies. We demonstrated that Treg counts were consistently decreased in all HIV infected group studied whatever the combination used. Such consistency was not observed when considering Treg percentages. Importantly, naïve and effector Treg subsets were differently affected depending on the stage of HIV infection. Primary HIV infected viremic patients exhibited significant decrease of both naïve and effector Treg. In contrast, during chronic stages, decreased Treg count essentially relies on effector Treg decline, whereas naïve Treg were mildly affected.

**METHODS**

**Study Participants**

Peripheral blood samples from 30 HIV-uninfected blood donors were obtained from the Etablissement Français du Sang (Hospital Saint Louis, Paris, France). With their informed consent, we collected samples from primary HIV-infected untreated patients enrolled in the French ANRS multicenter PRIMO cohort (Agence Nationale de Recherche sur le SIDA, CO06). Primary infection was defined by HIV RNA positivity and by a negative or emerging antibody response. We also studies 16 untreated chronically-infected patients which had been infected for a median of 48 months and are referred to as "untreated viremic" patients. We included in the study 18 ART treated aviremic individuals maintaining plasma HIV RNA levels <40 copies/mL on ART for more than one year. The remaining 22 patients were enrolled in the French HIV-Controllers cohort (ANRS CO18) [inclusion criteria: HIV infection >10 years, 90% of plasma HIV RNA values <400 copies/mL with no ART]. HIV RNA high sensitivity detection assay was performed for all samples (detection limit < 40 RNA copies/mL). Clinical and biologic characteristics of study participants are shown in Table 1. All groups were age-matched with the exception of HIV controllers, which by definition are long term HIV infected patients. As expected, primary HIV infected patients and untreated viremic patients exhibited significantly lower CD4 counts compared to healthy donor, ART treated patients and HIV controllers. HIV RNA viral load was not statistically different between primary infected and untreated viremic patients.

**Laboratory studies**

**Flow cytometry and regulatory T cells identification**

Absolute numbers of CD4+ T cells for healthy donors were determined in fresh whole blood by the use of TruCount tubes and CD3 FITC/CD4 PE/CD45 PerCP TriTest (BD) according to the manufacturer's instructions.

Conjugated antibodies for surface markers were purchased from BD Biosciences (San Jose, CA): CD3 (APC-H7), CD4 (PE-Cy7), CD45RA (FITC), CD25 (PE-Cy5) and eBioscience: CD127 (PE). Intracellular detection of FoxP3 with anti-hFoxP3 (APC, clone 236A/E7 [e-Bioscience]) was performed on fixed and permeabilized cells following manufacturer's instructions (e-Bioscience). Six-color flow cytometry was performed with a FACSCanto cytometer (BD Biosciences) and data files were analyzed using FlowJo software (Tree Star Inc.).

**Peptide-human leukocyte antigen class 1 multimers and activation profile**

HIV-specific CD8+ T cells were identified by using soluble PE-labeled or APC-labeled peptide-human leukocyte antigen (HLA) class 1 multimers (Proimmune, Oxford, United Kingdom; Beckman Coulter, Villepinte, France) derived from the HIV Gag, Nef, Pol and Env proteins. The following epitopes were used: the HLA-A*0201-restricted peptide ligands SLYNTVATL (Gag 77–85) and LKEPVHGV (Pol 476–484), the A*0301-restricted peptide ligands RLRPGKKK (Gag 20–28) and QVPLRPMTYK (Nef 73–82), the A*1101-restricted ligand AVDLSHFLK (Nef 84–92), the A*2402-restricted peptide ligand RYPLTFGWCY (Nef 134–143), the B*0702-restricted peptide ligand IPPIRIQGL (Env 848–856), the B*0801-restricted peptide ligands GEIYKRWII (Gag 259–267) and FLKEKGGL (Nef 90–97), the B*2705-restricted peptide ligand KRWIILGLNK (Gag 263–272), and the B*5701-restricted peptide ligands KAFSPEVIPMF (Gag 162–172), TSTLQEQIGW (Gag 240–249), and QASQDVKNW (Gag 308–316). T cell activation was assessed as the percentage of CD4+ expressing HLA-DR and CD8+ T cells expressing HLA-DR or co expressing HLA-DR and CD38.

**ELISPOT assay**
IFNγ -enzyme-linked immunosorbent spot assay was used to quantify ex vivo HIV-specific CD8+ T cell responses to peptides corresponding to optimal HIV-cytotoxic T lymphocyte (CTL) epitopes derived from the HIV-1 Env, Gag, Pol, and Nef proteins (National Institutes of Health HIV Molecular Immunology Database: http://www.hiv.lanl.gov/content/immunology/tables/optimal_ctl_summary.html). For each subject, optimal peptides were tested depending on the results of HLA typing. IFN-γ spot-forming cells were counted with a KS-ELISPOT system (Carl Zeiss Vision) and expressed as SFCs/10^6 PBMC. The number of specific spot-forming cells was calculated after subtracting the negative control value (i.e. mean of unstimulated wells). Positive responses were defined as greater than 50 spot forming cells per 10^6 PBMC and greater than 2 SD of unstimulated wells.

Statistical Methods

Statistical analysis was performed using GraphPad prism software. Groups were compared using the nonparametric Kruskal Wallis test followed by Dunn’s multiple comparison tests. Spearman’s rank test was used to determine correlations. Pearson correlation curve were indicated when correlations were significant and gaussian approximation validated.

RESULTS

Decline in Treg numbers in all HIV infected patients studied

Miyara and Sakaguchi have developed a novel strategy allowing naïve and effector Treg identification using differential expression of CD45RA and Foxp3 staining[29] (Fig. 1A). To fairly evaluate the relative advantages of both identification strategies in the context of HIV infection, we first analyzed “total” Treg defined by addition of naïve (CD45RA+Foxp3low) and effector (CD45RA−Foxp3hi) Treg compared to identification by Foxp3+CD25+CD127low expression on CD4 T cells (Fig. 1B and 1C, respectively). Regardless the identification strategy used, significant reduction in Foxp3 expressing CD4 T cells numbers was detected in all HIV infected groups compared to healthy donors. Quantification of Treg by CD45RA and Foxp3 combination appears more stringent, but does not drastically modify the conclusion usually drawn on total Treg numbers during HIV: decline in Treg cell numbers was consistently detected in all HIV patients studied, in accordance with studies demonstrating HIV infection affected both Treg and conventional CD4 T cells. Collectively our data demonstrate that disruption of the Treg pool is long lasting and occurs in all groups of HIV infected patients studied.

Tregs percentages are reduced in primary HIV infected patients and HIV controllers

Reduction of Treg numbers may reflect the impact of HIV on all CD4 T cells. To determine whether Tregs were differentially affected among CD4 T cells, we determined Treg percentages using each identification strategy. Percentages of Treg detected among CD4 T cells differed depending on the combination used: lower percentages were consistently recovered using CD45RA/Foxp3 combination compared to Foxp3+CD25+CD127low identification. However, both detection strategies revealed significant reduction in Treg percentages during primary infection and among HIV controllers compared to healthy donors (Fig. 2), the lowest percentage being observed during primary infection. No significant difference was observed in untreated viremic patients regardless the combination used. Observations differed in ART treated patients who exhibited significant reduction in CD127lowCD25+Foxp3+ Treg percentage but no significant differences when using CD45RA/Foxp3 detection strategy. These results confirm the discrepancy between Treg percentages among CD4 T cells and Treg cell numbers: whereas Treg numbers are consistently reduced in all HIV infected patients studied, Treg percentages are significantly altered in primary HIV infected patients and HIV controllers, but not in untreated and ART treated patients using the more stringent definition.

Effector Treg are preferentially affected during HIV infection

We next analyzed naïve (CD45RA+ Foxp3low) and effector (CD45RA− Foxp3hi) Treg fractions. Although a slight reduction of naïve Treg numbers was detected in all groups, statistical reduction of naïve Treg numbers was uniquely detected in primary HIV infected patients (Fig. 3A). Importantly, no statistical differences were detected in naïve Treg percentages in all groups of HIV infected patients compared to healthy donors. In contrast, effector Treg absolute numbers were statistically reduced in all groups studied (Fig. 3B). However percentages of effector Treg among CD4 T cells were solely statistically reduced in primary infected patients and HIV controllers. Collectively, analyses of naïve and effector Treg numbers suggested different outcome for naïve and effector Treg during HIV infection. Naïve Treg counts were affected during the early phase of infection (primary infected patients). In contrast, effector Treg numbers were consistently affected all along the course of HIV infection. Moreover, alteration in effector Treg numbers affected all groups of patients whereas alterations in percentages were less consistently detected. This suggests that quantification of Treg numbers is more sensible than quantification of percentages.

Correlation of effector Treg counts to CD4 count was lost in all HIV infected groups studied except in HIV controllers

To evaluate potential associations between Tregs and HIV related disease progression, we performed correlations between total, naïve and effector Tregs counts and CD4 count. When classical CD127lowCD25+Foxp3+ total Tregs identification was used, highly significant


definition.
correlation was obtained between Tregs and CD4 count in healthy donors (p<0.0001) as well as in aviremic HIV patients (p=0.0005 in ART treated and p=0.0003 in HIV controllers) (Fig. 4A). Conversely this correlation was lost in viremic patients both during primary infection (p=0.1727) and at the chronic stage (p=0.0628). When Treg subsets were considered (Fig. 4B and 4C), naïve or effector Treg counts were highly correlated to CD4 T cell count in healthy donors. In all groups of patients studied, correlation between naïve Treg count and CD4 count was observed (p=0.0306 PHI, 0.0152 VIR, 0.0037 ART and 0.0078 in HIC) (Fig. 4B). In contrast, a significant correlation between effector Treg numbers and CD4 count was detected in HIV controllers (p=0.0026) whereas it was lost in untreated viremic (p=0.5715), in primary infected (p=0.9483) and ART treated (p=0.1941) patients. Collectively, these data suggest that the relationship between naïve Treg and CD4 count is mildly affected during all HIV stages studied whereas effector Treg correlation to CD4 count is altered with the exception of HIV controllers.

**Naïve Treg but not effector Treg counts inversely correlate with viral load**

We next compared in viremic patients (including primary infected and untreated viremic patients) correlation obtained between CD127lowCD25+Foxp3+ Treg, naïve Treg and effector Treg counts and viral load. Using classical CD127lowCD25+Foxp3+ Treg identification, no significant correlation was obtained between Tregs and viral load (Fig. 5A). However, significant inverse correlation was detected between viral load and naïve Treg, but not effector Treg numbers (Fig. 5B and 5C). These data confirm that discriminating between naïve and effector Treg provide additional information compared to total Treg characterization.

**Inverse correlation between HIV specific CD8 T cell responses and effector Treg number**

We next investigated the direct impact of Treg on chronic immune activation evaluating HIV specific and non specific responses. No correlation was observed between naïve or effector Treg numbers and global T cell activation as assessed by determining percentages of HLA-DR+ CD38+ among CD4 and CD8 T cells in all groups of HIV infected patients nor in healthy donors (data not shown). However, in vitro experiments have clearly demonstrated the suppressive activity of Treg on HIV specific CD8 T cell responses [3–5]. We thus determined whether correlation exists between effector Treg numbers and HIV specific CD8 T cells responses. IFN-γ ELISPOT analyses following stimulation with HIV specific peptides were performed on HIV infected patients (n=27, including 7 PHI, 5 VIR, 5 ART and 10 HIV controllers) (Fig. 6A). No correlation between Treg count and HIV specific CD8 T cells were reached when considering "total" or " naï ve" Treg. In contrast, effector Treg count negatively correlated with spot-forming cells quantification of HIV specific CD8 T cells (p=0.0459). Regarding the viral status of HIV infected patients, negative correlation was significantly detected in viremic (p=0.0082) but not aviremic patients (p=0.6482) (data not shown). To confirm the specific impact of effector Treg on HIV specific CD8 T cell responses, we evaluated the ex vivo percentages of HIV specific CD8 T cell expressing CD38 and HLA-DR, two reliable markers of CD8 T cell activation in HIV infected patients (n=29: 8 PHI, 12 VIR, 5 ART, 4 HIV controllers) (Fig. 6B). An inverse correlation was statistically detected between effector Treg numbers and HIV specific HLA-DR+ CD38+ activated CD8 T cells (p=0.0028). Inverse correlation was also detected when considering viremic (p=0.0240) but not aviremic patients (p=0.3738) (data not shown). Importantly, these data demonstrate that effector Treg counts, but not naïve or total Treg directly impact on HIV specific responses and thus identify the predominant role of effector Treg on HIV specific responses.

**DISCUSSION**

Using Treg identification strategy as proposed by Miyara et al [28], we readdressed Treg quantification in the context of HIV infection analysing primary HIV infected, viremic, HAART treated, spontaneous controllers patients and healthy donors. Regardless the strategy considered, Treg numbers were consistently reduced in all groups of HIV infected patients studied when compared to healthy donors, as previously described [17,18,30]. Collectively, data obtained when using CD45RA/Foxp3 combination rather confirmed those observed with previous Treg identification strategy. Decrease in Treg percentages during primary HIV infection has been previously described [23] and may reflect Treg susceptibility to HIV infection [8,10] or recruitment to inflamed sites [31–33]. When considering HIV controllers, decrease in Treg percentages and numbers compared to healthy donors has been previously demonstrated [27].

We next evaluated whether naïve/effector Treg distinction provided novel insight on Treg biology during HIV infection. We showed that naïve and effector Treg count were differently affected during HIV infection. Initial decay of both naïve and effector Treg was observed during primary HIV infection. Interestingly, in all other groups of HIV infected patients, effector Treg decay was consistently observed whereas naïve Treg counts were mildly affected. The mechanisms involved in such specific effector Treg defects are unclear. Persistent effector Treg decay suggests low restoration and/or low persistence of effector Treg compartment. Defect of effector Treg does not appear to rely on the absence of naïve Treg precursors since naïve Treg numbers are essentially preserved/restored. Limited activation or conversely over activation leading to terminal differentiation and death may participate to such defects. Alternately, decay in effector Tregs that are especially sensitive to apoptosis may rely on highly defective survival. Effector Treg decay is especially striking in the context of HIV because high level of immune activation theoretically favours Treg survival. Treg survival has been shown to highly depend on IL-2 availability and IL-2 producing cells [34,35] but HIV infection is characterized by altered IL-2 production [36]. Effector Treg decay during chronic phases may thus reflect altered IL-2 production.
Dissecting naive and effector Treg allowed approaching functional suppressive capacity of Treg: naive Treg exhibit low in vivo suppressive activity, but upon in vitro stimulation, they exhibit high proliferation and survival capacity leading to suppressive functions. Conversely, effector Treg are directly suppressive, but exhibit low proliferative and survival capacity upon in vitro stimulation[37]. We favoured determination of Tregs absolute counts because Treg percentages among CD4 T cells depend on both regulatory and conventional CD4 T cells alterations. Secondly, as Tregs suppression affects CD4 T cells, but also CD8 T cells, B cells and innate cells[38], determining Treg percentages among CD4 T cells may preclude the observation of broader Treg effects. Demonstrating constant reduction in effector Tregs numbers modulate the understanding of Treg alteration during HIV infection. One may hypothesize CD8 immune responses are indeed less restricted due to reduced Treg pressure in the context of HIV infection and may participate to immune exhaustion. Unfortunately, we did not perform CD8 T cell count, thus precluding us to further discuss discrepancy between Treg/CD4 T cell ratio and Treg/CD8 T cell ratio.

We next attempted to evaluate the impact of effector Treg on HIV related disease progression. When considering CD4 count, effector Treg count positively correlated with CD4 T cell numbers in HIV controllers but not in primary infected, untreated or ART treated patients. Because positive correlation is also observed in healthy subjects, these data suggest that Treg homeostasis was less altered in HIV controllers compared to the other chronically infected patients. When considering viral load, we showed an inverse correlation between naive (but not effector) Treg and viral load, suggesting a limited impact of effector Tregs on viral control. Correlation of naive Treg with viral load is likely to represent the impact of virus on Treg compartment as early as the primary HIV infection rather than the impact of Treg on viral load. Collectively, these correlations rather identified Treg decay as a consequence of HIV infection. Secondly, we analyzed whether reduced effector Treg numbers directly affect HIV specific and non specific immune responses. Data addressing correlation between Treg and immune activation have been so far extremely heterogeneous and contradictory presumably due to variability in identification strategies and clinical status [5,24,27,39-42]. In the present study, no significant correlation between effector Treg numbers (nor percentages) and non specific chronic activation was detected (data not shown) supporting the hypothesis of a low impact of Treg on chronic immune activation. However, addressing chronic immune activation based on phenotypic characterization may not prove accurate enough. Indeed, Treg suppression does not necessarily abrogate T cell activation, but rather modulate the expansion/differentiation stage[38,43]. Finally, we determined whether correlation exist between effector Treg and HIV specific CD8 T cell responses. We observed that effector Treg (but not naive or total) Treg numbers correlated with the magnitude of HIV specific CD8 T cell responses in viremic patients as assessed by ELISpot assay following in vitro peptide stimulation and by phenotypic analysis. These results are in accordance with numerous in vitro suppression assays demonstrating the suppressive capacity of Treg on HIV specific responses [3-5].

In conclusion, our data demonstrate that naive and effector Treg were differentially affected during HIV infection. Effector Treg were consistently altered in all groups considered whereas naive Treg numbers were essentially affected during primary HIV infection. Although it is difficult to ascertain the causal or consequential link between effector Treg and HIV specific CD8 responses based on our current data, we identified the predominant association of effector Treg with HIV specific CD8 T cell response but not chronic immune activation.

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Footnotes:


References:

HIV-1 infection is characterized by profound depletion of CD161
cells and overexpression of CTLA-4 are linked to loss of antigen-specific CD4 T cells during primary human immunodeficiency virus type 1 infection. J Virol. 2006; 80: 10162 - 10172


11. Xing S, Fu J, Zhang Z. Increased turnover of Foxp3-high regulatory T cells is associated with hyperactivation and disease progression of chronic HIV-1 infection. J Acquir Immune Defic Syndr. 2010; 54: 455 - 462


17. Montes M, Lewis DE, Sanchez C. Foxp3+ regulatory T cells in antiretroviral-naive HIV patients. AIDS. 2006; 20: 1669 - 1671

18. Lim A, Tan D, Price P. Proportions of circulating T cells with a regulatory cell phenotype increase with HIV-associated immune activation and remain high on antiretroviral therapy. AIDS. 2007; 21: 1525 - 1534


23. Kared H, Leliivre JD, Donkova-Petriini V. HIV-specific regulatory T cells are associated with higher CD4 cell counts in primary infection. AIDS. 2008; 22: 2451 - 2460


32. Eppele HJ, Lodderenkemper C, Kunkel D. Mucosal but not peripheral FOXP3+ regulatory T cells are highly increased in untreated HIV infection and normalize after suppressive HAART. Blood. 2006; 108: 3072 - 3078


35. Almeida AR, Zaragoza B, Freitas AA. Indexation as a novel mechanism of lymphocyte homeostasis: the number of CD4+CD25+ regulatory T cells is indexed to the number of IL-2-producing cells. J Immunol. 2006; 177: 192 - 200


40. Prendergast A, Prado JG, Kang YH. HIV-1 infection is characterized by profound depletion of CD161+ Th17 cells and gradual decline in regulatory T cells. AIDS. 2010; 24: 491 - 502


Figure 1
Regulatory T cell counts
(A) Representative dot plots of CD45RA and Foxp3 expression on CD4 T cells recovered from HD, PHI and HIC are shown. (B, C) Graph representing total Treg counts assessed either by summing naïve (CD45RA+ Foxp3low) and effector (CD45RA− Foxp3hi) Treg (B) or classical CD127low CD25+ Foxp3+ staining (C) in PBMC from healthy donors (HD open circles) and HIV infected patients [PHI (filled red squares), VIR (open red squares), ART (open green triangles) and HIC (filled green triangles)]. Statistical significance was indicated as * when p value < 0.05, ** when p value < 0.01, *** when p value < 0.001 when detected.
Figure 2

Regulatory T cell percentages

Graph representing total Treg percentages among CD4 T cells assessed either by CD45RA/Foxp3 staining (A) or classical CD127low CD25+ Foxp3+ staining (B) in PBMC from healthy donors (HD open circles) and HIV infected patients (PHI (filled red squares), VIR (open red squares), ART (open green triangles) and HIC (filled green triangles). Statistical significance was indicated as * when p value < 0.05, ** when p value <0.01 when detected and *** when p value <0.001.
**Figure 3**
Persistent decline in effector Treg counts in HIV infected patients

Naive and effector regulatory T cell count were defined as CD45RA+ Foxp3low CD4 T cells and CD45RA− Foxp3hi CD4 T cells respectively as described in Fig. 1A. (A, B) Graph representing naïve (A) and effector (B) Treg count (left) and percentages (right) in all groups previously described: healthy donors (HD open circles) and HIV infected patients (PHI filled red squares, VIR open red squares, ART open green triangles and HIC filled green triangles). Statistical significance was indicated as * when p value < 0.05, ** when p value <0.01 when detected and *** when p value <0.001.
Figure 4
Effector Treg numbers correlated with CD4 count
Correlation between total CD127low CD25+ Foxp3+ (A), naïve (B) and effector (C) Treg counts and CD4 T cell counts were performed in all groups previously described: healthy donors (HD open circles) and HIV infected patients (PHI filled red squares, VIR open red squares, ART open green triangles and HIC filled green triangles). Statistical significance was indicated as * when p value < 0.05, ** when p value <0.01 when detected. Correlations were evaluated using a Spearman rank correlation coefficient test. Spearman r correlation and pearson correlation curve are indicated when correlations were significant.
Figure 5
Naïve but not effector Treg counts correlated with viral load

Graphs showing correlations between "total" (as defined by CD127low CD25+ Foxp3+ CD4 T cells) (squares) (A), naïve (circles) (B) and effector (circles) regulatory T cell counts (C) and viral load in viremic patients. Correlations were evaluated using a Spearman rank correlation coefficient test. Statistical significance was indicated as * when p value < 0.05. Spearman r correlation and pearson correlation curve are indicated when correlations were significant.
Figure 6
Inverse correlation between HIV specific CD8 T cell responses and effector Treg count
Correlations between "total" (as defined by CD127low CD25+ Foxp3+ CD4 T cells) (squares), naive (circles) and effector (circles) regulatory T cell counts and HIV specific CD8 T cell responses. (A) Correlations with ELISPOT SFC count upon HIV specific peptide stimulation of 27 HIV infected patients. (B) Correlation with percentages of activated CD8 T cells, defined by HLA-DR and CD38 co-expression on HIV specific CD8 T cell subsets detected in HIV infected patients (n= 29). Correlations were evaluated using a Spearman rank correlation coefficient test. Statistical significance was indicated as * when p value < 0.05. Spearman r correlation and pearson correlation curve are indicated when correlations were significant.
Table 1
Characteristics of HIV-Infected Patients and Healthy Controls

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No.</th>
<th>Age (years)</th>
<th>P Value</th>
<th>Viral Load (Log_{10} Copies/mL)</th>
<th>P Value</th>
<th>CD4 Count (Cells/μL)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy donors</td>
<td>30</td>
<td>37.5 (32-69)</td>
<td></td>
<td>...</td>
<td>...</td>
<td>1120 (763–2601)</td>
<td></td>
</tr>
<tr>
<td>Primary infection</td>
<td>19</td>
<td>32 (29–51)</td>
<td>NS</td>
<td>4.9 (4.7–6.3)</td>
<td>...</td>
<td>527 (421–1316)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>ART untreated</td>
<td>16</td>
<td>35 (32–56)</td>
<td>NS</td>
<td>4.4 (4.0–5.5)</td>
<td>NS</td>
<td>442 (358–897)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>ART treated</td>
<td>18</td>
<td>36 (32–55)</td>
<td>NS</td>
<td>&lt;1.6 (1.6–1.9)</td>
<td>NS</td>
<td>817 (757–1655)</td>
<td>NS</td>
</tr>
<tr>
<td>HIV controller</td>
<td>22</td>
<td>47.5 (45–62)</td>
<td>&lt;.05</td>
<td>&lt;1.6</td>
<td>&lt;.01</td>
<td>788 (656–1328)</td>
<td>NS</td>
</tr>
</tbody>
</table>

All data are median (25th–75th percentiles). In total, 105 subjects were included in this study, in 5 groups: healthy donors; HIV-infected individuals studied during primary HIV infection; HAART-naive viremic patients; aviremic patients (viral load <50 copies/mL) HAART-treated for >1 year; and HIV controllers, defined as those with spontaneous viral control for >10 years (viral load <400 copies/mL). P values were determined compared to healthy donors for age and CD4 counts, and P values for viral load were studied by comparison of HIV-infected patients with primary infection. Abbreviations: ART, antiretroviral therapy; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; NS, not significant.