Progressive CD127 down-regulation correlates with increased apoptosis of CD8 T cells during chronic HIV-1 infection

Shu-Ye Zhang*1, Zheng Zhang*2, Jun-Liang Fu2, Fu-Biao Kang3, Xiang-Sheng Xu2, Wei-Min Nie3, Chun-Bao Zhou2, Min Zhao3 and Fu-Sheng Wang1,2

1 Center for Infection and Immunity, Institute of Biophysics, Chinese Academy of Science, Beijing, P. R. China
2 Research Center for Biological Therapy, Beijing 302 Hospital, Beijing, P. R. China
3 Department of Infectious Diseases, Beijing 302 Hospital, Beijing, P. R. China

Chronic HIV-1 infection can induce a significant decrease in CD127 expression on CD8 T cells, but the underlying mechanisms and immunological consequences are unclear. In this study, we investigated CD127 expression on CD8 T cells from a total of 51 HIV-1-infected subjects and 16 healthy individuals and analyzed the association between CD127 expression and CD8 T-cell apoptosis in these HIV-1-infected subjects. We found that CD127 expression on total CD8 T cells was significantly down-regulated, which was correlated with the increased CD8 T-cell apoptosis and disease progression of chronic HIV-1 infection. The in vitro addition of IL-7 efficiently rescued the spontaneous apoptosis of CD8 T cells from HIV-1-infected individuals. IL-7 stimulation also transiently down-regulated CD127 expression, whereas some of the CD127− CD8 T cells regained CD127 expression soon after IL-7 was retracted from the incubation medium. Thus, IL-7 stimulation reduced apoptosis of both CD127+ and CD127− CD8 T cells to some degree. These data indicate that CD127 loss might impair IL-7 signaling and increase CD8 T-cell apoptosis during HIV-1 infection. This study, therefore, will extend the notion that IL-7 could be a good candidate for immunotherapy in HIV-1-infected patients.

Key words: Apoptosis · CD127 · CD8 T cells · HIV-1

Introduction

Chronic HIV-1 infection is often associated with progressive CD4 T-cell depletion and functional loss of the immune response against HIV-1. HIV-1 targeting CD4 T cells can directly cause massive CD4 T-cell depletion, but the majority of CD4 and CD8 T-cell death may be induced by bystander apoptosis (or loss), which is defined as a state of overall immune activation [1, 2]. Actually, both CD4 and CD8 T cells have been found to show an increased tendency to undergo spontaneous apoptosis in patients with chronic HIV-1 infection. The increased apoptosis not only results in T-cell depletion, but also leads to the regenerative exhaustion of the lymphoid system [3]. Therefore, immune interventions that aim to reduce apoptosis may mitigate the turnover rate of T-cell depletion.

One such agent under intensive evaluation is IL-7, which is a crucial cytokine involved in the generation, activation, and homeostasis of the T-cell compartment of the immune system [4, 5]. IL-7 is mainly produced by nonhemopoietic stromal cells in multiple organs, and its receptor consists of an α chain

*These authors contributed equally to this work.
(CD127) and a γ chain (γc, the common cytokine receptor, also known as CD132). While CD132 is expressed on the majority of hematopoietic cells, CD127 is exclusively expressed on T cells. IL-7R signaling is required for the homeostatic expansion of naïve and memory T-cell populations [6]. A recent study has shown that in vivo administration of rhIL-7 in humans significantly increases TCR repertoire diversity through the preferential expansion of naïve T-cell subsets, suggesting that IL-7 therapy could enhance and broaden immune responses, particularly in HIV-1-infected individuals with lymphocyte depletion [7].

Plasma IL-7 increases largely during HIV-1 infection, which has been found to be inversely correlated with CD4 T-cell counts and has been interpreted as a homeostatic response to lymphopenia [8]. Recent studies have indicated that IL-7 can promote Fas-induced T-cell apoptosis in nonactivated T cells [9]. At the same time, it can also increase Fas-mediated proliferative signals in suboptimally activated T cells [10], exerting a strong anti-apoptotic effect in vivo on T cells from HIV-1-infected subjects [11]. These data suggest that the increased plasma IL-7 may actively affect peripheral T-cell survival in HIV-1-infected individuals. However, the fact that the peripheral CD4 T-cell count remains low in the absence of anti-viral therapy suggests that IL-7 itself is unable to reverse the loss of CD4 T cells resulting from the effects of HIV viremia. This inability of plasma IL-7 to reverse the loss of CD4 T cells in vivo during the course of HIV-1 infection is still poorly understood. An alternative explanation is that the IL-7/CD127 axis may be impaired during HIV infection [12]. Actually, the IL-7 receptor, CD127, is an essential subunit for IL-7 signaling. In parallel with an increased IL-7 level in plasma, CD127 expression on T cells was significantly down-regulated and correlated with HIV-1 disease progression [12, 13]. Until now, little has been known regarding the underlying mechanisms of CD127 loss and the resulting immunological consequences during HIV infection, in particular, the role of CD127 in T-cell apoptosis in this disease.

In this study, we found that CD127 expression on CD8 T cells was associated with increased apoptosis of CD8 T cells in a cohort of HIV-1-infected individuals. The in vitro addition of IL-7 efficiently reduced the apoptosis of CD8 T cells in HIV-1-infected subjects. These findings not only confirm that the IL-7/CD127 axis regulates CD8 T-cell survival, but they also extend the notion that IL-7 may represent a novel immune therapeutic candidate in the treatment of HIV-1/AIDS.

**Results**

**CD127 down-regulation on CD8 T cells correlates with disease progression during chronic HIV-1 infection**

To define the pattern of CD127 expression on lymphocytes, our pilot experiments showed that CD127 was exclusively expressed on CD3⁺ T cells rather than CD3⁻ lymphocytes (data not shown). Next, we monitored the CD127 percentage on CD4 and CD8 T-cell populations in 51 HIV-1-infected individuals and 16 healthy controls (HC). We found that the CD127 percentage on both CD4 and CD8 T cells was significantly decreased in HIV-1-infected subjects compared with HC (Fig. 1A). In particular, the CD127 percentage on CD8 T cells was reduced by 50% in the HIV-1-infected subjects, compared with that of the HC; in contrast, the CD127 percentage on CD4 T cells was decreased less than 10% in HIV-infected subjects. Therefore, we mainly analyzed the CD127 expression by CD8 T cells and the associations of CD127 expression with CD8 T-cell apoptosis and the disease progression in the study (Fig. 1B).

We found that HIV-1-infected individuals displayed great differences in the CD127 percentage on CD8 T cells (Fig. 1B and C): HIV-1-infected subjects with more than 400 CD4 T-cells/µL exhibited a higher CD127 percentage on CD8 T cells than did those with fewer than 400 CD4 T-cells/µL. The CD127 percentage on CD8 T cells was even less in individuals with fewer than 200 CD4 T-cells/µL (Fig. 1C). The data clearly indicate that chronic HIV-1 infection may lead to progressive CD127 down-regulation on the overall CD8 T-cell population – particularly in patients with low CD4 T-cell counts and high viral loads.

We then compared CD127 percentage among 10 HIV-, 25 CMV-, and 16 influenza virus (Flu)-specific pentamer cells in the HIV-1-infected and HC subjects. It was found that only 20% of the HIV-, 10% of the CMV-, and 40% of the Flu-pentamer cells expressed CD127, indicating that CD127 percentage on these virus-specific CD8 T cells was at a low level during HIV-1 infection (Fig. 1D). More importantly, the CD127 percentage on both the CMV- and Flu-pentamer cells from HIV-1-infected subjects was significantly lower than that from HC subjects, suggesting that HIV-1 infection may cause CD127 down-regulation of the overall CD8 T-cell population rather than of only HIV-1-specific CD8 T cells.

Next, we examined the relationship between the CD127 percentage on CD8 T cells and two markers of HIV-1 disease progression, plasma viral load and the peripheral CD4 T-cell count. Our data indicated that the CD127 percentage on total CD8 T cells was positively correlated with peripheral CD4 T-cell count. Our data indicated that the CD127 percentage on total CD8 T cells was positively correlated with peripheral CD4 T-cell counts (Fig. 1E) but inversely correlated with plasma HIV-1 loads (Fig. 1F). These findings indicate that CD127 down-regulation on CD8 T cells was correlated with the disease progression of chronic HIV-1 infection.

**CD127 down-regulation defines a dysfunctional effector memory T-cell subset in HIV-1-infected individuals**

For this section, we first figured out the distribution of different memory CD8 T-cell pools in HIV-1-infected and HC individuals. According to CD45RA and CCR7 expression, CD8 T cells were generally divided into four memory subsets (Fig. 2A): naïve T (CD45RA⁺CCR7⁻, T naïve) cells, central memory T (CD45RA⁻CCR7⁺, T cm) cells, effector memory T (CD45RA⁻CCR7⁻, T em) cells, and effector T (CD45RA⁺CCR7⁺, T mna) cells. As shown in Fig. 2A, HIV-1-infected subjects retained the higher percentage of T cm (60.4%) and the lower percentage of T naïve (17.4%), in comparison with HC (18.1 and 49.0% for T cm and T naïve cells, respectively). The cellular subset data further

© 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim www.eji-journal.eu
Figure 1. CD127 down-regulation on CD8 T cells correlates with disease progression during chronic HIV-1 infection. (A) Percentage of CD127⁺ CD4 and CD8 T cells from individuals with chronic HIV-1 infection and HC subjects. (B) Representative dot plots of CD127 staining on T cells isolated from HC subjects and HIV-1-infected individuals with different CD4 T-cell counts (cells/µL) and viral loads (copies/mL). CD127 expression was determined by FACS gated on CD3 T cells. Values in the upper-right quadrant represent the percentage of CD8 T cells that express CD127. (C) Progressive loss of CD127⁺ CD8 T cells in HIV-1-infected subjects with different disease stages. n is the number of HIV-1-infected cases. (D) Total data (left) and representative dot plots (right) indicating the percentage CD127⁺ HIV-, CMV-, and Flu-specific CD8 T cells (SL9, NV9, and GL9, respectively) isolated from HC subjects and HIV-1-infected individuals. CD127 expression was determined by FACS gated on CD8 T cells. Values in the upper-right quadrant represent the percentage of virus-specific CD8 T cells that express CD127. (E and F) A significant correlation exists between the levels of CD127 expression on CD8 T cells and (E) peripheral CD4 T-cell counts or (F) plasma HIV-1 loads in HIV-1-infected subjects as evaluated using the Spearman rank correlation test. (A, C, and D) Each symbol represents one individual; horizontal bars represent the median values. Multiple comparisons were made using the Kruskal–Wallis H nonparametric test among the different groups. The Mann–Whitney U test was used to compare data from the HC and HIV-infected groups.
confirmed the observations that CD8 T\textsubscript{em} subsets were largely expanded during chronic HIV-1 infection (Fig. 2B). We further found that HIV-1-infected individuals also exhibited a great difference in the distribution of memory CD8 T-cell populations. HIV-1-infected subjects with fewer than 400 CD4 T-cells/μL displayed a higher percentage of T\textsubscript{em} (median, 70%) and a lower percentage of T\textsubscript{naïve} (<10%), compared with those with more than 400 cells/μL of CD4 T cells (48.7 and 18.6% for T\textsubscript{em} and T\textsubscript{naïve} cells, respectively) (Fig. 2B). This probably indicates that chronic HIV-1 infection led to a progressive decline in T\textsubscript{naïve} subsets but was with a continuous increase in T\textsubscript{em} subsets, which may have disturbed the distribution of the memory CD8 T-cell population.

Notably, we found that CD127 was more dominantly expressed in T\textsubscript{naïve} cells, and the CD127 percentage on the T\textsubscript{naïve}, T\textsubscript{em}, and T\textsubscript{emRA} populations was gradually decreased, regardless of disease status (Fig. 2C). In comparison with the HC, the CD127 percentage was down-regulated significantly on the four CD8 T-cell subsets in HIV-1-infected subjects, especially on T\textsubscript{em} subsets (Fig. 2D). This reduction of CD127 percentage on four CD8 T subsets was even more significant in HIV-1-infected subjects with CD4 T-cell counts smaller than 400 cells/μL; by contrast, patients with more than 400 CD4 T cells/μL exhibited a comparatively higher CD127 percentage on all of the CD8 memory T-cell subsets (Fig. 2D). Considering that CD8 T\textsubscript{em}...
subsets were largely expanded during chronic HIV-1 infection, CD127 loss in this subset might account for the majority of the loss in CD127 expression in HIV-1-infected subjects. Thus, these data indicate that HIV-1 infection led to a progressive CD127 loss, which subsequently defined an expansion of CD127\(^{-}\)CD8 T-cell subsets.

Next, we analyzed the association of CD127 with the differentiation markers (CD45RA, CCR7, and CD27), an activation marker (CD38), pre-apoptotic markers (CD57 and CD95), an exhaustion marker (PD-1), a proliferation marker (Ki67), and a functional marker (perforin) in both HIV-1-infected and HC subjects. As shown in Fig. 2E, CD127\(^{+}\)CD8 T-cell subsets from HCs represent a juvenile phenotype, as indicated by high levels of CCR7, CD45RA, and CD27 expression and low levels of CD57, CD95, PD-1, CD38, perforin, and Ki67 expression. In contrast, CD127\(^{-}\)CD8 T cells from these subjects represent a mature phenotype, as indicated by high levels of CD57 and perforin expression, medium levels of CD45RA and CD95 expression, and low levels of CCR7, CD27, CD45RA, and CD27 expression. Chronic HIV-1-infected subjects also displayed similar marker distribution in CD127\(^{+}\) and CD127\(^{-}\)CD8 T-cell subsets (Fig. 2E).

Notably, HIV-1 infection largely decreased the expression of differentiation markers and increased the expression of maturation markers such as CD38, CD57, CD95, PD-1, Ki67, and perforin among the CD127\(^{+}\)CD8 T-cell population. For the CD127\(^{-}\)CD8 T-cell population, HIV-1 infection only significantly increased CD38 and Ki67 expression and reduced perforin expression (Fig. 2E). This comprehensive analysis suggests that HIV-1 infection not only reduced CD127 expression but also significantly skewed the differentiation and maturation of both the CD127\(^{+}\) and CD127\(^{-}\)CD8 T cells. Particularly, these expanded CD127\(^{-}\)CD8 T-cell subsets displayed a highly activated state, expressing low levels of perforin and high levels of turnover phenotype, which in turn suggest that CD127\(^{-}\)CD8 T-cell subsets are prone to apoptosis in vivo.

CD127 down-regulation associated with increased apoptosis of CD8 T cells

Subsequently, we analyzed the spontaneous apoptosis of CD8 T cells from both HIV-1-infected and HC subjects in vitro during a 24 h incubation period (Fig. 3A). In general, freshly isolated CD8 T cells seldom underwent apoptosis in both HC and HIV-infected subjects. It was found that more CD8 T cells from HIV-infected patients became apoptotic during a prolonged incubation, e.g., more than 25% of CD8 T cells expressed annexin V during the in vitro 24 h incubation. By contrast, CD8 T cells from HC subjects expressed annexin V at a low level, indicating that few cells underwent apoptosis during the 24 h incubation. We further found that more than 30% of the CD8 T cells from HIV-infected individuals underwent spontaneous apoptosis while less than 5% of the CD8 T cells from HC subjects underwent apoptosis after the 24 h incubation in vitro (Fig. 3B). Notably, it was found that the apoptosis of CD8 T cells was significantly inversely correlated with CD127 expression on CD8 T cells in HIV-1-infected individuals (Fig. 3C). Importantly, we observed that CD127\(^{-}\)CD8 cells were clearly more prone to apoptosis than CD127\(^{+}\)CD8 cells.
T cells in HIV-1-infected individuals. According to CD127 expression levels, CD8 T cells were divided into three populations: CD127\textsuperscript{high}, CD127\textsuperscript{medium}, and CD127\textsuperscript{low}. It was clear that dramatically more CD127\textsuperscript{low} CD8 T cells underwent apoptosis than either CD127\textsuperscript{medium} or CD127\textsuperscript{high} CD8 T cells (Fig. 3D and E). These data indicate that CD127 expression on CD8 T cells was closely associated with apoptosis of CD8 T cells in HIV-1-infected subjects.

IL-7 reduces spontaneous apoptosis of CD8 T cells in HIV-infected subjects

We further examined the effect of IL-7 on spontaneous apoptosis of CD8 T cells from HIV-1-infected subjects in vitro and found that more than 40% of the CD8 T cells underwent spontaneous apoptosis during overnight culture, whereas the addition of IL-7 led to a significant reduction of CD8 T-cell apoptosis (Fig. 4A). Pooled data confirmed that IL-7 had a preventative effect on apoptosis of CD8 T cells from HIV-1-infected subjects (Fig. 4B). This effect was dose-dependent (data not shown). We also found that the IL-7-induced rescue of CD8 T-cell apoptosis in vitro could be maintained in a subsequent 5 day culture assay (Fig. 4C). These data strongly indicate that CD8 T cells have a relatively good response to IL-7 treatment in vitro, although HIV-1 infection has led to the marked reduction of CD127 on CD8 T cells.

We have found that CD127–CD8 T cells underwent more apoptosis than CD127\textsuperscript{+} CD8 T cells, and the addition of IL-7 largely reduced the apoptosis of CD8 T cells in vitro. Interestingly, not only can IL-7 reduce CD127\textsuperscript{+} CD8 T-cell apoptosis but it can also slightly decrease apoptosis of CD127\textsuperscript{−} CD8 T cells (although this rescue seemed to occur to a lesser degree than with the CD127\textsuperscript{+} CD8 T cells) (Fig. 4D). Pooled data further confirmed that more than 40% of CD127\textsuperscript{−} CD8 T cells were undergoing apoptosis in HIV-infected subjects, which is 15% more than CD127\textsuperscript{+} CD8 T cells. Addition of IL-7 in vitro could significantly reduce CD127\textsuperscript{−} CD8 T-cell apoptosis by 30%, while reducing CD127\textsuperscript{+} CD8 T-cell apoptosis by 50% (Fig. 4E). These data strongly indicate that IL-7 can reduce apoptosis in vitro not only for the CD127\textsuperscript{+} cells but also for CD127\textsuperscript{−} CD8 T cells in HIV-1-infected subjects.

Transient loss of CD127 on CD8 T cells upon IL-7 stimulation

We further sought the factors that led to CD127\textsuperscript{−} CD8 T-cell-sensing of the IL-7 signals. We found that CD127 expression on CD8 T cells from HC subjects remained at comparatively stable levels during a 24 h period in medium alone (spontaneous/Spo) or medium + IL-7 for 24 h. (C) Pooled data from the experiment described in (A) but after incubation for 5 days; mean and SD (n = 5). (D) Representative and (E) pooled data (n = 6) of the percentages of 7AAD- and annexin V-expressing CD127\textsuperscript{−} and CD127\textsuperscript{+} CD8 T cells isolated from an HIV-1-infected subject. *p<0.05, Wilcoxon matched-pairs T test.
remained at stable levels through 24 h of incubation in the presence of IL-7 in vitro. These data indicate that short-term IL-7 treatment results in only a transient loss of CD127 in a specific pattern and does not induce the differentiation of CD8 T cells.

We further purified peripheral CD127+/CD8 and CD127−/CD8 T-cell subsets from HIV-1-infected patients and serially investigated the CD127 expression on the two subsets in vitro in the absence of IL-7. The representative dot plots indicate that only 10% of the CD127−/CD8 T cells from an HIV-infected subjects regained CD127 expression, and there was no significant loss of CD127 on CD127+/CD8 T cells during the same incubation period (Fig. 5B). Thus, this CD127-regaining capability may, in part, explain the reason why CD127−/CD8 T cells were also responsive to IL-7, although we could not exclude low levels of CD127 molecules on CD8 T cells (levels below the detection limit of the FACS assay method).

Discussion

Increased apoptosis of T lymphocytes is a marked feature during chronic HIV-1 infection and usually correlates with disease progression [3]. It has been reported that the increase in plasma IL-7 might modulate peripheral T-cell survival during HIV-1 infection [11]; however, the relative mechanisms and immunological consequences remain unknown. In this study, we have provided new evidence that the down-regulation of IL-7 receptor (CD127) expression may contribute to increased apoptosis of CD8 T cells during chronic HIV-1 infection.

Previous studies have indicated that CD127 expression on CD8 T cells was significantly decreased during HIV-1 infection [13]. Our study comprehensively analyzed CD127 expression in HIV-1-infected subjects with various disease stages and found that progressive CD127 loss was closely correlated with HIV-1 disease progression. Our data also indicated that CD127 was...
down-regulated in a non-HIV-1-specific manner, since CD127 loss occurred in the overall CD8 T-cell population, including HIV-, CMV-, and Flu-specific CD8 T cells. Interestingly, we found that CD127 expression on CD4 T cells was also significantly decreased during HIV-1 infection. It is of significance to elucidate the influence of CD127 down-regulation on CD4 T-cell survival because CD4 T cells represent the pathologically relevant subset in this disease.

The underlying mechanism of CD127 down-regulation on these virus-specific CD8 T cells is not yet clear; some studies have suggested that CD127 down-regulation might be induced by direct contact with HIV-1 [14] or high levels of IL-7 [15] or that it might be an indirect effect of immune hyper-activation [16] during HIV-1 infection. In this regard, our data suggest that the high levels of immune activation that occur in HIV infection may lead to CD127 down-regulation on all CD8 T cells [16]. We found that the CD127 expression level was closely associated with activation marker CD38 levels on CD8 T cells (data not shown). In addition, persistent HIV-1 antigen stimulation might not be responsible for low CD127 expression on HIV-1-specific T cells because CD127 expression on Flu-specific CD8 T cells was also significantly down-regulated in these HIV-1-infected subjects, in whom Flu virus had indeed been cleared in vivo. Our data also did not support the view that a high level of IL-7 causes CD127 down-regulation, as we found that the addition of IL-7 causes only a transient, impermanent loss of CD127, and CD8 T cells easily regain CD127 expression at a later time. Thus, the mechanisms underlying the down-regulation of CD127 during HIV-1 infection are very complicated and require further study.

The immunological consequences of CD127 down-regulation are still unclear. Previous studies have indicated that CD127 loss alone or in combination with other phenotypes such as CD25, CD27, or CD45RA can serve as novel markers of the memory T-cell population during HIV-1 infection [17–19]. Our study extends these findings by showing that expanded CD127−/CD8 T cells indeed represent activated, pre-apoptotic, dysfunctional, and Tem-like subsets in these HIV-1-infected subjects. In contrast, CD127+/CD8 T cells displayed a high-turnover state, indicating that these cells were being driven to proliferate under the pressure of lymphopenia. More importantly, we found that CD127 down-regulation may be largely responsible for increased CD8 T-cell apoptosis during HIV-1 infection. First, CD127 loss was found to be correlated with increased CD8 T-cell apoptosis. Second, we found that CD127−/low T cells had undergone greater levels of spontaneous apoptosis than CD127+/high T cells in HIV-1-infected individuals. Finally, we found that the addition of IL-7 efficiently inhibited spontaneous apoptosis in CD8 T cells with in vitro incubation. Based on the essential role of CD127 in IL-7 signal transduction, this finding suggests that CD127 is not only a marker for apoptosis but also promotes T-cell survival by conducting the IL-7 signal.

Notably, plasma IL-7 was largely increased during chronic HIV-1 infection, which promoted peripheral T-cell survival in HIV-1-infected individuals to some extent [11]. However, this increased IL-7 per se could not yet reverse the progressive CD4 T-cell depletion during HIV infection. Different mechanisms may account for this inability. There are two possible explanations. The first is that the rate of CD4 T-cell destruction in advanced AIDS patients may exceed the regenerative capacity of IL-7. Alternatively, the pool of progenitor T cells may be irreversibly damaged or depleted. Indeed, the increase seen in plasma IL-7 levels in HIV-infected subjects is far lower than the dose of rIL-7 that was recently administered to humans [7]. The second is that the IL-7/IL-7 receptor axis may be impaired [12]. Our data supported the notion that CD127 expression was progressively lost during chronic HIV infection and might lead to poor responsiveness to IL-7 and increased apoptosis of T cells. Thus, T-cell homeostasis might be influenced by the balance between the increased IL-7 and down-regulated CD127 expression during HIV-1 infection. Future studies should elucidate the differential effects of the decreased CD127 and increased IL-7 on CD8 T cell survival in this disease.

Interestingly, we found that the addition of IL-7 reduced not only the number of CD127− T cells but also CD127−/CD8 T-cell apoptosis to some degree. This result suggests that some CD127−/CD8 T cells have the ability to receive the signal from IL-7. We therefore sought the underlying mechanisms, and although IL-7 transiently down-regulates CD127 expression in vitro, we found that some of the CD127−/CD8 T cells were able to regain CD127 expression soon after IL-7 was retracted from the incubation medium. These data further support a recent report in which IL-7 was said to possibly shed CD127 expression [20]. Alternatively, the recovered CD127 subsets in this study might be identical to newly identified naïve CD8 T cells that can rapidly restore CD127 expression, but the subsets generally display significantly lower levels of CD127 and higher levels of HLA-DR [18]. In addition, it seems very difficult to completely exclude the possibility that CD127 expression on the putative CD127− population was too low to be monitored using flow cytometry or recognized during cell sorting. Thus, it will be of interest to analyze the mechanism through which this small population of CD127− T cells can respond to IL-7.

In conclusion, our data suggest that CD127 expression on the overall CD8 T-cell population is progressively down-regulated. This loss of CD127 might impair IL-7 signaling and lead to an increase in CD8 T-cell apoptosis during HIV-1 infection. To some extent, in vitro addition of IL-7 is able to rescue the CD8 T-cell apoptosis. Therefore, this study supports the hypothesis that the IL-7/CD127 axis regulates CD8 T-cell survival and it extends the notion that IL-7 may serve as a novel candidate for immunotherapy in HIV-1-infected patients.

Materials and methods

Subjects

Fifty-one HIV-1-infected individuals – all of whom were paid blood donors and infected through blood transmission between...
1994 and 1995 – were enrolled in this study. None of these subjects had undergone antiretroviral therapy before sampling. Sixteen HC subjects were also recruited. All participants were antibody-negative to the hepatitis B and C viruses. The basic characteristics of the studied subjects are listed in Table 1. The study protocol was approved by the Ethics Committee of our department, and written informed consent was obtained from each subject. Some HIV-1-infected and HC subjects were serologically identified as having the HLA-A2 genotype.

### Cell isolation

PBMC were isolated from fresh blood samples. For some patients, CD3 T cells were directly obtained from peripheral blood by using a CD3 T-cell isolation kit (Stem Cell Technology, Canada). CD127^+^CD8 and CD127^−^CD8 T cells were then sorted by FacsAria (Becton Dickinson, San Jose, CA, USA). The purity of the CD127^+^CD8 and CD127^−^CD8 T cells was each $\geq 95\%$. Unless otherwise stated, freshly isolated cells were incubated in complete RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 20 mM HEPES, 100 U/mL penicillin, 100 μg/mL streptomycin, and $5 \times 10^{-5}$ M 2-mercaptoethanol.

### Antibodies and reagents

All antibodies were purchased from BD Biosciences (San Jose, CA, USA), except FITC- and PE-conjugated anti-CD127 (eBioscience, San Diego, CA, USA), FITC-conjugated anti-CCR7 (R&D Systems, Minneapolis, MN, USA), and allophycocyanin (APC)-conjugated major histocompatibility complex class I pentamers (ProImmune, Oxford, UK), which are reported to be frequently targeted by HIV-1 (p17 gag; SLYNTVAL, SL9) [21], CMV (pp65 495–503; NLVPMVATV, NV9), or Flu epitope (matrix 58–66; GILGFVFTL, GL9) [22]. Annexin V-FITC was purchased from Biovision (USA); 7-amino-actinomycin (7AAD) was purchased from Jingmei (Beijing, P. R. China). IL-7 and IL-15 were purchased from Peprotec (UK).

### Phenotypic analysis

For evaluation of CD127 expression, PBMC were stained with anti-CD3-FITC, anti-CD8-PerCP, pan-hematopoietic-APC, and anti-CD127-PE or incubated with antibody cocktails containing anti-CD45RA-APC, anti-CCR7-FITC, anti-CD127-PE, and anti-CD8-PerCP. To characterize the phenotypes of CD127^+^CD8 and CD127^−^CD8 T cells, PBMC were stained with anti-CD3-APC, anti-CD8-PerCP, anti-CD127-PE, and FITC-conjugated anti-CD38, anti-CD27, anti-CD28, anti-CD57, anti-CD95, anti-ki67, anti-perforin, and corresponding isotype antibodies, respectively. The cells were stained phenotypically and intracellularly according to our previously described protocols [22, 23]. The cells were then washed, fixed, and analyzed using FACSCalibur (Becton Dickinson) and FlowJo software (Tristar, USA).

### IL-7 treatment and apoptosis assay

PBMC, CD127^+^ and CD127^−^ CD8 T cells were incubated in 96-well plates in culture medium with or without IL-7 (50 ng/mL) for 24 h. Then the cells were harvested at different time points and evaluated for CD127 expression on CD8 T cells. Alternatively, PBMC, CD127^+^, and CD127^−^ CD8 T cells were incubated in 96-well plates in culture medium with or without IL-7 (50 ng/mL) for 5 days. Then the cells were also harvested at different time points and stained with annexin V-FITC and 7AAD to evaluate the effects of IL-7 on the spontaneous apoptosis of CD8 T cells.

### Plasma HIV-1 RNA monitoring

Plasma HIV-1 RNA levels were quantified according to our previously described protocols [21, 23]. The cut-off value was 500 copies/mL.

### Statistical analysis

All data were analyzed using SPSS software (SPSS, Chicago, IL, USA). The Kruskal–Wallis $H$ nonparametric test was performed for multiple comparisons among three or more groups. Statistical differences between the two groups were determined by using the Mann–Whitney nonparametric $U$ test. The data from the same individuals were compared by using the Wilcoxon matched-pairs $T$ test. Correlations between variables were evaluated using the Spearman rank correlation test. For all tests, a $p$ value of less than 0.05 is considered as a significant difference.

### Acknowledgements:

All authors express sincere thanks to all HIV-1-infected individuals and healthy participants in this study. This
Conflict of interest: The authors declare no financial or commercial conflict of interest.

References


Abbreviations: 7AAD: 7-aminoactinomycin · APC: allophycocyanin · HC: healthy control · Flu: influenza virus · T<sub>em</sub>: effector memory T-cell · T<sub>cd</sub>: central memory T-cell · T<sub>na</sub>: naïve T-cell · T<sub>eff</sub>: effector T-cell · T<sub>reg</sub>: regulatory T-cell · 7-AAD: 7-aminoactinomycin D · APC: allophycocyanin