Phenotype Alterations in Regulatory T-Cell Subsets in Primary HIV Infection and Identification of Tr1-like Cells as the Main Interleukin 10–Producing CD4+ T Cells

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Background. Conventional regulatory T cells (Tregs) can suppress human immunodeficiency virus type 1 (HIV-1)–specific immune responses but cannot control immune activation in primary HIV infection. Here, we characterized Treg subsets, using recently defined phenotypic delineation, and analyzed the relative contribution of cell subsets to the production of immunosuppressive cytokines in primary HIV infection.

Methods. In a longitudinal prospective study, ex vivo phenotyping of fresh peripheral blood mononuclear cells from patients with primary HIV infection was performed at baseline and month 6 of follow-up to characterize Treg subsets, immune activation, and cytokine production in isolated CD4+ T cells.

Results. The frequency of CD4+CD25+CD127low Tregs and the distribution between the naive, memory, and activated/memory Treg subsets was similar in patients and healthy donors. However, Tregs from patients with primary HIV infection showed peculiar phenotypic profiles, such as elevated FoxP3, ICOS, and CTLA-4 expression, with CTLA-4 expression strikingly increased in all Treg subsets both at baseline and month 6 of follow-up. The great majority of interleukin 10 (IL-10)–producing CD4+ T cells were FoxP3neg (ie, Tr1-like cells). In contrast to conventional Tregs, Tr1-like cells were inversely correlated with immune activation and not associated with lower effector T-cell responses.

Conclusion. FoxP3neg Tr1-like cells—major contributors to IL-10 production—may have a beneficial role by controlling immune activation in early HIV infection.

Keywords. primary/acute HIV infection; regulatory T cells; Treg subsets; activated Tregs; memory Tregs; immune activation; Tr1; IL-10.

Human immunodeficiency virus (HIV) infection is associated with a progressive depletion of CD4+ T lymphocytes and defective HIV-specific T-cell responses. Persistent immune activation plays a central role in driving CD4+ T-cell depletion and progression to AIDS [1, 2]. In that context, regulatory T cells (Tregs) were thought to be either beneficial, by suppressing generalized T-cell activation, or detrimental, by weakening HIV-specific responses and thus contributing to viral persistence. In primary HIV infection, it has been previously reported that conventional FoxP3+ Tregs could be able to suppress HIV-specific immune responses but not to control systemic immune activation [3, 4]. Several regulatory T-cell subsets have been described in humans but only conventional Tregs have been studied in primary HIV infection [5]. Moreover, the lack of a consensus phenotypic marker to reliably identify regulatory T cells has led to apparently discrepant results, leaving the role of Tregs in HIV infection under debate [5, 6].
Conventional Tregs are known to express CD25 and the transcription factor FoxP3 but also other cellular markers associated with suppression (eg, CTLA-4, ICOS or CD39) [7]. CD45RA expression allows the delineation of the FoxP3−CD4+ T-cell population into 2 subsets: naive CD45RA+ Tregs and effector CD45RA− Tregs [8]. Also, it was shown that ex vivo HLA-DR expression in CD4+CD25+ T cells identifies a functionally distinct regulatory T-cell population involved in contact-dependent suppression, whereas HLA-DR− Tregs were associated with interleukin 10 (IL-10) secretion [9]. Recently, a combination of the markers CD45RA and HLA-DR was reported to discriminate 3 functionally distinct Treg subpopulations: naive CD45RA+HLA-DR− Tregs, memory CD45RA−HLA-DR− Tregs, and memory/activated CD45RA−HLA-DR+ Tregs [10]. Only few studies delineate peculiar Treg subsets in HIV-infected patients [11], and no longitudinal data are available regarding Treg subsets during acute HIV infection.

Beside conventional Tregs, there are also other regulatory CD4+ T-cell subsets induced in the periphery, such as type 1 Tregs (Tr1) that do not express FoxP3 but produce IL-10 and Thelper type 3 (Th3) cells that secrete transforming growth factor β (TGF-β) [12]. These CD4+ Treg subsets lack specific phenotypic markers and have not yet been studied in the context of HIV infection. Tr1 cells may be of particular interest in the setting of chronic infectious diseases, as it was reported that chronic antigen stimulation can facilitate induction of Tr1-like cells, minimizing tissue damage due to excessive immune responses [13, 14].

The objectives of the present study were to characterize subsets of naive and memory HLA-DR− Tregs and memory/activated HLA-DR+ Tregs during primary HIV infection and to analyze the relative contribution of conventional Tregs and FoxP3− Tregs to the production of immunosuppressive cytokines (ie, IL-10 and TGF-β). In addition, we analyzed the relationship between Treg cell subsets and (a) T-cell activation and (b) effector Th1 cell responses.

**MATERIAL AND METHODS**

**Study Population**

Individuals with acute HIV infection were recruited in a prospective study conducted in 4 clinical sites in Paris, France. These patients have been described elsewhere [15, 16]. Acute HIV infection was defined by <3 bands on an HIV Western blot, detection of p24 antigenemia, and/or detection of plasma HIV RNA, with a negative or weakly positive result of an enzyme-linked immunosorbent assay. At baseline (ie, day 0 of enrollment), all patients were treatment naive. Some of the patients started combination antiretroviral treatment (cART) during follow-up, based on CD4+ T-cell counts and the decision of both physicians and patients. Written informed consent was provided by study participants, in accordance with French ethics laws. The ethics committee of Ile de France II approved the study. Blood specimens were collected from patients at baseline and at month 6 of follow-up. Plasma HIV-RNA levels were determined on site, using the locally available technique with a detection limit of 20 copies/mL. Blood specimens were also collected from healthy volunteers through the French blood bank (Etablissement Français du Sang). A written agreement was obtained for each donor to use the cells for clinical research, in accordance with French ethics laws.

**Flow Cytometry**

Peripheral blood was collected in ethylenediaminetetraacetic acid–containing tubes. Fresh peripheral blood mononuclear cells (PBMCs) were purified by density gradient centrifugation (Isopaque-Ficoll) 2–4 hours after blood sampling. Only freshly isolated cells were used in this study, to avoid any bias due to cryopreservation.

Cells were stained using multicolor panels and analyzed by flow cytometry (LSRII cytometer, Becton Dickinson) as described previously [4]. The following monoclonal antibodies (mAbs), conjugated to fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll protein–cyanine 5.5 (PerCP–Cy5.5), PE Texas Red (ECD), Alexa Fluor 488 (AF488), AF647, AF700, allophycocyanin (APC), APC-Hilite7 (H7), PE-Cy7, PE–Cy5, and eFluor 450 (eF450), were used at predetermined optimal concentrations: anti–CD3–ECD (Beckman Coulter); anti–CD4–PerCP–Cy5.5, anti–CD4–APC–H7, anti–CD8–AF488, anti–CD25–APC, anti–HLA-DR–PerCP–Cy5.5, anti–IL-2–FITC, and anti–IFN–γ–AF700 (BD Biosciences); anti–CD127–PE–Cy7, anti–IL-10–eF450, anti–FoxP3–APC, and anti–FoxP3–AF700 (eBiosciences); anti–TGF–β–PE (IQ Products); and anti–Ki-67–FITC (Dako). FcR Blocking Reagent (Miltenyi Biotec) was used to block unwanted binding of antibodies and increase the staining specificity of cell surface antigens. For intracellular staining of FoxP3, interleukin 10 (IL-10), transforming growth factor β (TGF-β), interleukin 2 (IL-2), interferon γ (IFN–γ), or Ki-67, cells were fixed and permeabilized using the FoxP3 Staining Buffer Set (eBioscience) according to the manufacturer’s recommendations. Analyses were performed using FlowJo software (TreeStar).

**Measurement of Cytokine-Secreting Tregs**

CD4+ T-cell enrichment was performed prior to density gradient centrifugation by incubating the blood with the RosetteSep human CD4+ T-cell enrichment antibody cocktail (Stem Cell Technologies), according to the manufacturer’s recommendations. Cells were then immunomagnetically separated, based on CD25 expression levels, using the EasySep human pan-CD25 positive selection and depletion kit, as well as the EasySep human CD4+CD25high T-cell isolation kit and the cell separator RoboSep (Stem Cell Technologies). CD25high cells were first isolated from CD4+ T cells. The remaining fraction was then...
separated into CD25\textsuperscript{+} and CD25\textsuperscript{neg} cells. Freshly isolated cell fractions (CD25\textsuperscript{neg}, CD25\textsuperscript{+}, and CD25\textsuperscript{high} CD4\textsuperscript{+} T cells) were stimulated with PMA (5 ng/mL) and ionomycin (1 µg/mL) at 37°C for 5 hours. After 2 hours of culture, brefeldin A (5 µg/mL; Sigma-Aldrich) was added. Intracellular cytokine staining was performed as described above.

**Statistical Analysis**

Data are described by medians and interquartile ranges (IQRs) for continuous variables. Nonparametric tests were used, to avoid the impact of potential outlier values in a small study. Comparisons between groups (patients vs healthy donors) were performed using the Mann–Whitney test. The Wilcoxon matched-pairs test was used to estimate the changes in the different variables throughout follow-up (baseline vs month 6). \(P\) values of <.05 were considered statistically significant.

**RESULTS**

**Study Population**

Twenty-eight patients who received a diagnosis early during primary HIV infection were prospectively enrolled in the study. Patients’ clinical characteristics at baseline and at month 6 of follow-up are depicted in Table 1. Most of these patients have been previously described [15]. Thirteen patients remained untreated during the study period. Eleven patients received cART just after baseline sampling; 2 patients were treated between months 3 and 6 of follow-up. Two patients were lost to follow-up.

**Heterogeneity of Conventional Treg Subsets**

The frequency of CD4\textsuperscript{+}CD25\textsuperscript{+}CD127\textsuperscript{low} Tregs (Figure 1A) was similar in patients and healthy donors and did not significantly change during follow-up (Figure 1B). Also, the distribution between naive, memory HLA-DR\textsuperscript{neg} and memory HLA-DR\textsuperscript{+} Tregs (Figure 1C) was similar between patients—whether treated or untreated—and healthy donors; however, a modest decrease in the proportion of HLA-DR\textsuperscript{neg} memory Tregs was observed between baseline and month 6 of follow-up, while the proportion of both other subsets did not significantly change. The contribution of each subset within Tregs was heterogeneous among individuals, and their dynamics varied between patients irrespective of ART initiation (Figure 1C). The expression of FoxP3 expression in CD4\textsuperscript{+}CD25\textsuperscript{+}CD127\textsuperscript{low} Tregs was higher in patients than in healthy donors (\(P = .021;\) data not shown); the highest FoxP3 mean fluorescence intensity (MFI) was found in memory HLA-DR\textsuperscript{+} Tregs; the FoxP3 MFI was also higher in memory HLA-DR\textsuperscript{neg} Tregs than in naïve Tregs, regardless of the group or time point studied. (Figure 1D).

**Peculiar ICOS and CTLA-4 Expression Profile in Treg Subsets From Patients With Primary HIV Infection**

In healthy donors, CTLA-4 expression (illustrated in Figure 2A) was low in naïve Tregs (median, 6.6%), while a median of 34% and 66% of HLA-DR\textsuperscript{neg} and of HLA-DR\textsuperscript{+} memory Tregs, respectively, expressed CTLA-4. Strikingly, CTLA-4 expression was strongly increased among all Treg subsets in patients at baseline, compared with healthy donors (median, 42% in naïve Tregs, 77% in HLA-DR\textsuperscript{neg} memory Tregs, and 93% in HLA-DR\textsuperscript{+} memory Tregs; \(P \leq .001\) for all comparisons with healthy donors). Moreover, CTLA-4 expression remained stable during follow-up, as the same high CTLA-4 expression was observed at month 6 of follow-up among all Treg subsets (Figure 2B).

ICOS expression was low in all Treg subsets from healthy donors. High ICOS expression was found in HLA-DR\textsuperscript{neg} and HLA-DR\textsuperscript{+} memory Tregs from patients at baseline (median, 10% and 20%, respectively, vs <2% in healthy donors). In contrast to CTLA-4, ICOS expression decreased at month 6 of follow-up in both memory subsets, although it remained higher than in healthy donors (Figure 2C).

![Table 1. Patients’ Characteristics](https://academic.oup.com/jid/article-abstract/211/5/769/2918024/769)

<table>
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<th>Time Point, ART Status</th>
<th>Patients, No.</th>
<th>HIV RNA Load, Log\textsubscript{10} Copies/mL</th>
<th>CD4\textsuperscript{+} T-Cell Count, Cells/mm\textsuperscript{3}</th>
<th>Ratio of CD4\textsuperscript{+} to CD8\textsuperscript{+} T Cells</th>
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<td>Baseline</td>
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<tr>
<td>All (untreated)</td>
<td>28</td>
<td>5.73 (4.66–6.41)</td>
<td>460 (335–613)</td>
<td>0.48 (0.27–0.77)</td>
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<td>Untreated at mo 6 of follow-up</td>
<td>13</td>
<td>4.74 (4.20–5.46)</td>
<td>620 (564–702)</td>
<td>0.51 (0.37–0.78)</td>
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<tr>
<td>ART at mo 6 of follow-up</td>
<td>13</td>
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<td>339 (315–386)</td>
<td>0.42 (0.25–0.82)</td>
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<td>Mo 6 of follow-up</td>
<td></td>
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<tr>
<td>Untreated</td>
<td>13\textsuperscript{a}</td>
<td>4.40 (3.50–4.80)</td>
<td>669 (457–725)</td>
<td>0.82 (0.62–1.02)</td>
</tr>
<tr>
<td>ART</td>
<td>13\textsuperscript{b}</td>
<td>1.30 (1.00–1.36)</td>
<td>724 (593–767)</td>
<td>1.31 (1.04–1.57)</td>
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Data are median (interquartile range), unless otherwise indicated.

Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus type 1.

\textsuperscript{a} For one patient experiencing an intercurrent episode associated with a high level of inflammation at month 6 of follow-up, clinical data from month 3 of follow-up were used.

\textsuperscript{b} Eleven patients received ART before day 15 of follow-up, and received ART before month 6 of follow-up. Two patients were lost to follow-up.
Relationship Between Conventional Treg Subsets and T-Cell Activation

We previously reported on a lack of correlation between the level of T-cell activation and the frequency of conventional Tregs in early primary HIV infection [4]. Here, instead of considering Tregs on the whole, we investigated the relationship between the proportion of Treg subsets and T-cell activation. None of the CD4+ Treg cell subsets were related to CD8+ T-cell activation (data not shown). No relationship was observed between the frequency of naive or memory HLA-DRneg Tregs and CD4+ T-cell activation, regardless of the activation marker used (Figure 3A and 3B). In contrast, we found a strong positive correlation between the proportion of memory activated HLA-DR+ Tregs and the level of CD4+ T-cell activation, whether assessed by the frequency of HLA-DR+CD4+ T cells or the frequency of Ki-67+CD4+ T cells (Figure 3C). Of note, the frequency of HLA-DR+CD4+ T cells, as expected, was positively correlated with the frequency of Ki-67+CD4+ T cells (R = 0.78; P < .0001). CTLA-4 and ICOS are markers associated with Treg suppressive function. However, the frequencies of CTLA-4+ Tregs and of ICOS+ Tregs were positively associated with the level of CD4+ T-cell activation (Figure 3D and 3E).

FoxP3negCD4+ T Cells: The Main Producers of IL-10 in Primary HIV Infection

At baseline, following PMA/ionomycin activation, a median of 10% (IQR, 6%–15%) and 11% (IQR, 4%–19%) of CD4+CD25+ FoxP3+ T cells produced IL-10 and TGF-β, respectively. This proportion did not significantly change during follow-up (data not shown). Of note, the median frequency of IL-10+ Tregs was 0.4% (IQR, 0.2%–0.5%), while the frequency of total IL-10-producing CD4+ T cells was 2.1% (IQR, 1.9%–3.5%).

Other CD4+ T cells that do not express FoxP3 can also produce IL-10. CD4+ T cells from patients were isolated according to the level of CD25 expression. As expected, higher median FoxP3 expression was found in CD25-expressing cells: 40% of

Figure 1. Subsets of conventional regulatory T cells (Tregs) during primary HIV infection (PHI). A, Representative flow cytometry–associated dot plots showing ex vivo expression of the indicated markers for identification of conventional Treg subsets in peripheral blood mononuclear cells. B, Frequencies of total Tregs (CD4+CD25+CD127low) in peripheral CD4+ T cells from healthy donors (HDs) and from patients with PHI at baseline (BL) and at month 6 of follow-up (M6). C, Distribution of Treg subsets: naive CD45RA+HLA-DRneg, memory CD45RAnegHLA-DRneg, and memory/activated CD45RAnegHLA-DR+ Tregs. Wilcoxon paired rank tests and Mann–Whitney tests were performed, and P values are indicated when difference was significant. D, FoxP3 expression levels, assessed as mean fluorescence intensities (MFIs), in each Treg subset. Patients who received antiretroviral therapy (ART) before M6 are represented as open circles.
CD25^{high} and 15% of CD25^{+} CD4^{+} T cells expressed FoxP3, while <1% of CD25^{neg} CD4^{+} T cells had detectable FoxP3 expression (Figure 4A). Although the proportion of IL-10–producing cells was lower in CD4^{+}CD25^{neg} T cells (median, 1.3%; IQR, 1.1%–3.1%) than in CD4^{+}CD25^{high} T cells (median, 6.9%; IQR, 5.2%–8.5%), most of CD4^{+} T cells that produce IL-10 within these CD4^{+} T-cell subpopulations were Foxp3^{neg}. Indeed, at baseline, among IL10–producing CD4^{+}CD25^{neg} T cells, a median of 93% (IQR, 90%–95%) were FoxP3^{neg} (ie, Tr1-like phenotype). Accordingly, as illustrated in Figure 4B, a median of 81% (IQR, 72%–83%) of IL-10–producing CD4^{+}CD25^{+} cells and of 75% (IQR, 67%–80%) of IL-10–producing CD4^{+}CD25^{high} cells were FoxP3^{neg}. Similar results were found at month 6 of follow-up (Figure 4B).

Although not specific, CD39 and ICOS have been suggested as makers for Tr1 cells [17, 18]. We thus assessed CD39 and ICOS expression in Tr1-like cells from patients with primary HIV infection at baseline (Figure 4C). The frequency of CD39-expressing cells was higher in Tr1-like cells, compared with IL-10^{neg}-FoxP3^{neg} CD4^{+} T cells (referred to as nonregulatory cells), but slightly lower than in conventional Tregs (Figure 4C). Moreover, ICOS expression levels were significantly higher in Tr1-like cells, compared with nonregulatory cells, but similar to levels measured in IL-10–expressing conventional Tregs (CD25^{FoxP3^{+}}), in line with previous results showing that ICOS-expressing conventional Tregs use IL-10 for their suppressive function [19].

**Association of IL-10–Producing Treg Subsets With Effector Th1 Responses and T-Cell Activation**

We next investigated the potential role of IL-10–producing Treg subsets in the control of immune activation and the suppression of effector CD4^{+} T-cell responses.

We did not find any relationship between IL-10–producing Treg subsets and the frequency of IFN-γ–producing CD4^{+} T
Figure 3. Correlations between conventional regulatory T cell (Treg) subset frequency and T-cell activation level in patients with primary HIV infection (PHI). The ex vivo frequencies of naive CD45RA+HLA-DRneg (A), memory CD45RAnegHLA-DRneg (B), memory/activated CD45RAneg HLA-DR+ (C), ICOS-expressing (D), and CTLA-4-expressing (E) conventional Tregs (CD4+CD25+CD127low) from patients with PHI at baseline were plotted as a function of CD4+ T-cell activation levels, defined by the percentage of CD4+ T cells expressing HLA-DR (upper panels) or Ki-67 (lower panels). Spearman rank correlation coefficients (R) and corresponding P values are indicated on each panel. Abbreviation: NS, not significant.
cells following PMA/ionomycin stimulation (data not shown). Noteworthy, the frequency of IL-10–producing CD25+FoxP3+ conventional Tregs negatively correlated with the proportion of IL-2–producing CD4+ T cells but was not related to T-cell activation (Figure 5A). In contrast, the frequency of IL-10–producing FoxP3neg Tr1-like cells did not correlate with the proportion of IL-2–producing CD4+ T cells, while it negatively correlated with CD4+ T-cell activation, as assessed by Ki-67 expression (Figure 5B).

**DISCUSSION**

Immune activation, which occurs early in primary HIV infection, is known to be a major contributor to HIV pathogenesis [2]. One of the main functions of Tregs during infections is to control collateral tissue damage associated with an inflammatory response [20]. However, conventional Tregs were found to be unable to control high levels of immune activation in viremic patients with acute or chronic infection [21]. Most studies investigated Tregs based on the classical phenotypic definition. We were interested to explore specific subsets of conventional Tregs, as well as other regulatory T-cell subsets. We first characterized Treg subsets on the basis of HLA-DR and CD45RA expression, characteristics that were recently reported as defining functionally distinct subpopulations of Tregs [10]. The relative frequencies of total, naïve, memory HLA-DRneg, and memory/activated HLA-DR+ Treg subsets were not altered in patients with primary HIV infection, compared with healthy donors. However, Tregs from patients were found to upregulate the Treg-associated markers FoxP3, ICOS, and CTLA-4. There was no evidence that a specific subset of conventional Tregs may control immune activation in patients with primary HIV infection, as we found either no relationship or a positive correlation between CD4+ and CD8+ T-cell activation and the...
proportion of any conventional Treg subset (Figure 3 and data not shown). A recent study showed that HIV-infected Tregs downregulate genes critical to regulatory function, which could account for their impaired capacity to control generalized immune activation [22]. ICOS expression was only elevated in memory Treg subsets and decreased between baseline and month 6 of follow-up in the same way as systemic T-cell activation [15]. Thus, the transient increase in ICOS expression detected in Tregs from patients with primary HIV infection may only reflect ICOS upregulation on bulk activated CD4+ T cells. Indeed, ICOS can be considered as a T-cell activation marker because it is expressed on T cells following activation and then confers effector functions to activated T cells [23]. Accordingly, ICOS+ Tregs were positively associated with markers of T-cell activation.

In contrast to ICOS, CTLA-4 expression was tremendously elevated in all conventional Treg subsets—while not on double negative regulatory T cells [4]—from patients with primary HIV infection, compared with healthy individuals, and remained high after 6 months of follow-up, even in patients who started ART. An increase in CTLA-4 expression was previously reported in Tregs and bulk CD4+ T cells in other infectious diseases, such as malaria [24]. In HIV infection, strong CTLA-4 upregulation was particularly described on effector HIV-specific CD4+ T cells, with the highest levels found in primary HIV infection [25, 26]. In contrast, CTLA-4 was found to be either not or slightly increased in Tregs from patients with chronic HIV infection [27, 28]. These discrepancies may be related to differences in the stage of infection and/or the staining protocols (ie, surface vs intracellular staining).

A number of Tregs have been described to constitutively express CTLA-4, which is involved in their immunoregulatory functions [29, 30]. CTLA-4 is crucial for the ability of Tregs to inhibit the potency of antigen-presenting cells to activate T cells [30]. Hence, the strong CTLA-4 upregulation in Tregs from patients with primary HIV infection may contribute to the impaired functional capacity of dendritic cells reported in HIV-infected patients [31, 32]. Moreover, CTLA-4 contributes...
to the generation of peripheral Tregs, because (1) CTLA-4 engagement can promote Foxp3+ T-cell generation both in vitro and in vivo [33], and (2) CTLA-4+ Tregs induce IDO expression in dendritic cells [34], which, in turn, induce Treg generation [35]. Therefore, the high frequency of CTLA-4+ Tregs reported here in patients with acute HIV infection may support the increased frequency of Tregs consistently reported in chronic HIV infection [5].

To gain insight into functional properties of regulatory T cells, we assessed expression of immunosuppressive cytokines in conventional Tregs, as well as in Foxp3negCD4+ T cells. Interestingly, the great majority of IL-10–producing cells were found in the Foxp3neg cell population (ie, Tr1-like cells that may have comparable regulatory function to conventional Tregs) [36]. It was reported that plasma levels of IL-10 were elevated in patients with primary HIV infection [37], and this may be attributed in part to Tr1-like cells. Indeed, it was shown that IL-10 expression was upregulated in HIV-infected patients, particularly in CD4+ T cells but also in B cells, natural killer cells, and double-negative T cells [4, 38]. Elevated plasma levels of TGF-β found during primary HIV infection [37] may contribute to the rise in IL-10–producing cells [39]. Of note, ICOS signaling is associated with IL-10 induction [19, 40] and promotes differentiation of Tr1 cells [41]. The upregulation of ICOS expression that we found may be involved in the generation of IL-10–producing cells. More generally, IL-10 can be induced in Foxp3negCD4+ T cells when and where inflammation occurs [42]. The identification of regulatory cell subsets able to decrease immune activation without suppressing effector Th1 responses is of great interest in chronic viral infections. Interestingly, we found that Tr1-like cells seem in line with these properties, as opposed to conventional Tregs, which have been found to be unable to dampen high immune activation while inhibiting HIV-specific responses via cell-cell contact mechanisms [4, 27, 43–45]. Tr1 cells mostly function through the release of IL-10 to directly regulate the inflammation at peripheral mucosal surfaces [46]. This suggests that the suppressive effect of regulatory Tr1 cells mostly occurs in peripheral inflamed tissues, where HIV replication occurs preferentially. Thus, Tr1-like cells in patients with primary HIV infection may be more prone to control general immune activation, including CD4+ activation/proliferation (as assessed by Ki-67 expression), than CD4+ T-cell effector function (as assessed by IL-2 production). Moreover, we found that conventional Tregs expressed the highest levels of CD39, an immunoregulatory ectoenzyme that had been associated with HIV progression [27] and, interestingly, with negative regulation of IL-2 production in effector CD4+ T cells [47]. This is in line with our observation that conventional Tregs were inversely related to the frequency of IL-2–producing CD4+ T cells.

Of interest, Tr1-like cells are known to be involved in intestinal homeostasis [42] and therefore may limit HIV-associated gut damages resulting in microbial translocation, a major cause of chronic immune activation [48]. Interplay between Foxp3+ Tregs, Tr1 cells, and Th17 cells has been described in the intestinal mucosa [42]. Impairment of the intestinal barrier integrity may thus be associated with alterations in the balance between these cell subsets. Dysregulation in the Th17 to Treg balance has previously been associated with progressive HIV infection [49, 50], and the Th17/Treg ratio was found to predict T-cell activation in patients with primary HIV infection [15]. However, relationships between Tr1 and Th17 cells are less well defined and have never been investigated in the context of HIV infection.

Altogether, these data provide a comprehensive analysis of different subsets of regulatory T cells in early HIV infection. While repartition of conventional Treg subsets was rather similar to what is found in healthy donors, upregulation of CTLA-4 was the most striking phenotype alteration observed in all Treg subsets. None of the conventional Treg subpopulations seem able to control HIV-induced pathogenic immune activation. However, our data suggest that, in addition to double-negative regulatory T cells [4], Tr1-like cells, as opposed to conventional Tregs, could be considered a beneficial regulatory T-cell population in HIV disease. To our knowledge, Tr1-like cells had never been investigated in HIV infection and deserve to be further studied. Their potential role needs to be confirmed in larger cohorts of patients with treated and untreated HIV-infection. Tr1 cell–based therapeutic strategies—a new research area—are currently under investigation and seem to be safe, at least in patients with severe Crohn disease [3, 19]. However, therapies specifically designed to induce expansion of immunoregulatory cells in HIV infection raise substantial safety concerns. Nonetheless, as an expansion of Tr1 cells may be a beneficial asset, it could be of interest when testing immune-based therapies for HIV infection to investigate their potential effect on Tr1 cells.

Notes
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Potential conflicts of interest. All authors: No reported conflicts.

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