CD127 and CD25 Expression Defines CD4⁺ T Cell Subsets That Are Differentially Depleted during HIV Infection

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CD127 and CD25 Expression Defines CD4⁺ T Cell Subsets That Are Differentially Depleted during HIV Infection¹

Richard M. Dunham,!* Barbara Cervasi,!* Jason M. Brenchley,‡ Helmut Albrecht,§ Amy Weintrob,§ Beth Sumpter,‡ Jessica Engram,§ Shari Gordon,*† Nichole R. Klatt,*† Ian Frank,* Donald L. Sodora,§ Daniel C. Douek,‡ Mirko Paiardi,*, and Guido Silvestri2*†

Decreased CD4⁺ T cell counts are the best marker of disease progression during HIV infection. However, CD4⁺ T cells are heterogeneous in phenotype and function, and it is unknown how preferential depletion of specific CD4⁺ T cell subsets influences disease severity. CD4⁺ T cells can be classified into three subsets by the expression of receptors for two T cell-tropic cytokines, IL-2 (CD25) and IL-7 (CD127). The CD127⁺CD25low⁻⁻ subset includes IL-2-producing naive and central memory T cells; the CD127⁻CD25⁻ subset includes mainly effector T cells expressing perforin and IFN-γ; and the CD127lowCD25high subset includes FoxP3-expressing regulatory T cells. Herein we investigated how the proportions of these T cell subsets are changed during HIV infection. When compared with healthy controls, HIV-infected patients show a relative increase in CD4⁺CD127⁺CD25⁻ T cells that is related to an absolute decline of CD4⁺CD127⁻CD25low⁻⁻ T cells. Interestingly, this expansion of CD4⁺CD127⁻ T cells was not observed in naturally SIV-infected sooty mangabeys. The relative expansion of CD4⁺CD127⁺CD25⁻ T cells correlated directly with the levels of total CD4⁺ T cell depletion and immune activation. CD4⁺CD127⁻CD25⁻ T cells were not selectively resistant to HIV infection as levels of cell-associated virus were similar in all non-naive CD4⁺ T cell subsets. These data indicate that, during HIV infection, specific changes in the fraction of CD4⁺ T cells expressing CD25 and/or CD127 are associated with disease progression. Further studies will determine whether monitoring the three subsets of CD4⁺ T cells defined based on the expression of CD25 and CD127 should be used in the clinical management of HIV-infected individuals. The Journal of Immunology, 2008, 180: 5582–5592.

IV infection is associated with a progressive decline of CD4⁺ T cells and loss of immune function, which manifests clinically as an increased susceptibility to opportunistic infections and neoplasm. Early studies showed that HIV uses the CD4 molecule as an entry receptor and that infected CD4⁺ T cells are directly killed by the virus, providing a logical explanation for the progressive decline of these cells in HIV-infected individuals (1, 2). However, other studies have shown that, during chronic HIV infection, more CD4⁺ T cells die than can be accounted for by direct infection (3), and that, in fact, an increased death rate is observed even in nontarget cells, such as CD8⁺ T cells (4–8). This bystander loss of uninfected T cells is thought to be related to a state of generalized immune activation that is consistently associated with HIV infection (9). Based on these findings, it has been proposed that chronic immune activation plays a key role in the HIV-associated CD4⁺ T cell depletion (8, 10–13). Consistent with this model is the finding that the expression of activation markers on T cells is a strong predictor of disease progression in HIV-infected individuals (14, 15).

During HIV infection, the dynamics of CD4⁺ T cells and their subsets are influenced by several factors, including the level of virus replication and the involvement of these cells in the immune response to HIV. Virus replication occurs preferentially in effector memory CD4⁺ T cells expressing the viral co-receptor CCR5 and thus results in a net loss of these cells that is particularly rapid and pronounced at the level of MALT (16–18). On the other hand, the HIV-associated chronic immune activation results in a constant recruitment of CD4⁺ T cells from the central memory and naive pools into the effector pool, thus creating a strain on the homeostatic mechanisms of CD4⁺ T cell maintenance (8, 10–12). As such, the net effect of HIV infection on the relative proportions of CD4⁺ naïve (Tₙ),³ memory (Tₘ), and effector (Tₑ) T cells is far from understood and likely depends greatly on the stage of disease.

The level of HIV-associated chronic immune activation (and its related impact on CD4⁺ T cell homeostasis) is currently assessed by measuring the expression of markers of T cell maturation (CD45RA, CD62L, CCR7), activation (HLA-DR, CD38), and proliferation (Ki67) that are not, per se, suitable targets for immune-based interventions. In this study we sought to investigate the dynamics of the CD4⁺ T cell compartment following HIV infection by focusing on functional markers that could also serve as targets for immunomodulatory therapies. Expression of the receptors for IL-2 and IL-7 controls naïve and memory T cell homeostasis, proliferation, and differentiation (19). IL-2 and IL-7 are members of a

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Abbreviations used in this paper: Tₙ, naïve T; Tₘ, memory T; Tₑ, effector T; Treg, regulatory T cell; SM, sooty mangabey.
family of cytokines that utilize a common $\gamma$-chain (CD132) in combination with high-affinity receptors: IL-2R$\beta$ (CD122) and IL-2R$\alpha$ (CD25) for IL-2, and IL-7R$\alpha$ (CD127) for IL-7. IL-2R$\alpha$ signaling is required for T cell development and to support Ag-specific T cell responses (19). Additionally, the role of IL-2 in promoting regulatory T cell (Treg) function is demonstrated by the finding that both IL-2$^{-/-}$ and IL-2R$\alpha^{-/-}$ mice develop autoimmune and lymphoproliferative disorders (19). While the low-affinity IL-2R is constitutively expressed by T cells, TCR-mediated signaling, thus priming the newly activated T cell response to viral infection, a small number of effector phenotype (49, 50). A general down-regulation by Ag or IL-7 binding, IL-7R$\alpha$-chain and the assembly of the high-affinity heterotrimeric IL-2R complex. In contrast, Tregs constitutively express the high-affinity complex (CD25$^{high}$), while several studies have shown abnormalities of the CD4$^+$/CD25$^+$ Treg subset during chronic HIV infection by expanding T N or TM and, in the case of IL-7, may rebalance the observed expansion of Treg subset during chronic HIV infection (20–30), it is still unclear to what extent these cells play a role in the immunopathogenesis of AIDS, particularly in terms of suppressing HIV-specific T cell responses and mitigating the HIV-associated chronic immune activation. Interestingly, the fraction of CD4$^+$/CD25$^+$ T cells is dramatically increased after exogenous administration of IL-2 (31).

IL-7 signals are vital to T cell development, as IL-7$^{-/-}$ or IL-7$\alpha^{-/-}$ mice exhibit severe T cell deficiency (32, 33). The binding of IL-7 to its high-affinity receptor CD127 results in up-regulation of antiapoptotic molecules such as bcl-2 and enhancement of survival and/or proliferation is contingent upon expression of CD25$^+$ and the University of Pennsylvania.

**Animals**

Peripheral blood was sampled from 110 SIV-infected and 30 SIV-uninfected sooty mangabeys (Cercopithecus asytis) housed at the Yerkes National Primate Research Center (Atlanta, GA). All experiments were approved by the Institutional Animal Care and Use Committees of Emory University and the University of Pennsylvania.

**Antibodies**

Anti-CD127-PE was purchased from Beckman Coulter. Anti-CD3 (clone SP34-2)-Pacific Blue or Alexa700, anti-CD4 (clone L200 or SK3 or RPA-T4)-PerCP or PerCP-Cy5.5 or Alexa700, anti-CD8 (clone SK1 or RPA-T8)-PerCP or FITC or APC or PE-Cy7, anti-perforin (clone dC9)-FITC, anti-Ki67 (clone B56)-FITC, anti-CD25 (clone 2A3 or M-A251)-APC or FITC or APC-Cy7, anti-CD38-APC (HIT-2), anti-IL-2 (clone MQ1-17H12 or 5344.111)-FITC, anti-IFN-γ (clone 25723.11)-APC, anti-CD62L (SK1)-FITC, anti-CD27 (clone L128)-FITC or PE, anti-CCR7 (clone 3D12)-PE-Cy7, anti-CD45RA (HI-100)-APC, anti-HLA-DR (L243)-APC or CyChrome, anti-CD28 (CD82.2)-CyChrome, anti-CCR5 (2D7/CCR5)-CyChrome and annexin V FITC were purchased from BD Biosciences. Anti-CD45RA (clone MEM-56)-Alexa405 was purchased from Caltag Laboratories. Anti-FoxP3 (PCH-101)-FITC was purchased from eBioscience.

**Flow cytometry**

PBMCs were isolated from whole blood by density gradient centrifugation using standard procedures. For intracellular Ags, cells were permeabilized using BD FACSperm2 (BD Biosciences) according to the manufacturer’s instructions. PBMCs were analyzed by four-to-eight-color fluorescent Ab staining. Flow cytometric acquisition and analysis were performed on at least 50,000 acquired events (gated on lymphocytes) on a FACS caliber flow cytometer driven by the CellQuest software (BD Pharmingen) or an LSR II flow cytometer driven by FACSdiva software (BD Biosciences). Data analysis was performed using FlowJo software (Tree Star). Cell sorting experiments were performed on a FACSaria (BD Biosciences) after staining with fluorescent Abs specific for CD4, CD127, CD25, and CD45RA. Cells were sorted into RPMI 1640 containing 10% FBS, and the purity of sorted populations was always >98%. Absolute CD4$^+$ T cell counts were calculated by multiplying the fraction of CD3$^+$CD4$^+$ cells within the forward light scatter-area/side light scatter-area-defined lymphocyte region by the total lymphocyte count derived from the complete blood count. This value was then used to calculate the absolute counts of the three studied CD4$^+$ T cell subsets by multiplying the absolute CD4$^+$ T cell count by the fractions of CD127$^+$CD25$^{high-w}$, CD127$^+$CD25$^+$, and CD127$^-$CD25$^{low-w}$ cells (gated as in Fig. 1A).

**In vitro cytokine production**

For intracellular cytokine staining, whole blood was centrifuged over a Cellgro (Mediatech) Lymphocyte Separation Medium density gradient, and mononuclear cells were harvested. These cells were then incubated for 6 h with PMA (10 ng/ml), the calcium ionophore A23187 (200 ng/ml), and monensin (10 nM) (all from Sigma-Sldrich). Surface staining was performed as described above. Cells were permeabilized using BD Cytofix/Cytoperm (BD Biosciences) according to the manufacturer’s instructions and stained for IL-2 or IFN-γ at 4°C for 20 min.

**Apoptosis**

Lymphocytes were stained for the relevant surface markers and annexin V-FITC either immediately after isolation or after 24 or 48 h at 37°C in RPMI 1640 containing 10% FBS. For these experiments all blood samples were collected by venipuncture.

**HIV cell-associated DNA content**

Quantification of HIV gag DNA in sorted CD4$^+$ T cells was performed by quantitative real-time PCR by the 5'-nucleate (TaQman) assay with an ABI7700 system (PerkinElmer) as previously described (60, 61). To quantify cell number in each reaction, quantitative PCR was performed simultaneously for albumin gene copy number as previously described (62). Standards were constructed for absolute quantification of gag and albumin copy number, and they were validated with sequential dilutions.
of 8E5 and Ach2 cell lysates that contain one copy of gag per cell. Duplicate reactions were run and template copies were calculated using ABI7700 software.

**Statistical analysis**

Statistical analysis was performed using GraphPad Prism v4.0c for Mac OS X. Matched pairwise comparisons were made using the Wilcoxon matched pairs test. One-factor multiple comparisons were computed with the Friedman test. Two-factor multiple comparisons were calculated using the two-way ANOVA. Correlations were performed using the Spearman rank correlation coefficient test. In all tests $\alpha$ was set to 0.05.

### Results

**Expression of CD127 and CD25 define three subsets of CD4+ T cells**

Three subsets of CD4+ T cells are defined by the expression of IL-2Rα (CD25) and IL-7Rα (CD127): CD127+CD25low, CD127+CD25+, and CD127lowCD25high (Fig. 1A). In the healthy HIV-uninfected individuals enrolled in this study ($n = 20$), the majority (average 88.2 ± 2.87%) of CD4+ T cells were included in the CD127+CD25low subset, with the CD127lowCD25high and the

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</table>

* C indicates Caucasian; AA, African American.
FIGURE 1. Expression of CD127 and CD25 defines three subsets of CD4+ T cells. A, Gating strategy for defining subsets of CD4+ T cells using CD127 and CD25. Plots shown are gated on lymphocytes, then CD3+, CD8+, and CD4+. B, Comparison of the average fraction of CD4+ T cells within each subset in HIV-infected (filled bars) and uninfected (open bars) individuals. ***p < 0.001 (two-way ANOVA). C, Comparison of the absolute number of CD4+ T cells within each subset in HIV-infected individuals (filled bars) and uninfected (open bars) controls.

CD127+CD25− subsets containing relatively few cells (average 7.45 ± 1.64% and 3.65 ± 2.67%, respectively) (Fig. 1B). When the relative proportions of the same three CD4+ T cell subsets were examined in HIV-infected individuals (n = 48, average CD4+ T cell count of 237 ± 184 cells/mm³), we observed a significant increase in the size of the CD127+CD25− subset (17.77 ± 13.0%) and a concomitant decline in the fraction of CD4+CD127+CD25low− T cells (70.9 ± 15.79%) (Fig. 1B). The relative size of the CD127low/CD25high subset was only slightly elevated in HIV-infected individuals compared with controls (10.3 ± 7.27%).

In terms of absolute numbers of cells per cubic millimeter of blood, we observed a dramatic decline of the CD4+CD127−CD25− low−/− T cell subset in HIV-infected individuals (175 ± 148 cells/mm³) compared with controls (461 ± 318 cells/mm³) (p < 0.001, Fig. 1C). Similarly, the absolute number of CD4+CD127low/CD25high T cells was decreased in HIV-infected individuals when compared with controls, although in a less drastic fashion (21 ± 15 vs 43 ± 30 cells/mm³, p = NS, Fig. 1C). In contrast, the absolute number of CD4+CD127+CD25− T cells was maintained, and, in fact, even slightly increased in HIV-infected patients (41 ± 45 vs 22 ± 14 cells/mm³, p = NS, Fig. 1C). These data indicate that the relative increase in size of the CD4+CD127+CD25− T cell subset associated with HIV infection is not due to an increased absolute number of CD127−CD25− cells but rather to a substantial and remarkably selective loss of CD127+CD25low−/− cells (and, to a lesser extent, of CD127low/CD25high cells). These findings are in stark contrast to the changes observed in the expression of CD127 on CD8+ T cells during HIV infection, where a major expansion in the absolute number of CD8+CD127+ T cells was associated with normal numbers of CD8+CD127− cells (49).

Phenotypic and functional characteristics of the three subsets of CD4+ T cells identified by expression of CD25 and CD127

We next performed a detailed phenotypic and functional characterization of the three subsets of CD4+ T cells. We first used eight-color flow cytometry on PBMCs derived from both HIV-infected individuals and controls to assess the expression of CD45RA and CD62L and thus characterize the state of differentiation (i.e., naive vs central memory vs effector memory) of the CD4+ T cell subsets (63). As shown in Fig. 2A, we found that the CD127+CD25low−/− subset is relatively equally divided between CD45RA+CD62L+ naive cells and CD45RA−CD62L− central memory cells, that the CD127+CD25− subset includes the vast majority of the CD62L− effector memory cells, and that the CD127low/CD25high subset mostly expresses markers of central memory cells (CD45RA−CD62L+). Interestingly, while the phenotype of the CD127+CD25low− and CD127low/CD25high subsets with respect to CD45RA and CD62L expression was similar between HIV-infected individuals and controls (Fig. 2A), the CD127+CD25− subset included a higher fraction of CD4+CD62L−CD45RA+ and CD4+CD62L−CD45RA− T cells in HIV-infected patients. Consistent with recent reports (57–59), >80% of the CD4+CD25low/CD25high T cells expressed Foxp3+ (not shown). This finding, together with the expression of a central memory phenotype (CD45RA−CD62L−) by these cells, prompted us to consider them as Tregs. Figure 2B shows the absolute numbers/mm³ of the three CD4+ T cell subsets included in the naive (CD45RA−CD62L−), central memory (CD45RA−CD62L−), and effector memory (CD62L−) populations.

We next measured the expression of markers of T cell activation within the subsets of CD4+ T cells defined by CD25/CD127 expression. Although all three subsets expressed each of the activation markers analyzed to some degree, the CD127+CD25− subset contained, in both HIV-infected patients and controls, the highest fraction of cells expressing the proliferation Ag Ki67, the activation marker HLA-DR, as well as lower levels of CD27 and CD28 (Fig. 2C). These findings are consistent with the previous observation that CD4+CD127+CD25− T cells are enriched in CD62L− cells (Fig. 2A). Importantly, note that HIV-infected patients did not show an increased fraction of cells expressing activation markers within any of the studied subsets, but rather a relative increase of the CD4+CD127+CD25− T cells that express the highest levels of activation markers in both HIV-infected patients and controls was shown.

To investigate the functional properties of the described CD4+ T cell subsets, we next measured the production of IL-2 and IFN-γ by PBMCs isolated from HIV-infected individuals and controls using intracellular cytokine staining after stimulation with PMA/ionomycin (A23187). Because stimulation through the TCR and its downstream signaling pathways can modulate the expression of cytokine receptors, we conducted preliminary experiments to sequentially measure CD25 and CD127 expression over the course of 6 h of PMA/ionomycin stimulation. We found that while CD127 expression on CD4+ T cells remained constant, CD25 expression was up-regulated by 4 h of stimulation (not shown). We thus focused our assessment of cytokine production on CD4+CD127+ and CD4+CD127− T cells. As shown in Fig. 2D, CD4+ T cells from both HIV-infected individuals and controls show a particular
pattern of cytokine production after 4 h of PMA + A23187 stimulation characterized by production predominantly of IL-2 by CD4^+ CD127^+ T cells and IFN-γ by CD4^+ CD127^- T cells. The results of this experiment thus further support our previous phenotypic characterization of the CD4^+ CD127^+CD25low/- T cell subset as including mainly naive and central memory cells and the CD4^+ CD127^- T cell subset as including mainly effector memory or terminally differentiated cells.

We next measured the in vitro susceptibility of each of the CD4^+ T cell subsets to both spontaneous and activation-induced apoptosis. To this end, we measured annexin V binding to lymphocytes derived from healthy donors and HIV-infected patients under two different experimental conditions: immediately postisolation (baseline apoptosis), and after 24 or 48 h incubation in the absence of any stimulation (spontaneous apoptosis). Fig. 2E shows that, while baseline apoptosis levels were slightly elevated in all CD4^+ T cell subsets from HIV-infected individuals, the levels of spontaneous apoptosis were significantly higher for the CD127^-CD25^-CD45RO^+ subset as compared with the CD127^-CD25low/-CD45RO^- subset. The relative expansion of CD4^+ CD127^-CD25^- T cells is correlated with markers of disease progression in HIV-infected individuals.

During chronic HIV infection, the prevailing level of immune activation is a strong predictor of disease progression (64, 65). As the
relative expansion of CD4+CD127−CD25− T cells in HIV-infected patients appears to reflect an increased fraction of effector memory CD4+ T cells, we next sought to determine whether any of the changes in the CD4+ T cell subsets that we observed during HIV infection correlated with markers of disease progression. We first observed a direct correlation between the fraction of CD4+CD127−CD25− (but not the CD4+CD127+CD25low− T cells) and viral load (Fig. 3A), indicating that the relative shift toward the CD4+CD127−CD25− T cell subset is likely related to the prevailing level of virus replication. Interestingly, we found a significant inverse correlation between the proportion of CD4+CD127−CD25− T cells and the overall percentage of circulating CD4+ T cells, as well as a significant direct correlation between the proportion of CD4+CD127−CD25low− T cells and the overall percentage of CD4+ T cells (Fig. 3B). As shown in Fig. 3C, we also observed a direct correlation between the fraction of CD4+CD127−CD25low− T cells and the absolute CD4+ T cell count, while no correlation was observed between the percentage of CD4+CD127−CD25− T cells and the absolute CD4+ T cell count. These correlations suggest that the HIV-induced progressive decline of circulating CD4+ T cells is consistently associated with a relative expansion of CD4+CD127−CD25− T cells within the residual pool of CD4+ T cells.

Given the known association between chronic T cell activation and disease progression in HIV-infected individuals (14, 64, 65), we also measured the relationship between the relative proportions of the three CD4+ T cell subsets and the level of immune activation, assessed as the fraction of CD8+ T cells that have lost the expression of CD28 (66) or CD127 (49). We observed a significant direct correlation between the fraction of CD4+CD127−CD25− T cells and both the fraction of CD8+CD28− T cells and CD8+CD127− T cells (p = 0.0279 and p = 0.0222, respectively, from Spearman rank correlation; data not shown). Taken together, these findings indicate that the relative expansion of CD4+CD127− T cells observed in HIV-infected individuals is correlated with both the level of CD4+ T cell depletion and the degree of vivo T cell activation.

Nonpathogenic SIV infection of sooty mangabeys is not associated with changes in the proportions of CD4+ T cell subsets defined by CD127 and CD25

In a series of previous studies, we and others have shown that natural SIV infection of sooty mangabeys (SM), a nonhuman primate species from West Africa, is typically nonpathogenic and is associated with maintenance of CD4+ T cell homeostasis despite chronically high levels of virus replication (67–71). We thus proposed that, in naturally SIV-infected SMs, the presence of typically low levels of immune activation is instrumental in the preservation of peripheral CD4+ T cell counts and the maintenance of the disease-free state (72).
As such, we sought to determine whether natural SIV infection of SMs is associated with changes in the relative proportions of CD4⁺ T cell subsets defined based on CD127/CD25 expression. Fig. 4 shows averages of the CD4⁺ T cell subsets assessed in 110 SIV-infected and 30 uninfected SMs. No significant differences in the relative proportions of CD4⁺CD127⁺CD25low/−, CD4⁺CD127lowCD25bighi, or CD4⁺CD127⁺CD25⁻ T cell subsets were observed in SIV-infected as compared with uninfected SMs. Since natural SIV infection is typically characterized by a dissociation between virus replication and T cell activation, these findings further support the possibility that the imbalance of CD4⁺ T cell subsets observed in HIV-infected individuals is related to the chronic immune activation state rather than being a direct consequence of virus replication.

**CD4⁺ CD127⁺ CD25⁻ T cells are not preferentially infected during chronic HIV infection**

The in vivo death of CD4⁺ T cells that occurs during chronic HIV infection is caused by two major, non-mutually exclusive mechanisms: 1) direct virus infection and 2) increased apoptosis of uninfected cells that is thought to be related to chronic immune activation (11–13). To directly assess the impact of direct virus infection on the described imbalance of CD4⁺ T cell subsets defined by CD127/CD25 expression, we measured the cell-associated HIV-DNA content in sorted CD4⁺ T cells. We first FACSpurified CD4⁺ T cells into the subsets defined above (CD127⁺CD25low/−, CD127⁻CD25⁺, CD127lowCD25bighi) and additionally subdivided the CD4⁺CD127⁺CD25low/⁻ T cell subset using CD45RA to differentiate between T₅ (CD45RA⁻) and T₅B (CD45RA⁺) cells. This subdivision of the CD4⁺CD127⁺CD25low/⁻ T cell subset was conducted to take into account that the presence of CD4⁺ T₅ cells, which are infrequently infected by HIV (60), within the CD4⁺CD127⁺ T cells would skew our assessment of the role of direct HIV infection in determining the relative expansion of CD4⁺CD127⁺CD25⁻ T cells. In these experiments, HIV gag copy number in each sorted subset was measured in cellular DNA extracts by quantitative real-time PCR using gag-specific primers as described (60, 61). As expected, the CD4⁺CD127⁺CD25low/⁻CD45RA⁻ naive CD4⁺ T cell subset contained the lowest median HIV gag copies per cell (49 copies/cell, interquartile range of 10–185 copies/cell). Importantly, the remaining three subsets contained similarly high quantities of HIV gag DNA (CD127⁺CD25low/⁻CD45RA⁺ cells: 234 copies/cell, interquartile range of 100–1080 copies/cell; CD127⁻CD25⁺ cells: 243 copies/cell, interquartile range of 103–647 copies/cell; and CD127lowCD25bighi cells: 404 copies/cell, interquartile range of 102–855 copies/cell) (Fig. 5A). These data indicate that differences between CD4⁺ T cell subsets with respect to their susceptibility to direct virus infection are unlikely to be the mechanism underlying the relative expansion of CD4⁺CD127⁺CD25⁻ T cells that we found associated with chronic HIV infection.

To further define the potential role of susceptibility to HIV infection of specific CD4⁺ T cell subsets, we next measured the expression of CCR5, the major co-receptor for HIV and a marker of T cell activation. As shown in Fig. 5B, we report that the expanded CD4⁺CD127⁺CD25⁻ T cell subset contained the highest fraction of cells expressing CCR5 when compared with CD4⁺CD127⁻CD25⁺ and CD4⁺CD127lowCD25bighi T cells. These findings are not unexpected, as CCR5 is preferentially expressed on effector memory CD4⁺ T cells (73) that are overrepresented in the subset of CD4⁺CD127⁺CD25⁻ T cells (Fig. 2B). Together with the finding that CD4⁺CD127⁺CD25⁻ T cells are not selectively spared from direct HIV infection, these CCR5 data suggest that the preferential loss of CD4⁺CD127⁺CD25low/⁻ T cells reflects the ongoing chronic immune activation rather than an exquisite susceptibility to HIV-mediated killing. As such, these results further emphasize the pathogenic role of chronic T cell activation in determining the HIV-associated immune dysfunction.

**FIGURE 4.** SIV infection of sooty mangabeys does not modulate the proportions of CD4⁺ T cell subsets defined by CD127 and CD25. Average fraction (left) and number per cubic millimeter of blood (right) of CD4⁺ T cells found in the CD127/CD25 subsets in peripheral blood of 110 SIV-infected (filled bars) and 30 uninfected (open bars) sooty mangabeys.

**FIGURE 5.** CD4⁺CD127⁺CD4⁺ T cells are not preferentially infected during chronic HIV infection. A, Quantification of cell-associated HIV DNA content using quantitative, real-time PCR within the CD127/CD25 subsets of CD4⁺ T cells expressed as copies of HIV gag per 10,000 cells (normalized by copies of albumin). Friedman’s test was used to measure statistical significance. B, Comparison of the average fraction of each CD127/CD25 CD4⁺ T cell subset expressing CCR5 in HIV-infected (filled bars) and uninfected (open bars) individuals.
Discussion

Despite more than 25 years of intense study, the mechanisms by which HIV causes AIDS remain relatively poorly understood. The observation that HIV infection is followed by a progressive depletion of circulating CD4+ T cells has been traditionally interpreted as resulting from the selective infection and killing of CD4+ T cells (74). However, the HIV-associated immune dysfunction goes above and beyond the numeric loss of CD4+ T cells, as it involves functional abnormalities of the CD4+ T cells that remain as well as impaired function of many other immune cell types (75–78). While some of the non-CD4+ T cell dysfunction has been proposed to be a secondary consequence of the overall loss of CD4+ T cell-mediated “help”, it has become apparent that both the loss of CD4+ T cells and the generalized immune abnormalities are related to a state of chronic immune activation (8, 10–12). Crucial evidence in favor of the pathogenic role of the HIV-associated immune activation includes 1) the observation that T cell activation is a better predictor of disease progression than is virus replication (14, 64, 65), and 2) the discovery that SIV infection of natural hosts is typically nonpathogenic despite high-level virus replication in the context of minimal immune activation (69–71).

In this study, we sought to address the pathogenic role of functional abnormalities within the pool of residual CD4+ T cells and/or changes in the relative proportions of specific CD4+ T cell subsets in HIV-infected patients. We thus assessed the HIV-associated qualitative changes within the CD4+ T cell compartment by applying a recently proposed phenotypic classification of CD4+ T cell subsets based on the expression of CD127 and CD25 (57–59). According to these studies, circulating CD4+ T cells can be divided into three phenotypically and functionally distinct subsets: 1) CD127+CD25low− cells that include naive and central memory CD4+ T cells, 2) CD127−CD25− cells that show characteristics of effector memory CD4+ T cells, and 3) CD127lowCD25high cells that express markers of regulatory T cells. Importantly, this classification may prove clinically useful as expression of CD127 and CD25 is linked to specific CD4+ T cell functions (i.e., responsiveness to IL-7 and IL-2, respectively) that may be targeted as part of immune-based interventions for HIV infection.

In this study, we found that during HIV infection the relative proportion (but not absolute number) of the CD4+CD127+CD25− subset was significantly and consistently expanded, due to a selective absolute loss of the CD127−CD25low− subset. These results indicate that, within the overall CD4+ T cell pool, the well-known increase in the fraction of cells showing signs of immune activation (such as up-regulation of HLA-DR and loss of CD28 expression (66, 79)) is not due to a generalized modification of the activation (such as up-regulation of HLA-DR and loss of CD28 expression) but rather due to the CD4+ T cell population being depleted of Tγδ and Tcells (80) that rarely express markers of activation irrespective of HIV infection. Importantly, we found that the relative expansion of the CD4+CD127+CD25− T cell subset correlates with markers of disease progression such as the depletion of CD4+ T cells and the level of immune activation, suggesting a role for the described changes in the proportions of the CD4+ T cell subsets in the pathogenesis of HIV infection and AIDS. The current study examined only peripheral blood-derived CD4+ T cells. Although studies of other tissues are difficult to perform in humans, it will be important, in future work, to determine whether a similar increase of CD4+CD127+ T cells is present in lymph nodes (where most CD4+ Tγδ and Tcells reside).

While other studies have suggested that chronic HIV infection is associated with dysfunction of the Treg subset, either in the direction of an increased (21, 24–26, 30) or reduced (23, 27) ability to regulate T cell responses, our study failed to reveal any significant change in the relative proportion of CD4+CD127lowCD25high T cells in HIV-infected individuals compared with uninfected controls. As we have not investigated the Treg function in detail, our findings are compatible with the possibilities that Treg function is affected by HIV infection in a way that does not involve major changes in the proportion of these cells within the circulating CD4+ T cell compartment, or that, in fact, changes in Treg activity are not a key factor in AIDS pathogenesis.

The association between the relative expansion of CD4+CD127+ T cells and both decreased CD4+ T cell levels and increased immune activation can be explained in several ways. First, it could be hypothesized that CD4+CD127+CD25− T cells are selectively spared by the virus, perhaps as a consequence of lower CCR5 expression. However, the data presented here show clearly that the level of cell-associated virus is similar in this subset of CD4+ T cells when compared with other CD4+ T cell subsets, and that CCR5 expression on CD4+CD127+CD25− T cells is, in fact, higher than on other subsets of CD4+ T cells. Another potential interpretation is that CD4+CD127+CD25− T cells are intrinsically less prone to the apoptosis of bystander, uninfected T cells that is typically associated with HIV infection (81, 82). In this study, the direct measurement of ex vivo spontaneous apoptotic cell death did not indicate any preferential apoptosis of CD4+CD127+ T cells. Note, however, that this work has not ruled out a differential susceptibility of CD4+CD127−CD25− T cells to Fas-mediated apoptosis, a possibility suggested by the observation that IL-7 increases susceptibility to Fas-mediated apoptosis (83). A further explanation for the loss of IL-7Rα expression on CD4+ T cells that is associated with HIV infection is that increased levels of circulating IL-7 result in active down-regulation of CD127 expression on a subset of CD4+ T cells (37). While we have not directly addressed this possibility in the current study, it should be noted that postligand binding down-regulation of a receptor (as occurs for CD127 on IL-7-exposed Tγδ and Tcells) is a relatively transient phenomenon that is conceptually and biologically distinct from the permanent loss of expression of a given receptor that occurs as a result of cellular differentiation (as occurs for CD127 on Tγδ) (37). Consistent with this view are recent reports showing a significant increase in the proportion of CD4+CD127+ and CD8+CD127− T cells in hepatitis C virus infection in absence of any significant change in plasma IL-7 concentration (84). A final and perhaps more likely possibility is that the expansion of CD4+CD127+CD25− T cells reflects, both as a determinant and as a consequence, the HIV-associated chronic immune activation. In this perspective, it is conceivable that the CD4+CD127+CD25− T cells that show features of activated effectors, including production of IFN-γ but not IL-2 (as demonstrated here), could be key promoters of the overall level of immune activation and thus contribute to AIDS pathogenesis. The proportional expansion of these CD4+CD127+CD25− T cells is in turn promoted by the level of immune activation and its detrimental effects on the development of naive T cells in the thymus and the homeostasis of memory T cells in the periphery. Taken as a whole, these results demonstrate that apoptosis and direct HIV infection cannot account for the imbalance of the CD4+ T cell compartment with respect to subsets of cells expressing CD127, highlighting the role of immune activation in driving HIV pathogenesis.

From a clinical point of view, the definition of subsets of CD4+ T cells that are selectively preserved or depleted during HIV infection may provide some additional predictive value for when and how CD4+ T cell depletion leads to full-blown AIDS. Clinicians involved in the management and therapy of HIV-infected individuals have long known that similarly low absolute CD4+ T cell levels result in elevated viral load and a greater risk of opportunistic infections.
counts can be associated with highly variable times to progression to AIDS. In this context, our current findings suggest that the relative expansion of CD4+CD127-CD25+ T cells represents an additional marker of immune dysfunction during HIV infection. Note that CD4+CD127-CD25+ T cells do not include naive or central memory cells, thus suggesting that a relative expansion of these CD4+CD127- cells has virtually no role in protecting from new or recall antigenic challenges. This fact, together with the basic premise that CD4+CD127- T cells are resistant to IL-7-mediated mechanisms of immunological homeostasis, provides a logical explanation for the hypothesis that the relative expansion of CD4+CD127- T cells is an index of CD4+ T cell dysfunction in HIV-infected individuals. As such, measurement of the fraction of CD4+CD127- T cells may complement the information provided by CD4+ T cell counts in assessing the risk of developing AIDS. Consistent with the hypothesis that the relative increase of the CD4+CD127-CD25+ T cell subset is a marker of disease progression during HIV infection, the observation is that, in naturally SIV-infected SMs (in which virus replication is associated with neither immunodeficiency nor generalized immune activation), the level of CD4+CD127-CD25+ T cells is comparable to that observed in uninfected animals. While a larger prospective study is needed to confirm the clinical utility of measuring CD127 and CD25 expression on CD4+ T cells, our current work provides the rationale for further exploring the possibility that monitoring the fraction of specific CD4+ T cell subsets could be helpful to identify patients at high risk to progress to AIDS and who would thus be candidates for more aggressive therapeutic and/or prophylactic regimens.

Finally, the demonstration of a selective increase in the fraction of CD4+ T cells that do not express CD127 provides some indirect hints as to the potential role of IL-7 as an immunological therapy for HIV infection. The limitations of the currently available anti-viral regimens (cost, side effects, inability to eradicate infection), as well as increasing evidence that immune-mediated mechanisms (i.e., chronic immune activation) play an active role in AIDS pathogenesis, provide the rationale for exploring new therapeutic strategies aimed at directly correcting the HIV-induced immune dysfunction. IL-7 appears particularly promising due to its potential ability to increase the survival and expansion of the subsets of mature T cells expressing its high-affinity receptor, CD127 (i.e., naive and central memory). Additionally, IL-7 has the ability to enhance T cell development at the level of bone marrow and thymus, thus leading to increased production of naive T cells (85). The observation that, in both HIV-infected individuals and SIV-infected rhesus macaques, IL-7 treatment results in an expansion of naive and memory CD4+ T cells gives further foundation for this intervention (56, 86–90). In this context, measurement of CD127 expression on CD4+ T cells might prove useful to predict the type of immunological response to IL-7, and thus identify HIV-infected patients who would be most likely to benefit from this treatment.

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References


