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Programmed Death-1 Is a Marker for Abnormal Distribution of Naive/Memory T Cell Subsets in HIV-1 Infection

Gaelle Breton,*†‡§ Nicolas Chomont,*†‡§ Hiroshi Takata,§ Rémi Fromentin,§ Jeffrey Ahlers,§ Abdelali Filali-Mouhim,§ Catherine Riou,*†‡ Mohamed-Rachid Boulassel,§ Jean-Pierre Routy,*,†§ Bader Yassine-Diab,*†‡§ and Rafick-Pierre Sékaly*,†‡§

Chronic activation of T cells is a hallmark of HIV-1 infection and plays an important role in disease progression. We previously showed that the engagement of the inhibitory receptor programmed death (PD)-1 on HIV-1–specific CD4+ and CD8+ T cells leads to their functional exhaustion in vitro. However, little is known about the impact of PD-1 expression on the turnover and maturation status of T cells during the course of the disease. In this study, we show that PD-1 is upregulated on all T cell subsets, including naive, central memory, and transitional memory T cells in HIV-1–infected subjects. PD-1 is expressed at similar levels on most CD4+ T cells during the acute and the chronic phase of disease and identifies cells that have recently entered the cell cycle. In contrast, PD-1 expression is dramatically increased in CD8+ T cells during the transition from acute to chronic infection, and this is associated with reduced levels of cell proliferation. The failure to downregulate expression of PD-1 in most T cells during chronic HIV-1 infection is associated with persistent alterations in the distribution of T cell subsets and is associated with impaired responses to IL-7. Our findings identify PD-1 as a marker for aberrant distribution of T cell subsets in HIV-1 infection. The Journal of Immunology, 2013, 191: 2194–2204.

Acute HIV-1 infection (AHI) leads to the massive depletion of mucosal CD4+ T cells and progressive dysfunction of cellular and humoral immune responses in most infected individuals. Rapid replication of virus in CD4+ T cells, including Th17 cells in mucosal lymphoid tissue, concomitant with inflammatory cytokine and chemokine production leads to disruption of the gut mucosal barrier, permitting commensal bacteria to enter into the bloodstream, resulting in systemic activation of innate immune cells by microbial products (1).

Chronic activation of the immune system is characterized by 1) high levels of expression of activation markers, including HLA-DR, CD38, and the nuclear Ag Ki67 on both CD4+ and CD8+ T cells (2–6); 2) increased susceptibility to activation-induced cell death (7); 3) increased plasma levels of inflammatory cytokines and type I IFNs; and 4) accumulation of terminally differentiated CD4+ and CD8+ T cells as shown by the loss of expression of the CD28 costimulatory molecule and increased levels of CD57 expression (9–11). Chronic immune activation is now recognized as the sine qua non for HIV-1 pathogenesis and is a better predictor of HIV-1 disease progression than the extent of viral replication (6, 12–14). Cross-sectional studies of immune function in HIV-1–infected individuals, including global gene expression analysis of innate and adaptive cell populations, suggest that the host immune response cannot contain virus replication and that the cumulative effects of type I IFNs and proinflammatory and profibrogenic cytokines can explain, in large part, the ensuing global defects in T and B cell function, homeostasis, and cell death (15–25).

The programmed death (PD)-1/PD-ligand (PD-L)1 co-inhibitory pathway plays a significant role in the regulation of the immune response and dysfunction in chronic infectious diseases and cancer (26). The increased levels of expression of PD-1 molecules on Ag-specific T cells in persistent infection, including HIV-1–specific T cells, provide a signature of functional T cell exhaustion. PD-1, a T cell activation marker, is upregulated upon TCR triggering and accumulates at the immunological synapse (27). Upon engagement by its ligand PD-L1 or PD-L2, PD-1 signaling enforces an inhibitory program that blocks further TCR-induced T cell proliferation and cytokine production (28, 29). In a typical acute viral infection this mechanism constitutes a feedback loop that limits the increase in absolute numbers of effector cells during the expansion phase of the immune response. However, under conditions of chronic Ag exposure, this pathway impairs the responsiveness of T cells, ultimately leading to the exhaustion of these cells. Studies from a number of groups have shown that during chronic HIV-1 infection (CHI) PD-1 expression on HIV-1–specific T cells correlates with viral load and that blocking PD-1 engagement restores T cell effector functions in vivo (30–34). Additionally, in vivo PD-1 blockade in chronic SIV infection restored CD8+ T cell function, reduced viral load levels, and increased survival of SIV-infected macaques (35).
Although the role of PD-1 in T cell exhaustion during CHI is well established, its function during AHI is much less clear. Therefore, we performed a cross-sectional analysis of PD-1 expression in CD4+ and CD8+ T cell subsets in a total of 51 individuals at all stages of disease, including individuals receiving highly active antiretroviral therapy (HAART) and uninfected donors.

Materials and Methods

Study population

For phenotypic characterization, 13 subjects with CHI, 8 individuals with AHI, 15 subjects receiving HAART, and 15 HIV-1 seronegative control individuals were enrolled. A summary of these patients’ clinical data is shown in Table I. To assess the capacity of memory T cells to respond to IL-7 stimulation, eight subjects with CHI and five HIV-1 seronegative control individuals were enrolled. A summary of these patients’ clinical data is shown in Table II. None of the HIV-1 acute or chronic infected patients was on antiretroviral therapy at the time of this study. The following guidelines proposed by the Acute HIV Infection and Early Disease Research Program sponsored by the National Institutes of Allergy and Infectious Disease Division of AIDS (Bethesda, MD) were used to estimate the date of infection: 1) the date of the first positive HIV RNA test or p24 Ag assay available on the same day as a negative standard HIV enzyme immunoassay test minus 14 d; 2) the date of onset of symptoms of an acute retroviral syndrome minus 14 d; 3) the date of the first indeterminate Western blot minus 35 d; 4) the date of any positive test for HIV antibodies or p24 Ag assay available on the same day as a negative standard HIV enzyme immunoassay test minus 14 d. All patients signed informed consent approved by the Royal Victoria Hospital and Centre de Recherche du Centre Hospitalier de l’Université de Montréal Review Boards.

Preparation of PBMCs

PBMCs were isolated from heparinized blood by density gradient centrifugation (Ficoll-Paque) and cryopreserved in liquid nitrogen.

Abs and reagents

For phenotypic characterization, the following monoclonal mAbs were used: anti–CD3-A700 (UCHT1), anti–CD4-BV420 (RPA-T4), anti–CD8-PE (HI100), anti–CD28-PE-Cy7 (BD3), anti–CD45RA-allophycocyanin (HIL00), anti–CCR7-PE-Cy7 (ID21), anti–CD27-allophycocyanin-Cy7 (M-T271), anti–PD-1-PE (MH4), anti–CD57-FITC (H-1K), and anti–Ki67-FITC (B56). For Phosflow analysis, the following mAbs were used: anti–CD3-PE-Cy7 (UCHT1), anti–CD4-PerCP-Cy5.5 (RA4–T4), anti–CD8-PE-Cy7 (HI100), anti–CD28-PE (RPA-T8), anti–CD45RA-ECRD (2H4LDH1LDDB), anti–CCR7-PE (150503), anti–CD27-Qdot655 (CLB-27/1), anti–PD-1-PE (J105), Live/Dead Aqua, and anti–Stat5a-FITC (Bcl-2/100). Recombinant human IL-7 was purchased from R&D Systems.

Phenotypic characterization by flow cytometry

Cells from all donors were simultaneously stained with two nine-color, 11-parameter staining cocktails (cocktail 1: CD3, CD4, CD8, CD45RA, CCR7, CD27, CD28, PD-1, and CD57; cocktail 2: CD3, CD4, CD8, CD45RA, CCR7, CD27, CD28, PD-1, and Ki67). FACS staining was performed on ex vivo whole PBMCs. Titrated Abs were added to 2 million cells in 50 μL PBS for 20 min at 4°C. Intracellular staining for Ki67 was conducted by incubating cells stained for cell surface markers with FACS permeabilizing solution (BD Biosciences) for 20 min at room temperature. Cells were then stained in permeabilization buffer (PBS containing 0.05% saponin) with Ki67 Ab or isotype control for 30 min at room temperature. Washed cells were fixed in 2% formaldehyde and stored at 4°C until analysis, which was performed using an LSR II flow cytometer (BD Biosciences), and flow cytometry data were analyzed using FlowJo 8.9 software (Tree Star).

Bcl-2 intracellular staining

PBMCs were labeled with surface markers for 30 min at 4°C. The cells were washed twice with PBS/2% FCS and fixed in 2% formaldehyde for 20 min at room temperature. The cells were permeabilized in PBS containing 0.1% saponin and 10% FCS for 10 min at 4°C and then stained with anti–Bcl-2 for 30 min at room temperature. Finally, cells were washed and fixed in 2% formaldehyde. For the analysis, cells were acquired on an LSR II flow cytometer (BD Biosciences), and the isotype-matched Ab was used as a negative control. All flow cytometry data were analyzed using FlowJo 8.9 software (Tree Star).

Statistical analysis

Correlations were performed using the Spearman test, and statistical significances between groups were calculated by a Mann–Whitney U test using Prism 3.0 software (GraphPad Software). We used the receiver operating characteristic (ROC) and the area under the ROC curve to evaluate the performance of the Fisher linear discriminant analysis classifier to predict the HIV status (acute/chronic) in one- and two-variable models using the leave-one-out cross-validation method. We generated ROC curves by calculating the classifier’s true positive rate (sensitivity) and false positive rate (100 – specificity) at various class membership probability scores yielded by the classifier. Each value of the score yields a single point on the ROC curve. The area under the ROC curve provides a measure of how well the predictor variables tested could distinguish between two groups (acute/chronic). The class prediction analysis was performed using CMA, a package from Bioconductor (http://www.bioconductor.org), an open-source software library for the analysis of genomic data based on R, a language and environment for statistical computing and graphics (http://www.r-project.org).

Results

HIV-1 infection is associated with abnormal distribution of T cell subsets specific to each stage of the disease and incompletely restored by HAART

To study the impact of HIV-1 infection on the distribution of T cell subsets, we first measured the frequencies of CD4+ and CD8+ T cell subpopulations in individuals at three different stages of the disease (Table I). The combined use of the CD45RA, CCR7, and CD27 markers allowed us to identify five phenotypically and functionally distinct populations in both CD4 and CD8 compartments: naive (T_N: CD45RA+, CCR7+, CD27+), central memory (T_CM: CD45RA+, CD27+, CCR7+), transitional memory (T_TM: CD45RA−, CD27+, CCR7+), effector memory (T_EM: CD45RA−, CD27−, CCR7+), and effector CD8+ T cells and terminally differentiated CD4+ T cells (T_E and T_TM: CD45RA−, CD27−, CCR7−) (Fig. 1A).

AHI was characterized by increased frequencies of CD8+ T_TM and T_EM cells (p < 0.0001 and p < 0.05, respectively) concomitant with decreased frequencies of CD8+ T_N and T_CM cells (p < 0.0001 and p < 0.0001, respectively) when compared with control donors (Fig. 1B). Remarkably, CD8+ T_TM cell expansion during AHI is dramatic (2.3-fold increase). Additionally, increased frequencies of CD4+ T_EM cells (p < 0.001) were also noted in acutely infected subjects when compared with uninfected individuals (Fig. 1B). This shift toward a more differentiated T cell phenotype observed in AHI likely reflects strong priming of naive T cells and their differentiation into a large population of end-stage effector cells, a hallmark of acute viral infections (37). Although CD4+ T_EM and CD8+ T_EM cells were found at high frequencies in both AHI and CHI subjects, the transition from the acute to the chronic phase was accompanied by an increased frequency of T_N cells in both compartments (p < 0.05 for the CD4 compartment and p < 0.001 for the CD8 compartment), whereas...
the frequencies of CD4+ TCM, CD4+ TTM, and CD8+ TTM cells decreased ($p < 0.05$, $p < 0.05$, and $p < 0.0001$, respectively) (Fig. 1B). These results confirm previous findings (24) that showed a more differentiated phenotype of CD4+ and CD8+ T cells in chronically infected subjects when compared with HIV-1 donors. A similar analysis performed in aviremic-treated individuals showed that HAART was able to restore the distribution of all memory CD4+ T cell subsets to the frequencies observed in healthy donors. However, HAART failed to restore normal CD8+ T cell subset frequencies, with persisting disequilibrium shown by lower numbers of TCM ($p < 0.05$) and higher numbers of TEM cell subsets ($p < 0.05$) compared with uninfected subjects (Fig. 1B).

Thus, despite effective antiretroviral treatment, even for a prolonged period of time, the CD8+ TCM and TEM cell compartments are not fully restored to the homeostatic levels found in uninfected controls. Collectively, our results indicate that HIV-1 infection leads to a disequilibrium in the frequency of CD4+ and CD8+ T cell subsets in both acutely and chronically infected patients. The distribution of most if not all T cell subsets in both CD4 and CD8 compartments is affected. More importantly, differences observed are specific to each stage of disease and response to HAART.

HIV-1 infection is associated with altered differentiation of T cell subsets and senescence

To characterize the extent of differentiated T cell populations in HIV-1–infected individuals and controls we assessed the relative levels of expression of CD28 and CD57 on both CD4+ and CD8+ T cell subsets. A number of groups have shown that the loss of CD28 expression as well as increased levels of CD57 expression are associated with a state of replicative senescence that correlates with HIV-1 disease progression (9, 38, 39).

As shown in Fig. 2A, loss of CD28 expression was observed in uninfected individuals in the late differentiated TEM and TTD cell subsets in the CD4 compartment whereas CD28 expression in the CD8 compartment progressively decreased with differentiation to $\sim$43% of total effector cells.

Table I. Clinical characteristics of HIV-1–infected subjects

<table>
<thead>
<tr>
<th>Patient</th>
<th>Viral Load$^a$</th>
<th>CD4 Count$^b$</th>
<th>CD8 Count$^c$</th>
<th>Treatment$^d$</th>
<th>Time of Infection$^e$</th>
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<tr>
<td>VR DRPI 025</td>
<td>218,113</td>
<td>296</td>
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<td>425</td>
<td>Unre 12 mo</td>
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<td>Median</td>
<td>28,594</td>
<td>487</td>
<td>763</td>
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<tr>
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<td>3,221,835</td>
<td>257</td>
<td>1007</td>
<td>Unre 43 d</td>
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<tr>
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<td>366,646</td>
<td>483</td>
<td>930</td>
<td>Unre 51 d</td>
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<td>ACU 011</td>
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<td>594</td>
<td>692</td>
<td>Unre 52 d</td>
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<tr>
<td>ACU 375</td>
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<td>378</td>
<td>779</td>
<td>Unre 72 d</td>
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<tr>
<td>ACU DRPI 039</td>
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<td>1499</td>
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<tr>
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<tr>
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<td>475</td>
<td>640</td>
<td>Unre 57 d</td>
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<tr>
<td>Median</td>
<td>28,594</td>
<td>487</td>
<td>763</td>
<td></td>
<td></td>
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<tr>
<td>Chronic-treated</td>
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<td></td>
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<tr>
<td>ST 101002</td>
<td>&lt;50</td>
<td>662</td>
<td>1051</td>
<td>DDI D4T EFV 19 mo</td>
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<tr>
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<td>463</td>
<td>376</td>
<td>NFT D4T 3TC 20 mo</td>
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<tr>
<td>ST 310</td>
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<td>650</td>
<td>392</td>
<td>D4T 3TC IND 64 mo</td>
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<td>604</td>
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<td>IND COM 53 mo</td>
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<td>883</td>
<td>336</td>
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<td>443</td>
<td>322</td>
<td>RIT COM KAL 18 mo</td>
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<td>673</td>
<td>COM NEV 57 mo</td>
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<td>COM NEV 100 mo</td>
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<td>583</td>
<td>3TC EFV ABC 165 mo</td>
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<tr>
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<td>492</td>
<td>582</td>
<td>RIT REY KIV 166 mo</td>
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<tr>
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<td>IND AZT 3TC 86 mo</td>
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<td>715</td>
<td>D4T ATA 139 mo</td>
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<tr>
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<td>671</td>
<td>1120</td>
<td>3TC ABC KAL 242 mo</td>
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<tr>
<td>ST 017</td>
<td>&lt;50</td>
<td>510</td>
<td>765</td>
<td>COM RIT 61 mo</td>
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<tr>
<td>Median</td>
<td>&lt;50</td>
<td>563</td>
<td>613</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$Copies of HIV-1 RNA/ml of plasma at the time of study.

$^b$Peripheral CD4+ T lymphocytes count at the time of study.

$^c$Peripheral CD8+ T lymphocytes count at the time of study.

$^d$Antiretroviral drug abbreviations: ABC, abacavir; ATA, atazanavir; AZT, zidovudine; COM, combivir; DDI, didanosine; DEL, delavirdine; D4T, stavudine; EFV, efavirenz; IND, indinavir; KAL, kaletra; KIV, kivexa; NEV, nevirapine; NFV, nelfinavir; REY, reyataz; RIT, ritonavir; 3TC, lamivudine.

$^e$Duration of infection at the time of study.
The percentage of CD8+ T cell expressing CD28 was most significantly reduced in the AHI individuals compared with uninfected individuals (TCM, TTM, and TEM cells, \( p < 0.0001 \); TE cells, \( p < 0.001 \)). The frequencies of CD8+ T cells, TCM, and TTM cell subsets were also more pronounced during AHI than during CHI \(( p < 0.05 \) for all subsets). Importantly, we found a significant reduction in the frequency of CD8+ TCM cells that expressed CD28 in both acute and chronic infection, which is consistent with the lower frequency of cells in this subset. When compared with the control group, CHI and AHI subjects showed significantly lower percentages of CD4+CD28+ cells. Chronically infected individuals showed significant reduction in the frequencies of CD4 cells expressing CD28 in activated TCM, TTM, and TTD cell subsets \(( p < 0.001 \)) as well as in the TCM cell subset \(( p < 0.05 \)) compared with untreated controls, whereas the percentage of CD4+CD28+ cells was similar between the acutely infected and untreated groups. CHI and AHI subjects showed an increased percentage of CD57+ cells when compared with the control group, CHI and AHI subjects showed significantly increased percentages of CD4+CD28+ cells. Chronically infected individuals showed significant reduction in the frequencies of CD4 cells expressing CD28 in activated TCM, TTM, and TTD cell subsets \(( p < 0.001 \)) as well as in the TCM cell subset \(( p < 0.05 \)) compared with untreated controls, whereas the percentage of CD4+CD28+ cells was similar between the acutely and chronically infected individuals. Lastly, the frequencies of CD4+CD28+ cells in HAART-treated subjects never fully recovered to levels observed in uninfected individuals (TCM cells, \( p < 0.001 \); TTM and TE cells, \( p < 0.05 \)) whereas the only significant differences in CD4+CD28+ expression between HAART-treated subjects and uninfected individuals occurred in the TEM cell pool \(( p < 0.001 \)).

Because CD57 expression has been associated with replicative senescence and apoptosis in HIV-infected individuals \((40)\), we next assessed the percentage of T cells that expressed CD57 in each of the CD4 and CD8 subpopulations (Fig. 2B). As expected, the upregulation of CD57 expression occurred mainly in the most differentiated TEM and TTD/E cell subsets in both CD4+ and CD8+ T cells and exhibited a wide range of individual responses within each group.

With the exception of CD4+ Tn cells, all CD4+ subsets from CHI subjects showed an increased percentage of CD57+ cells when compared with controls (TCM cells, \( p < 0.05 \); TTM, TEM, and TTD cells, \( p < 0.001 \)). All CD8+ subsets also displayed an increased percentage of CD57+ cells \(( p < 0.05 \); TCM cells, \( p < 0.0001 \); TTM, TEM, and TE cells, \( p < 0.05 \)). It appears that T cells start to express CD57 very early in AHI, as all the subsets show significantly increased numbers of CD57+ cells compared with untreated subjects with CD4+ TCM \(( p < 0.05 \)), CD4+ TTM \(( p < 0.001 \)), CD4+ TEM \(( p < 0.001 \)), CD4+ TTD \(( p < 0.001 \)), and CD8+ TEM \(( p < 0.001 \); CD8+ TCM \(( p < 0.0001 \); CD8+ TTM \(( p < 0.05 \); and CD8+ TE cells \(( p < 0.001 \).

![FIGURE 1](http://www.jimmunol.org/) Disruption of T cell subset distribution in HIV-1 infection is specific to each disease stage. (A) Seven-parameter flow cytometry gating strategy to identify CD4+ and CD8+ T cell subsets. Tn, TCM, TTM, TEM, and TTD/TE cell subsets are identified based on their CD45RA, CCR7, and CD27 expression. (B) Distribution of blood CD4+ and CD8+ T cell subpopulations in acute-untreated \(( n = 8 \); black symbol), chronic-untreated \(( n = 13 \); red symbol), chronic-treated \(( n = 15 \); blue symbol), and uninfected \(( n = 15 \); green symbol) subjects. Each symbol represents one subject. Means are shown as horizontal bars. Statistical significance was determined using the Mann–Whitney U test. * \( p < 0.05 \), ** \( p < 0.001 \), *** \( p < 0.0001 \).
Importantly, note that we did not see any significant differences in CD57 expression between CHI and AHI. Virally suppressed subjects also displayed abnormally high frequencies of CD57+ cells in the CD4+ TEM (p, 0.05), CD8+ TCM (p, 0.05), and CD8+ T cell subsets (p, 0.05) compared with uninfected controls, once again indicating that HAART does not completely restore the replicative capability and clonal turnover of effector and effector memory cell populations. Consistent with this hypothesis, we found a strong inverse correlation between the percentage of CD57+ cells and the percentage of CD28+ cells in individual CD4+ and CD8+ naive/memory subsets, suggesting that the loss of CD28 expression was accompanied by the upregulation of the CD57 marker (data not shown).

Collectively, our results demonstrate that during HIV-1 infection CD28+ cells and CD57+ cells can be found in most CD4+ and CD8+ subsets in addition to the terminally differentiated senescent cell population usually described in chronically infected individuals. Markers associated with senescence were mainly attributed to memory subsets in the CD4 compartment and found in all subsets in the CD8 compartment. Importantly, we found no significant differences in the frequencies of T cell subsets expressing either CD28 or CD57 between CHI and AHI, indicating that functional impairment of T cell responses reaches a plateau within 1–3 mo after infection (Table I).

To better assess the extent to which HIV-1 infection drives CD4+ and CD8+ T cell replicative senescence, we measured the fraction of proliferating (i.e., Ki67+) cells in all T cell subsets from all subjects enrolled in this study (Supplemental Fig. 1A). Ki67 is a commonly used and reliable marker of T cell turnover, thereby reflecting the level of immune activation in HIV-1 pathogenesis (2–6). In untreated HIV-1–infected subjects, irrespective of the disease status (acute or chronic), all T cell subsets showed significantly higher frequencies of Ki67+ cells when compared with uninfected controls (p, 0.0001 for all CD4+ and CD8+ subsets in AHI and CHI) (Fig. 3). Interestingly, even TN cells expressed higher levels of Ki67 in both chronically and acutely HIV-1–infected subjects compared with uninfected controls (p, 0.0001). The increased percentages of CD4+Ki67+ TN cells in acute infection but unaltered expression of CD28 and CD57 suggest that cell death and lymphopenia are perturbing normal homeostatic proliferation at the earliest stages of HIV-1 infection.

CD8+ T cell subsets during AHI also showed higher frequencies of Ki67+ cells compared with CHI (CD8+ TN and TCM cells, p, 0.001; CD8+ TTM and TEM cells, p, 0.05) in contrast to similar frequencies of CD4+Ki67+ T cells throughout infection. This observation can be explained by high levels of virus replication in acute infection driving CD8+ T cell activation and expansion that occur in response to virus replication and the emergence of escape variants.

**FIGURE 2.** Differentiation toward senescent stages of all T cell subsets characterized by loss of CD28 expression and increased CD57 expression in HIV-1 infection. (A) Percentage of ex vivo CD28-expressing cells in CD4+ and CD8+ T cell subsets in HIV-1–infected individuals and controls. (B) Percentage of ex vivo CD57-expressing cells in CD4+ and CD8+ T cell subsets in HIV-1–infected individuals and controls. Each symbol represents one subject: acute-untreated (n = 8; black symbol), chronic-untreated (n = 13; red symbol), chronic-treated (n = 15; blue symbol), and uninfected (n = 15; green symbol) subjects. Means are shown as horizontal bars. Statistical significance was determined using the Mann–Whitney U test. *p < 0.05, **p < 0.001, ***p < 0.0001.

**FIGURE 3.** Increased percentage of Ki67+ proliferating cells in CD4+ and CD8+ T cell subsets during HIV-1 infection. Bars represent mean values of acute-untreated (n = 8; black bar), chronic-untreated (n = 13; red bar), chronic-treated (n = 15; blue bar), and uninfected (n = 15; green bar) subjects. Statistical significance was determined using the Mann–Whitney U test. *p < 0.05, **p < 0.001, ***p < 0.0001.
variants. When compared with uninfected controls, frequencies of Ki67\(^{+}\) cells remained significantly higher in all CD4\(^{+}\) and CD8\(^{+}\) T cell subsets with the exception of CD8\(^{+}\) TN cells in subjects receiving suppressive HAART (CD4\(^{+}\) T\(_N\) cells, \(p < 0.05\); CD4\(^{+}\) T\(_{CM}\) cells, \(p < 0.001\); CD4\(^{+}\) T\(_{TM}\), T\(_{EM}\), and T\(_{TD}\) cells, \(p < 0.0001\); CD8\(^{+}\) T\(_{CM}\) and T\(_{TM}\) cells, \(p < 0.001\); CD8\(^{+}\) T\(_{EM}\) and T\(_{E}\) cells, \(p < 0.0001\)), suggesting that residual viremia in treated subjects could induce low levels of CD8\(^{+}\) T cell proliferation and CD4\(^{+}\) T cell homeostatic proliferation in response to persistent CD4\(^{+}\) T cell depletion.

All T cell subsets in both AHI and CHI show increased PD-1 expression

To investigate whether the expression of the coinhibitory receptor PD-1 on T cells correlated with perturbations in CD4\(^{+}\) T and CD8\(^{+}\) T cell subsets that we observed throughout HIV-1 infection, we analyzed levels of PD-1 expression on all T cell subsets in our different cohorts (Supplemental Fig. 1B). We observed significantly higher levels of PD-1 expression on total CD4\(^{+}\) and CD8\(^{+}\) T cells in both acutely (\(p < 0.0001\) for both subsets) and chronically (CD4\(^{+}\) T cells, \(p < 0.0001\); CD8\(^{+}\) T cells, \(p < 0.001\)) infected subjects when compared with uninfected donors (Supplemental Fig. 2A). Interestingly, levels of PD-1 expression on CD4\(^{+}\) T cells were not statistically different between acute and chronic infection whereas PD-1 levels significantly increased on CD8\(^{+}\) T cells during chronic infection (\(p < 0.05\)). The initiation of HAART led to a marked decrease in levels of PD-1 expression in both CD4 and CD8 compartments (1.6- and 2.0-fold decrease, respectively; Supplemental Fig. 2A).

AHI was characterized by a marked increase in the levels of PD-1 expression in all CD4\(^{+}\) T cell subsets (\(p < 0.0001\) for T\(_N\), T\(_{CM}\), T\(_{TM}\), and T\(_{TD}\) cell subsets and \(p < 0.001\) for T\(_{EM}\) cell subsets; range in fold increase, 1.6-2.0) whereas this upregulation was found exclusively in T\(_N\) (\(p < 0.001\)), T\(_{CM}\) (\(p < 0.001\)), and T\(_{TM}\) cell subsets (\(p < 0.05\)) in the CD8 compartment (range in fold increase, 1.3-1.9). In contrast, the transition from AHI to CHI was accompanied by significantly increased levels of PD-1 expression in all CD8\(^{+}\) T cell subsets (\(p < 0.05\) for T\(_N\), T\(_{EM}\), and T\(_{E}\) cell subsets; \(p < 0.001\) for T\(_{CM}\) cell subset; \(p < 0.001\) for T\(_{TM}\) cell subset; range in fold increase, 1.4-1.7); the levels of PD-1 expression were even further augmented in the three CD8\(^{+}\) T cell subsets that already expressed PD-1 at high levels during acute infection. Alternatively, PD-1 expression levels were comparable in AHI and CHI in all CD4\(^{+}\) T cell subsets (with the exception of the CD4\(^{+}\) T\(_N\) subset; \(p < 0.05\)) (Fig. 4A). Importantly, we observed a positive correlation between PD-1 expression and viral load in the CD4 compartment in CHI but not in AHI, suggesting differential association between PD-1 and viral load in AHI and CHI subjects (Supplemental Fig. 3). Although our group and others reported a correlation between viral load and PD-1 expression in the CD8 compartment in CHI in previous studies (30, 31), we did not observe such a correlation in the current study, most probably due to the limited number of subjects included.

To investigate the effectiveness of HAART to reduce levels of PD-1 expression in CD4\(^{+}\) and CD8\(^{+}\) T cell subsets, we then compared the expression levels of PD-1 in T cells from subjects receiving suppressive HAART and uninfected controls. Our results showed that PD-1 remained upregulated in the late differentiated CD4 subsets (\(p < 0.05\) for T\(_{EM}\) cells and \(p < 0.001\) for T\(_{TD}\) cells) and in the less differentiated CD8 subsets (\(p < 0.001\) for T\(_{E}\) cells and \(p < 0.05\) for T\(_{CM}\) cells) even after prolonged antiviral treatment (Fig. 4A).

Taken together, our results show that 1) HIV-1-infected patients express higher levels of PD-1 than do uninfected individuals in all naive/memory subsets with the exception of late differentiated CD8\(^{+}\) T\(_{EM}\) and T\(_{E}\) cell subsets; 2) PD-1 expression is higher in all CD8 subsets during CHI when compared with AHI; and 3) HAART treatment can restore PD-1 expression to the levels observed in uninfected donors in most but not all CD4 and CD8 subsets.

Expression of PD-1 is associated with a reduced ability of CD8\(^{+}\) T cells to proliferate in CHI but not in AHI

Because we showed that PD-1 was associated with a skewed distribution of naive and memory CD8\(^{+}\) T cell subsets during the course of the disease, we extended our analysis to determine the relationship between PD-1 upregulation and T cell turnover as monitored by the expression of Ki67. We focused our analysis on CD8\(^{+}\) T\(_N\), T\(_{CM}\), T\(_{TM}\), and T\(_{EM}\) cell subsets that showed reduced levels of Ki67 associated with proliferative capability during the chronic stage of infection (Fig. 3). We found that the reduction in proliferative potential was accompanied by an increased expression of PD-1 (Fig. 4). Interestingly, when examining Ki67 and PD-1 coexpression, AHI and CHI subjects segregated from each other, with the acute infected cohort expressing both PD-1 and Ki67, whereas the chronically infected subjects expressed only PD-1 (Fig. 5A). Using a class prediction algorithm to predict HIV disease status (acute/chronic), we observed that combining Ki67 and PD-1 improves the prediction performance in comparison with Ki67 and PD-1 tested individually (Supplemental Table I), and this allowed us to discriminate acute from CHI with high confidence. Thus, the expression of low levels of PD-1 expression together with high frequencies of Ki67\(^{+}\) on CD8 T\(_{CM}\) cells was the best predictor of acute infection (area under the ROC curve of 0.98). Importantly, a similar analysis performed on CD4\(^{+}\) T cells could not distinguish AHI and CHI subjects (data not shown), suggesting that this phenotype of PD-1\(^{low}\)Ki67\(^{+}\) was a unique feature of functional CD8\(^{+}\) T cells during acute infection whereas the phenotype PD-1\(^{high}\)Ki67\(^{low}\) identified a population of exhausted CD8\(^{+}\) T cells.

**FIGURE 4.** PD-1 upregulation on all T cell subsets in both acute and chronic infection. Each symbol represents one subject: acute-untreated (\(n = 8\); black symbol), chronic-untreated (\(n = 13\); red symbol), chronic-treated (\(n = 15\); blue symbol), and uninfected (\(n = 15\); green symbol) subjects. Means are shown as horizontal bars. Statistical significance was determined using the Mann–Whitney U test. *\(p < 0.05\), **\(p < 0.001\), ***\(p < 0.0001\).
To further delineate between PD-1 as an activation marker and PD-1 as an exhaustion marker, we assessed the levels of expression of PD-1 on Ki67+ versus Ki67− cells in total CD4+ and CD8+ T cells (Fig. 5B). Ki67+ and Ki67− CD4+ T cells from control donors as well as successfully treated subjects expressed similar levels of PD-1. Alternatively, CD4+Ki67+ T cells from both chronically (p < 0.0001) and acutely (p < 0.001) HIV-1–infected individuals expressed significantly higher levels of PD-1 than did their Ki67− counterparts, suggesting that PD-1 expression reflects the continuous proliferation and differentiation of CD4+ T cells during AHI and CHI. Moreover, Ki67+PD-1highCD4+ T cells were found at similar frequencies during AHI (3.5%) and CHI (2.8%) (Supplemental Fig. 2B) despite the different distributions of CD4+ T cell subsets observed between the two disease stages (Fig. 1B). This observation indicates that a small pool (2–4%) of proliferating PD-1highKi67+CD4+ T cells are present at constant frequencies throughout the disease progression.

We performed a similar analysis on total CD8+ T cells from HIV-1–infected individuals and observed a completely different pattern. Indeed, both proliferating and nonproliferating CD8+ T cells expressed similar or lower levels of PD-1 during acute infection. In contrast, proliferating cells from chronically infected subjects showed higher levels of PD-1 when compared with nonproliferating CD8+ T cells (p < 0.0001), and this was accompanied by a dramatic decrease in the frequency of proliferating CD8+ T cells when compared with AHI (from 16 to 8%; p < 0.001) (Supplemental Fig. 2B). This suggests that the high levels of PD-1 expression observed on Ki67+CD8+ T cells during CHI were associated with reduced clonal turnover whereas the low levels of PD-1 expression during AHI and as well as in treated and uninfected donors did not affect the capacity of CD8+ T cells to proliferate.

Collectively, our results clearly identify a dual role for PD-1 as both an activation marker during AHI and an exhaustion marker in CD8+ T cells during CHI as measured by the different levels of expression of this receptor in combination with inverse relative levels of Ki67 expression.

Expression of PD-1 is associated with impaired survival and IL-7 responsiveness in CD4+ and CD8+ memory T cells isolated from chronically HIV-1–infected subjects

To determine whether PD-1 expression identifies cells that show impaired capacity to survive, we measured the baseline expression levels of the survival factor Bcl-2 in ex vivo total CD4+ and CD8+ memory T cells expressing high and low levels of PD-1 (Fig. 6A). Basal Bcl-2 levels were significantly lower in PD-1high cells when compared with their PD-1low counterparts in the CD4+ (p < 0.01 for CHI subjects) as well as in the CD8+ (p < 0.05 for CHI subjects and controls) memory compartment (Fig. 6B). This suggested that PD-1 is preferentially expressed by cells endowed with reduced survival capacity in infected as well as in uninfected individuals.

We next evaluated the capacity of memory CD4+ and CD8+ T cells isolated from control individuals and CHI subjects to respond to IL-7 stimulation in vitro by measuring Stat5a phosphorylation upon short-term exposure to the cytokine (Fig. 6C). Memory CD8+ and CD4+ T cells from CHI subjects, irrespective of their level of PD-1 expression, showed lower levels of Stat5a phosphorylation upon IL-7 stimulation when compared with uninfected controls (p < 0.05 with the exception of the PD-1high CD4+ memory cells), indicating that HIV-1 infection is associated with impaired T cell response to IL-7 stimulation. Of note, this defect being observed in the PD-1high and PD-1low fractions indicates that, in addition to PD-1, other markers may identify cells impaired in their capacity to respond to the homeostatic signal conferred by IL-7 during CHI. More importantly, PD-1high memory CD8+ T cells from all study subjects (controls and CHI) displayed lower levels of Stat5a phosphorylation upon IL-7 stimulation when compared with their PD-1low counterparts, indicating that PD-1 identifies memory CD8+ T cells with impaired response...
that PD-1 is already significantly upregulated on CD4+ and CD8+ T cells during CHI (30–34). In the present study we demonstrate that PD-1 expression on CD45RA+ T cells and PD-1 expression in PD-1high and PD-1low CD45RA−CD4+ or CD8+ T cells of HIV-1-infected individuals and controls. Each symbol represents one subject: chronic-untreated (n = 8; red symbol) and uninfected (n = 5; green symbol) subjects. Means are shown as horizontal bars. Statistical significance was determined using the Mann–Whitney U test. *p < 0.05, **p < 0.001, ***p < 0.0001. (C) Representative dot plot analysis showing pStat5a expression in PD-1high and PD-1low CD45RA−CD4+ or CD8+ T cells. (D) pStat5a expression in PD-1high and PD-1low CD45RA−CD4+ or CD8+ T cells of HIV-1–infected individuals and controls. Each symbol represents one subject: chronic-untreated (n = 8; red symbol) and uninfected (n = 5; green symbol) subjects. Means are shown as horizontal bars. Statistical significance was determined using the Mann–Whitney U test. *p < 0.05, **p < 0.001, ***p < 0.0001.

FIGURE 6. Baseline Bcl-2 and IL-7 induced Stat5a phosphorylation in PD-1high and PD-1low CD45RA− memory T cells. (A) Representative dot plot analysis showing Bcl-2 expression in PD-1high and PD-1low CD45RA−CD4+ or CD8+ T cells. (B) Bcl-2 expression in PD-1high and PD-1low CD45RA−CD4+ or CD8+ T cells of HIV-1–infected individuals and controls. Each symbol represents one subject: chronic-untreated (n = 8; red symbol) and uninfected (n = 5; green symbol) subjects. Means are shown as horizontal bars. Statistical significance was determined using the Mann–Whitney U test. *p < 0.05, **p < 0.001. (C) Representative dot plot analysis showing Bcl-2 expression in PD-1high and PD-1low CD45RA−CD4+ or CD8+ T cells. (D) Bcl-2 expression in PD-1high and PD-1low CD45RA−CD4+ or CD8+ T cells of HIV-1–infected individuals and controls. Each symbol represents one subject: chronic-untreated (n = 8; red symbol) and uninfected (n = 5; green symbol) subjects. Means are shown as horizontal bars. Statistical significance was determined using the Mann–Whitney U test. *p < 0.05, **p < 0.001, ***p < 0.0001.

Discussion
We and other groups had previously shown that the upregulation of PD-1 on CD4+ and CD8+ T cells leads to their functional exhaustion during CHI (30–34). In the present study we demonstrate that PD-1 is already significantly upregulated on CD4+ and CD8+ T cells during AHI. Moreover, our results indicate that PD-1 is upregulated on all T cell subsets, including naive cells, which have not encountered their cognate Ag yet, as reported in other studies investigating PD-1 expression on T cell subsets during chronic viral infections (41–43). A number of Ag-dependent and -independent mechanisms have been proposed for the global upregulation of PD-1 on T cells during CHI, including residual levels of viral replication (44), inflammatory cytokines (16), and acute homeostatic proliferation of T cells (45). One additional mechanism was proposed by Kinter et al. (46) who showed that homeostatic cytokines such as IL-2, IL-7, and IL-15 induce the upregulation of PD-1 expression on purified T cells. This cytokine-induced PD-1 expression does not impair cytokine-driven events such as proliferation or survival whereas it limits cytokine production after TCR engagement. Moreover, the authors showed that IL-2 administration in successfully treated subjects did not interfere with the ability of the PD-1+ cells to proliferate in vivo (46). However, in chronically infected individuals, the dose and multiplicity of homeostatic cytokines as well as the constant triggering of homeostatic mechanisms may have induced higher levels of PD-1 expression on T cells. These higher levels of PD-1 could reach a value that will impair cytokine-driven proliferation as shown by the decrease in Ki67+ T cells.

The size of the peripheral T cell pool is remarkably stable, reflecting precise regulation of cellular proliferation, survival, and apoptosis to maintain anatomical niches and the functional diversity needed to respond to pathogenic threats. Homeostasis refers to the capacity of the immune system to preserve its internal equilibrium in T cell subset number, diversity, and distribution, allowing it to return to a normal steady-state following perturbation such as infections and lymphopenia. In the present study, we observed a breakdown of T cell homeostasis at all stages of the disease. Acutely infected individuals showed an expansion of highly differentiated TEM cells in both the CD4 and the CD8 compartment, which is the signature of most acute viral infections, but also a dramatic diminution in frequencies of CD8+ TN cells and the massive expansion of CD8+ TEM cells. Chronically infected subjects clearly showed a more differentiated profile having lower frequencies of TEM cells and higher frequencies of CD8+ TEM cells in both the CD4 and CD8 compartments. PD-1 upregulation and continuous PD-L1 engagement during HIV-1 infection could impact peripheral blood T cell subset homeostasis by impairing T cell survival, homeostatic self-renewal, differentiation capacity, de novo priming, as well as the functional effector responses to Ag (30–34, 47, 48). PD-1 is a sensitive marker of TCR activation as well as tolerance induction following sustained activation by PD-L1/L2, and in this study we found that it represents a marker for the breakdown of naive/memory T cell subset homeostasis in HIV-1 infection.

Focusing more specifically on the transition from AHI to CHI, our findings provide further insight into potential mechanisms by...
which immune control in infected individuals might be compromised. Strikingly, HIV-1 chronically infected individuals have an accumulation of naive cells in both the CD4 and CD8 compartments when compared with acutely infected individuals. It has been well documented that chronically infected individuals have defective thymic function, and this suggests that the accumulation of naive cells is due to impaired priming and not to the overproduction of recent thymic emigrant cells from the thymus (22, 49). This could have a huge impact on the diversity of the T cell repertoire of chronically infected individuals, which may result in a less polyclonal repertoire than primary infected individuals. This is consistent with the observation that chronically infected individuals also display much more viral diversity than do acutely infected individuals (50, 51). A reduction in the diversity of the Ag-specific repertoire might contribute to an inability to control viral infection in chronically infected subjects. Notably, PD-1 upregulation on naive T cells might contribute to progressive immunodeficiency in HIV-1 disease and might limit the effectiveness of therapeutic vaccine strategies in HIV-1 infection by preventing the activation and expansion of protective T cell responses. Furthermore, chronically infected individuals also have lower numbers of TTM cells than do acutely infected individuals. Our finding that both the CD4 and CD8 TTM populations express the highest levels of PD-1 is significant because TTM cells constitute a pool of highly proliferative and polyfunctional cells capable of generating high numbers of effector cells and therefore capable of boosting and sustaining the immune response. Importantly, TTM cells of chronically infected individuals express much higher levels of PD-1 than do acutely infected individuals, suggesting that those PD-1high cells may be more susceptible to PD-1-mediated apoptosis (32) or may be impaired in their proliferative capacity. Thus, the accumulation of TN cells and diminished frequency of TTM cells might explain the lack of self-renewal and nonfunctional antiviral CD8+ T cells associated with the loss of virus control during CHI compared with AHI.

We next assessed in vivo proliferation of T cell subsets by measuring Ki67 expression during HIV-1 infection. In the CD4 compartment, the percentage of Ki67+ cells in all naive/memory subpopulations is similar in early and late HIV-1 infection. In the CD8 compartment, there are much higher frequencies of Ki67+ proliferating cells in AHI than in CHI, with this being true for total CD8+ T cells but also for each naive/memory T cell subset. We observed that levels of PD-1 were the same on CD8+Ki67+ T cells and CD8+Ki67− T cells in uninfected and primary infected individuals. In contrast, in chronically infected individuals PD-1 expression was higher on CD8+Ki67− T cells than on CD8+Ki67+ T cells. The increased levels of PD-1 found on CD8+Ki67+ T cells in chronically infected individuals could be associated with decreased in vivo proliferative capacity and may partly explain the diminution in percentage of Ki67+ cells in CHI compared with AHI. Of note, our study did not allow us to distinguish between homeostatic and Ag-driven proliferative capacity of Ki67+ cells. Nevertheless, in vitro IL-7 stimulation of PD-1high memory CD8+ T cells showed that these cells have impaired responsiveness to homeostatic cytokines in chronically infected subjects. This suggests that PD-1 expression identifies cells impaired in their capacity to respond to homeostatic signals, in addition to their well-documented defect to respond to antigenic stimulation (30–34). In conclusion, PD-1 upregulation on proliferating CD8+ T cells may contribute to their loss of function by altering their proliferative capacity and further limit their ability to expand in sufficient magnitude to exert any control on virus replication. Therefore, PD-1 constitutes a marker for the breakdown of T cell distribution in HIV-1 infection.

Our results also show that in addition to the abnormal distribution and loss of T cell function, CD4+ and CD8+ T cells in HIV-1–infected subjects undergo progressive differentiation toward late stages (i.e., CD57+ cells and CD28− cells). In these individuals both CD4+ and CD8+ T cells express CD57 and lose CD28 expression before they have reached late differentiation stages. This abnormal senescence of all naive/memory subsets clearly contributes to functional immune deficiency. Interestingly, we could not find any significant correlation between PD-1 upregulation and loss of CD28 or CD57 overexpression in any of the study groups. Thus, during HIV-1 infection, the accumulation of highly differentiated cells within subsets that usually encompass nondifferentiated cells did not correlate with PD-1 upregulation and could partly explain the inability to control viremia.

Recurrent differences were observed between the CD4+ and CD8+ T cell compartments at all stages of the disease. Those include cellular subset distribution, differentiation/senescence status, loss of function, and/or reconstitution after HAART treatment. In the CD4 compartment, we observed barely any differences between acute and chronic infections, suggesting that functional impairment reaches a plateau within 1–3 mo after infection. Importantly, our data show that initiation of HAART results in the normalization of almost all parameters in the CD4 compartment of virally suppressed subjects who showed restoration of their CD4+ T cells counts (>400 cells/μL). Of note, the downregulation of PD-1 expression in HAART-treated individuals to expression levels seen in uninfected controls has been proposed as a sensitive marker of immune reconstitution in successfully treated subjects (52). Regarding the CD8 compartment, major differences are observed between acute and chronic infections, with the most obvious one being the loss of proliferative capacity. Moreover, HAART only partially restores the CD8 compartment, suggesting that longer treatment duration may be needed to reach normalization. This could be explained by the fact that the CD8 compartment is not depleted but is highly impaired in function. Additionally, persistent low levels of viral replication in anatomical reservoirs may sustain CD8+ T cell activation and dysfunction (44).

In conclusion, this study provides a comprehensive characterization of phenotypic changes in T cell subsets during HIV-1 infection that reflect different mechanisms involved in the loss of CD4+ and CD8+ T cell function during the acute and chronic stages of HIV-1 infection. Specifically, changes in CD8+ T cell subset profiles that begin in acute infection and that become fixed in chronic infection, even after initiation of HAART, reveal the profound loss of functional effector cells and the proliferative capability of naive and memory subsets that could contribute to the eventual failure to control virus replication. Future work to identify the Ag specificities of CD8+PD-1high TTM cells that are functionally exhausted and the role of PD-1-PD-L1 signaling for the establishment of latency in CD4+ T cells will be crucial for the design of therapeutic strategies aimed at blocking PD-1 signaling and other coinhibitory molecules (53). Our findings in this study strongly suggest that therapeutic interventions should be initiated early in acute infection before homeostatic mechanisms become deregulated and immune paralysis ensues.

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Disclosures

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References


