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The investigation of CD4+T-cell functions in primary HIV infection with antiretroviral therapy

Yu Sun, MD, Yajing Fu, PhD, Zining Zhang, PhD, Tian Tang, BS, Jing Liu, MD, Haibo Ding, MD, Xiaoxu Han, PhD, Junjie Xu, PhD, Zhenxing Chu, MD, Hong Shang, PhD*, Yongjun Jiang, PhD*

Abstract

Human immunodeficiency virus (HIV) infection leads to reduced CD4+T-cell counts and immune dysfunction. Initiation of antiretroviral therapy (ART) in HIV primary infection has been recommended to achieve an optimal clinical outcome, but a comprehensive study on restoration of CD4+T-cell function in primary HIV-infected individuals with ART still needs to be eluciated. We investigated longitudinal changes in the CD4+T-cell counts, phenotypes, and functions in HIV-infected individuals with early ART (initiated within 6 months after HIV infection) or later ART (initiated more than 12 months after HIV infection). Patients from early ART and later ART groups had received ART for at least 1 year. Individuals with early ART had more CD4+T cells, a faster rate of CD4+T-cell recovery than those receiving later ART; the levels of CD4+T-cell activation and senescence were lower in early ART compared to those with later ART ($P = .031$; $P = .016$), but the activation was higher than normal controls (NC) ($P = .001$); thymic emigrant function was more upregulated in early ART than in later ART ($P = .015$), but still lower than NC ($P = .027$); proliferative capacity and interferon-γ secretion of CD4+T cells were significantly decreased in primary infection ($P < .001$; $P = .029$), and early ART restored these CD4+T-cell functions, there is no difference with NC, later ART could partially restore the functions of CD4+T cells, but it remained lower than that of NC ($P = .005$; $P = .019$). Early ART could better improve CD4+T-cell function.

Abbreviations: ART = antiretroviral therapy, CFSE = carboxyfluorescein diacetate succinimidyl ester, CHI = chronic HIV infection, HIV = human immunodeficiency virus, HLA-DR = human leukocyte antigen DR, IFN-γ = interferon-γ, IRS = immune reconstitution syndrome, NC = normal control, PBMC = peripheral blood mononuclear cell, PHI = primary HIV infection, RT-PCR = reverse transcriptase polymerase chain reaction, SD = standard deviation, T_{CM} = central memory T cell, T_{EM} = effector memory T cell, T_N = naive T cell.

Keywords: CD4+T-cell function, early ART, HIV infection, later ART

1. Introduction

One feature of human immunodeficiency virus (HIV) infection is the major reduction in CD4+T-cell counts and immune dysfunction.[1,2] Previous studies have revealed that there is an imbalance in the distribution of the subsets of CD4+T cells, the levels of activation and senescence are increased, and the functions of CD4+T cells are impaired.[3–7] Administration of antiretroviral therapy (ART) can significantly reduce the HIV viral loads and restore the CD4+T-cell counts, improving the life expectancy of HIV-infected individuals.[6,8–11] Initiation of ART in HIV primary infection has been recommended to achieve an optimal clinical outcome, but a comprehensive study on CD4+T-cell functions still needs to be conducted. Although there are several studies assessing the recovery of CD4+T-cell counts, much less is known about the restoration of CD4+T-cell function during early ART (initiated within 6 months after HIV infection, determined by Fiebig stages, the last HIV-negative date and high-risk behavior).

The recovery of CD4+T-cell counts is the focus of investigation after ART. Previous studies revealed that CD4+T-cell counts were higher in those participants who initiated ART earlier in comparison with those initiated ART later.[12,13] The number of CD4+T cells is maintained by the balance between their production and destruction, that is, when destruction exceeds production, the CD4+T-cell counts declines.[14,15] Decelerated thymic emigrant function and an accelerated level of senescence may both lead to a reduction in the CD4+T-cell counts.[3,5,15] Thymic emigrant function is evaluated by the level of CD31+ naive T cells ($CD31^+T_N$) and T-cell senescence was characterized by expression of CD57, a surface marker indicating proliferative

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Authors' contribution: YS performed the experiments of CD4+T-cell phenotypes and functions, analyzed, and interpreted the data and wrote the manuscript. YF analyzed and interpreted the data. ZZ and TT performed experiments of CD4+T-cell count. JL and HD performed antiretroviral therapy to patients. XH performed experiments of plasma Viral Loads. JX and ZC collected epidemiological information. HS designed and supervised the study. YJ designed the study, analyzed and interpreted the data, and wrote the manuscript.

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Department of Laboratory Medicine, Key Laboratory of AIDS Immunology of National Health and Family Planning Commission, The First Hospital, China Medical University, Shenyang, China.

* Correspondence: Hong Shang and Yongjun Jiang, Department of Laboratory Medicine, Key Laboratory of AIDS Immunology of National Health and Family Planning Commission, The First Hospital, China Medical University, No 155, Nanjingbei Street, Heping District, Shenyang 110001, Liaoning Province, China (e-mails: hongshang100@hotmail.com [HS] and jiangjun55555@163.com [YJ]).

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history and poor proliferative capacity.[5,16–18] Previous studies indicated thymic emigrant function and the senescence level could not be completely recovered even after ART was initiated in chronic HIV infection (CHI).[18,19] It remains unknown whether thymus dysfunction and/or abnormal senescence can be prevented in early ART. Meanwhile, chronically generalized activation is a major reason for the reduction in the $CD4^+$T-cell counts in HIV infection,[20] and it also influenced the recovery of $CD4^+$T-cell counts in HIV infection with ART.[21–23] The level of activation was reduced in patients receiving ART in CHI,[22,24] while it might remain high level of activation if immune reconstitution syndrome (IRS) happens usually in HIV-infected individuals with low mean $CD4^+$T-cell counts before ART initiation.[3,25] It was reported that early ART was associated with a reduced degree of T-cell activation, but the activation level still remained higher compared to HIV-negative controls.[13,21,26] The kinetic changes in the $CD4^+$T-cell activation level and activation of $CD4^+$T-cell subsets in patients receiving early ART have been less extensively investigated. In addition to the changes in the $CD4^+$T-cell counts, HIV infection causes $CD4^+$T-cell function damage.[7] Previous studies indicated that later ART (initiated more than 12 months after HIV infection) may partially restore $CD4^+$T-cell function,[27,28] but the proliferative function and interferon-γ (IFN-γ) secretory function of $CD4^+$T cells in early ART have rarely been reported.

The aim of this study was to determine whether early ART has a better role in recovery of T-cell functions compared with later ART, and we explored the restoration of $CD4^+$T-cell counts, the reversal of $CD4^+$T-cell abnormal activation, senescence, and dysfunction. Our data indicated that early ART played an important role in restoration of $CD4^+$T-cell functions, but thymic emigrant function cannot be restored.

2. Methods

2.1. Subjects

HIV-infected persons from the First Hospital of China Medical University were recruited and subdivided into 4 groups: primary HIV infection group (PHI, 82 participants) which was defined as persons infected with HIV for less than 6 months without receiving ART; CHI group (101 participants) which was defined as untreated subjects who had been infected with HIV for more than 1 year; early ART group (early ART, 45 participants) which was defined as patients initiated ART within the first 6 months after HIV infection; and later ART group (later ART, 83 participants) which was defined as patients initiated ART more than 12 months after HIV infection. The infection day was estimated by an integrated analysis of fiebig stages (determined by HIV viral ribonucleic acid, HIV specific antibody, and p24 antigen), last negative date and their high-risk behavior. All subjects of this study decided when to initiate therapy by themselves. All patients were treated with regimens including 2 nucleoside reverse transcriptase inhibitors as backbone, and 1 nonnucleoside reverse transcriptase inhibitor was added in 40 patients (88.9%) in early ART and 80 patients (96.4%) in later ART, or 1 protease inhibitors was added in 5 patients (11.1%) in early ART and 3 patients (3.6%) in later ART. At the time of sample collection, patients from early ART and later ART groups had received ART for at least 1 year, with viral loads less than 20 copies/mL. Thirty-one HIV-negative healthy participants were chosen to be the normal controls (NC) (Table 1).

We provided written informed consent to each participant in the study. Our study was approved by the Research and Ethics Committee of The First Hospital of China Medical University. The whole process of the study was conducted according to the principles of the Declaration of Helsinki.

2.2. Antibodies used for flow cytometry

Anti-CD3-PE-cy7, anti-CD45RA-FITC, anti-CD3-FITC/CD8-PE/CD45-PerCP/CD4-APC, and anti-CD38-PE reagents were purchased from BD Biosciences (San Jose, CA). Anti-CD4-APC-cy7, anti-CCR7-PerCP-cy5.5, anti-human leukocyte antigen DR-APC, and anti-IFN-γ-APC reagents were purchased from BD PharMingen (San Diego, CA). Anti-CD31-APC and anti-CD57-PE were purchased from BioLegend (San Diego, CA). Anti-CCR7-APC was purchased from R&D Systems (Minneapolis, MN).

2.3. Measurement of absolute $CD4^+$T-cell counts and plasma viral loads

A single-platform lyse-no-wash procedure was performed with TruCOUNT tubes (BD Biosciences) and anti-CD3-FITC/CD8-PE/CD45-PerCP/CD4-APC reagent (BD Biosciences). $CD4^+$T-cell counts were analyzed by a FACS Calibur flow cytometer (BD Biosciences) and MultiSET software (BD Biosciences, San Jose, CA).

Plasma Viral Loads were detected by reverse transcriptase polymerase chain reaction with the COBAS Amplicor HIV Monitor 1.5 (Roche Molecular Systems, Branchbury, NJ). Undetectable limitation was defined as plasma viral loads <20 copies/mL.

2.4. Immunostainings of T-cell subsets

Fresh whole blood samples were stained using fluorochrome-conjugated antibodies, and we determined the T-cell subsets, defined as naive T cells (T_N, $CD45RA^+CCR7^+$), effector memory

Table 1

Characteristics of participants.

Variable	No. of participant	Male, no., %	Age, mean (rang, SD), y	Viral load at sampling, log_{10} copies/mL, mean (SD)	$CD4^+$T-cell counts at sampling, mean (SD), cells/μL	Length of time receiving ART, mean (SD), mo	Time from infection onset to initiation of ART mean (SD), d
PHI	82	81 (98.8%)	29 (18–56,9.32)	5.12 (0.97)	422.96 (204.02)	–	–
CHI	101	95 (94.06%)	37 (18–73,11.64)	4.61 (0.61)	275.02 (158.02)	–	–
Early ART	45	45 (100%)	32 (18–54,11.1)	<1.3	574.59 (184.45)	20.56 (7.15)	70 (43.02)
Later ART	83	74 (89.16%)	43 (22–76,12.27)	<1.3	388.01 (144.69)	33.72 (15.91)	1593 (965.858)
NC	31	27 (87.10%)	34 (25–65,8.19)	–	758.29 (279.74)	–	–

ART = antiretroviral therapy, CHI = chronic HIV infection, NC = normal controls, PHI, primary HIV infection, SD = standard deviation.

T cells (T_{EM}, $CD45RA^+CCR7^-$), and central memory T cells (T_{CM}, $CD45RA^-CCR7^+$). HLA-DR and CD38 were used to determine the activation of T cells. The phenotypes of T cells were detected by flow cytometry (LSRII; BD Biosciences) and analyzed with BD FACSDiva software (BD Biosciences, San Jose, CA).

2.5. Proliferation assays

Fresh peripheral blood mononuclear cells (PBMC) were labeled with the CellTrace carboxyfluorescein diacetate succinimidyl ester (CFSE) Cell Proliferation Kit (Invitrogen, Carlsbad, CA) at a final concentration of 2 μmol/L in serum-free RPMI 1640 (Hyclone, Logan, UT) for 15 minutes at 37°C in 5% CO_2. Stained cells (2×10^6 cells/mL) were incubated with anti-CD3 (0.5 μg/mL, BD Biosciences) and anti-CD28 (1 μg/mL, BD Biosciences) in R-10 medium (RPMI 1640 supplemented with 10% fetal bovine serum) for 5 days. On day 5, cells were stained with anti-CD3-PE-cy7 and anti-CD4-APC-cy7 and detected by flow cytometry.

2.6. Intracellular cytokine staining

Fresh PBMC (2.5×10^6 cells/mL) were incubated with anti-CD28 (2 μg/mL) and anti-CD3 (1 μg/mL) for 18 hours, then Golgi stop (BD PharMingen) was added and incubated for an additional 6 hours. The cells were stained with anti-CD3-PE-cy7 and anti-CD4-APC-cy7, then permeabilized with Cytofix/Cytoperm (BD PharMingen) for 30 minutes and stained with anti-IFN-γ-APC (BD PharMingen). Cells were detected by flow cytometry.

2.7. Statistical analysis

Statistical analyses were performed using SPSS 17.0. Mann–-Whitney *U* was used to compare between 2 groups of subjects. The Kruskal–Wallis and Mann–Whitney *U* tests were used to compare among the multiple groups of subjects. All tests were 2-tailed, and $P < .05$ was considered significant.

3. Results

3.1. Trajectories of $CD4^+$T-cell counts during early ART and later ART

To investigate whether the recovery of $CD4^+$T-cell counts was better in early ART, we examined the kinetic changes of $CD4^+$T-cell counts both in early ART and later ART. After 30 months of ART, $CD4^+$T-cell counts of all the 78 patients increased from 243 to 413 cells/μL. The elevation in $CD4^+$T-cell counts was most prominent during the first 3 months after ART initiation (mean change 136 cells/μL). After the first 3 months, $CD4^+$T-cell counts became relatively stable (Fig. 1A).

Compared with later ART, $CD4^+$T-cell counts in early ART were always higher (Fig. 1B). Monthly changes of $CD4^+$T-cell count from baseline ($\triangle CD4^+$T/month) in early ART were higher than those in later ART during the 1st month (143 ± 142 vs 82 ± 73 cells/μL, $P = .012$); in addition, at the 3rd and 12th month, $\triangle CD4^+$T/month count in the early ART were significantly higher than those in later ART (57 ± 44 vs 37 ± 24 cells/μL, $P = .011$; 18 ± 12 vs 12 ± 9 cells/μL, $P = .008$) (Fig. 1C).

Furthermore, after 12 months of ART, patients with $CD4^+$T-cell counts change from baseline ($\triangle CD4^+$T) greater than 300 cells/μL, were observed in 33% of the early ART cases but only 9% in those with later ART; in contrast, $\triangle CD4^+$T less than 100 cells/μL were detected in 21% of the early ART cases but almost double that number (38%) when the ART was initiated later (Fig. 1D and E). In summary, individuals with ART that had been initiated in the primary stage had not only a greater $CD4^+$T-cell count, but also a faster rate of $CD4^+$T-cell recovery than those in whom ART started later.

3.2. Early ART restores more $CD4^+T_N$ cells

In addition to cell count, another important factor that contributes to $CD4^+$T-cell functioning is the subset construction. We analyzed the $CD4^+$T-cell subsets in patients receiving either early or later ART. By using CD45RA and CCR7, 3 $CD4^+$T-cell subsets could be identified in human peripheral blood samples: T_N, T_{EM}, and T_{CM} (Fig. 2A). The percentage of $CD4^+T_N$ in early ART was higher than that observed in later ART ($P = .023$), and it was similar to the situation in NC, and the percentage of $CD4^+T_N$ in later ART and CHI were lower than those detected in the NC ($P = .001$; $P = .04$, respectively); in comparison with CHI, $CD4^+T_N$ in PHI was higher ($P = .036$) (Fig. 2B). The percentage of $CD4^+T_{EM}$ in CHI was higher than the corresponding values in PHI and NC ($P = .013$; $P = .003$, respectively), and the percentage of $CD4^+T_{EM}$ in the early ART was not different from the NC; however, the percentage of $CD4^+T_{EM}$ in later ART was higher than that in NC ($P = .003$) (Fig. 2D). The percentage of $CD4^+T_{CM}$ in later ART was higher than the corresponding values in CHI and NC ($P = .003$; $P = .047$, respectively) (Fig. 2C). HIV-infected individuals receiving early ART had higher $CD4^+T_N$ values and lower $CD4^+T_{EM}$ in comparison with later ART.

3.3. Early ART preserves higher thymic emigrant function

The thymus is the major source of $CD4^+T_N$. We studied the thymic emigrant function of those patients receiving either early or later ART, by determining the expression of CD31 on $CD4^+T_N$. In comparison with the NC, the $CD31^+CD4^+T_N$ counts were lower in the following groups: early ART, later ART, PIH, and CHI ($P = .027$; $P < .001$; $P = .005$; $P < .001$, respectively); $CD31^+CD4^+T_N$ counts were higher in PHI when compared with CHI ($P = .038$); $CD31^+CD4^+T_N$ counts in early ART were higher than in later ART ($P = .015$) (Fig. 2E). This indicated that early ART may achieve better thymic emigrant function than later ART, but the improvement was still lower than the value in NC.

3.4. Early ART causes lower activation of $CD4^+$T cells

To investigate the different changes of the activation level of $CD4^+$T cells in patients with early ART compared with later ART, we assessed the kinetic changes in $CD4^+$T-cell activation in these 2 group. During 12 months of ART, the activation ($HLA\text{-}DR^+CD38^+$) of $CD4^+$T was decreased in individuals receiving either early ART or later ART. The decline in activation occurred most prominently during the first 3 months after ART; this was the case in both early and later ART, after that time, the change started to stabilize. Importantly, the activation of $CD4^+$T cells in early ART was always lower than those occurring in later ART (Fig. 3A).

The activation of $CD4^+$T cells was higher in the PHI and CHI groups as compared to NC ($P < .001$; $P < .001$); the $CD4^+$T-cell activation status in early ART and later ART was lower than those in PHI and CHI. (early ART vs PHI: $P < .001$; later ART vs CHI: $P = .001$), but still higher than NC (early ART vs NC: $P = .001$; later ART vs NC: $P < .001$); the level of $CD4^+$T-cell activation was lower in early ART in comparison to the situation in patients receiving later ART ($P = .031$) (Fig. 3B). We further

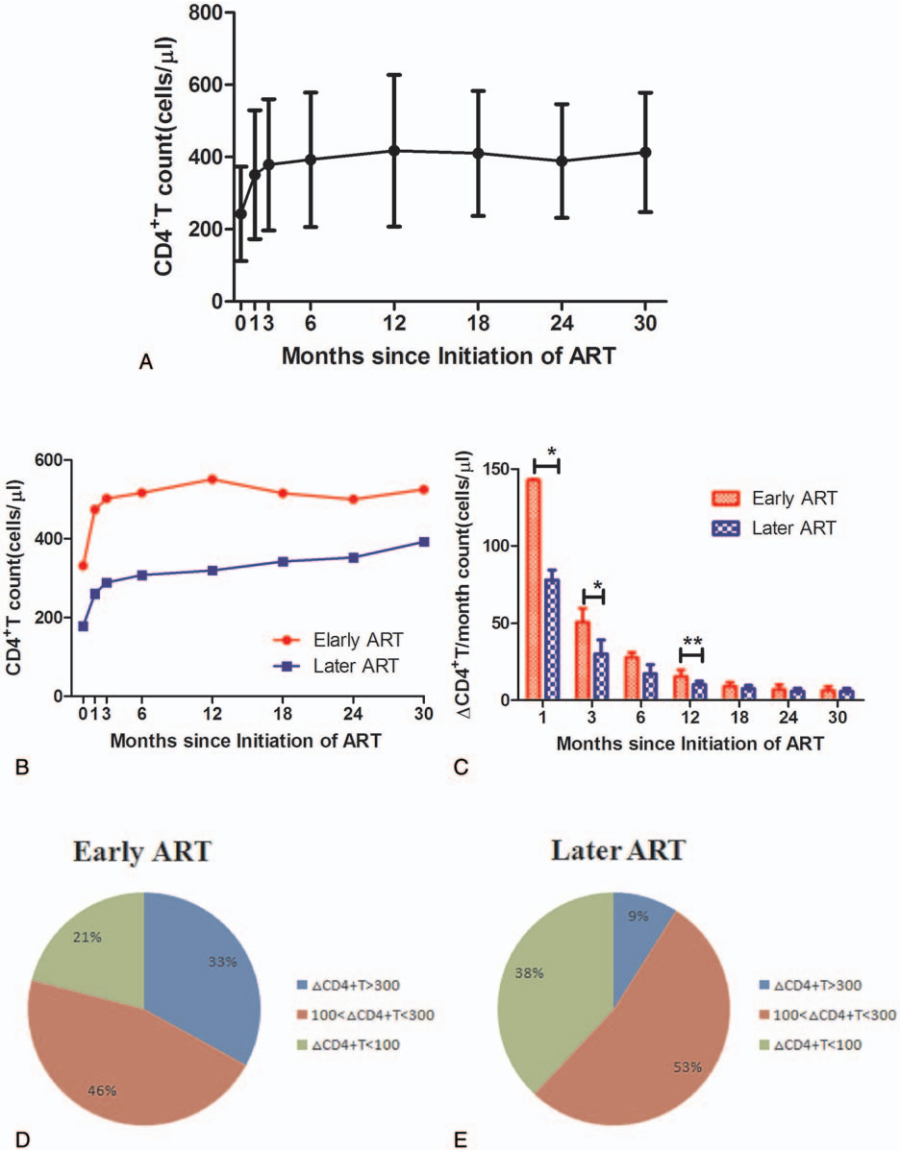

Figure 1. Trajectories of CD4$^+$T-cell counts during early antiretroviral therapy (ART) and later ART. (A) Trajectory of CD4$^+$T-cell counts in patients with ART (n = 78, mean values ± standard deviation (SD) are shown in the figure). (B) Trajectories of CD4$^+$T-cell counts in individuals initiating ART ≤ 6 months of infection (early ART, red, n = 33), or ≥1 years after initial infection (later ART, blue, n = 45, mean values are shown in figure). (C) Monthly changes of CD4$^+$T-cell counts (△CD4$^+$T/month) were compared between early ART (red, n = 33) and later ART (blue, n = 45) (mean values ± SD are shown in figure). Contribution of individuals with different CD4$^+$T-cell changes (△CD4$^+$T) to the overall total after 12 months of early ART (D) or later ART (E). $^*P < .05$, $^{**}P < .01$.

tested the activated levels of CD4$^+$T-cell subsets, and it showed the similar results (Fig. 3C and D). This indicated that individuals receiving early ART had a lower activation level of CD4$^+$T cells than in later ART, but the value remained higher than in NC.

3.5. Reduced senescence of CD4$^+$T cell in early ART

We measured the expression of CD57 molecule to evaluate senescence of CD4$^+$T cells in HIV-infected individuals. The percentage of CD57$^+$CD4$^+$T cell was higher in CHI than in 3 other groups that is, later ART, PHI, and NC ($P = .01$; $P < .001$; $P < .001$, respectively), and the percentages of CD57$^+$CD4$^+$T cell in early ART were lower than those in later ART ($P = .016$) (Fig. 4A). We further tested the senescence of CD4$^+$T-cell subsets, and it showed the similar results (Fig. 4B). Thus, the senescence of CD4$^+$T cell was reduced in patients with early ART in comparison with the later ART group.

3.6. Early ART improves the proliferative capacity of CD4$^+$T cells

In addition to the counts and phenotype of CD4$^+$T cell, we studied whether therapy initiated at different time exerted different effects on the recovery of CD4$^+$T-cell functions. We used CFSE to determine proliferative capacity and used CFSE^{low} to reflect CD4$^+$T-cell proliferation (Fig. 5A). The percentages of proliferating CD4$^+$T cells in PHI group, CHI group, and later ART group were lower than those in NC ($P < .001$; $P = .001$; $P = .005$, respectively), and the percentage of proliferating CD4$^+$T cell was higher in individuals with early ART than in either PHI or later ART ($P = .002$; $P = .037$) (Fig. 5B). It is concluded that proliferative capacity of CD4$^+$T cells was better in early ART in comparison with later ART.

IFN-γ secretion is another important function for some CD4$^+$T cells. In comparison with the NC, the percentages of IFN-γ

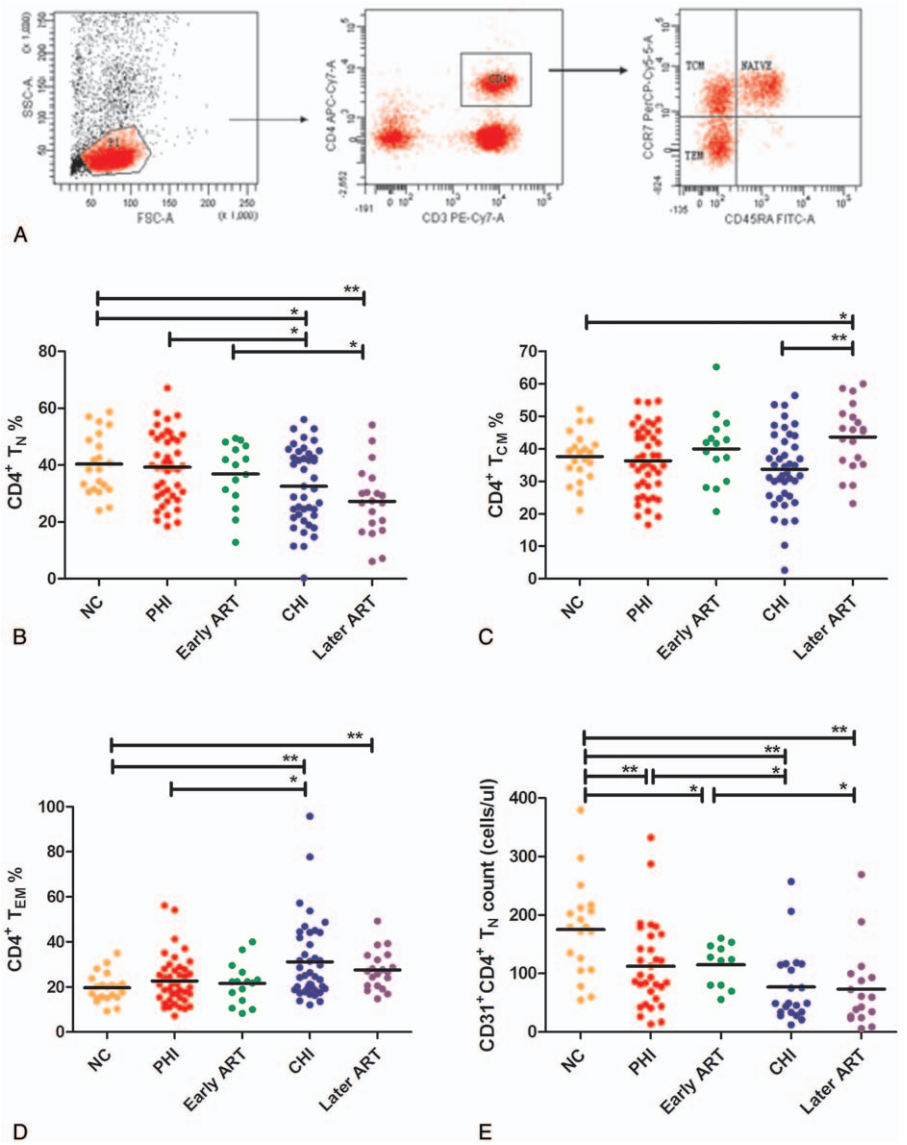

Figure 2. Percentage of $CD4^+$T-cell subsets and $CD31^+CD4^+$ naive T cell (T_N) counts in patients receiving early antiretroviral therapy (ART) or later ART. (A) The gating strategy involved a FS-SS gate for lymphocytes, $CD3^+CD4^+$ gate for $CD4^+$T cells; $CD45RA^+CCR7^+$ gate for naive $CD4^+$T cell (T_N); $CD45RA^-CCR7^+$ and $CD45RA^-CCR7^-$ gates for central memory and effector memory T cells. The percentages of $CD4^+$T subsets naive (B), central memory T cell (C), and effector memory T cell (D) were detected by flow cytometry (LSRII; BD Biosciences) and analyzed with BD FACSDiva software. The data are presented for all 5 groups: HIV-negative normal controls (NC, yellow, n = 21), primary HIV infections (PHI, red, n = 43), early ART (early ART, green, n = 15), chronic HIV infections (CHI, blue, n = 42), and later ART (later ART, purple, n = 20). Thymic emigrant function was determined by measuring absolute count of $CD31^+CD4^+T_N$ (E) in NC (n = 19), PHI (n = 31), early ART (n = 11), CHI (n = 21), and later ART (n = 17). Horizontal lines represent mean values. $^*P < .05$, $^{**}P < .01$.

secreting $CD4^+$T cells were lower in the following groups: PHI group, CHI group, and later ART group ($P = .029$; $P = .023$; $P = .019$, respectively), and the percentage of IFN-γ secreting $CD4^+$T was higher in early ART when compared with PHI ($P = .038$), but not significantly different from the NC (Fig. 6A and B). It is concluded that the secretory capacity of $CD4^+$T cells in the early ART group was better than those in later ART.

4. Discussion

The decline of $CD4^+$T-cell counts is the most prominent and representative feature of HIV infection and an elevated $CD4^+$T-cell counts is known to be a critical factor in immune recovery.[12,29,30] Our data showed that $CD4^+$T-cell counts in patients receiving early ART were always higher than those in whom ART was initiated later. This is consistent with recent studies,[1,31–33] but we further found early ART may restore more percentage of T_N than later ART, quite different from the study of Amu et al[34], which showed a similar level of $CD4^+$T-cell counts and the percentage of T_N between early and later ART. The reason for these differences between the 2 studies is possibly due to the $CD4^+$T-cell counts at initiation of later ART is higher than that in early ART in Amu et al study.

The restoration of $CD4^+$T-cell counts may be quite related with initial $CD4^+$T-cell counts. In order to remove this bias, we detected the monthly changes of $CD4^+$T-cell counts ($\triangle CD4^+T$/month) and $CD4^+$T-cell growth after 12 months of ART ($\triangle CD4^+T$). As far as we are aware, this type of calculation has not been previously reported in early ART. We found that $\triangle CD4^+T$/month and $\triangle CD4^+T$ were also higher in patients with

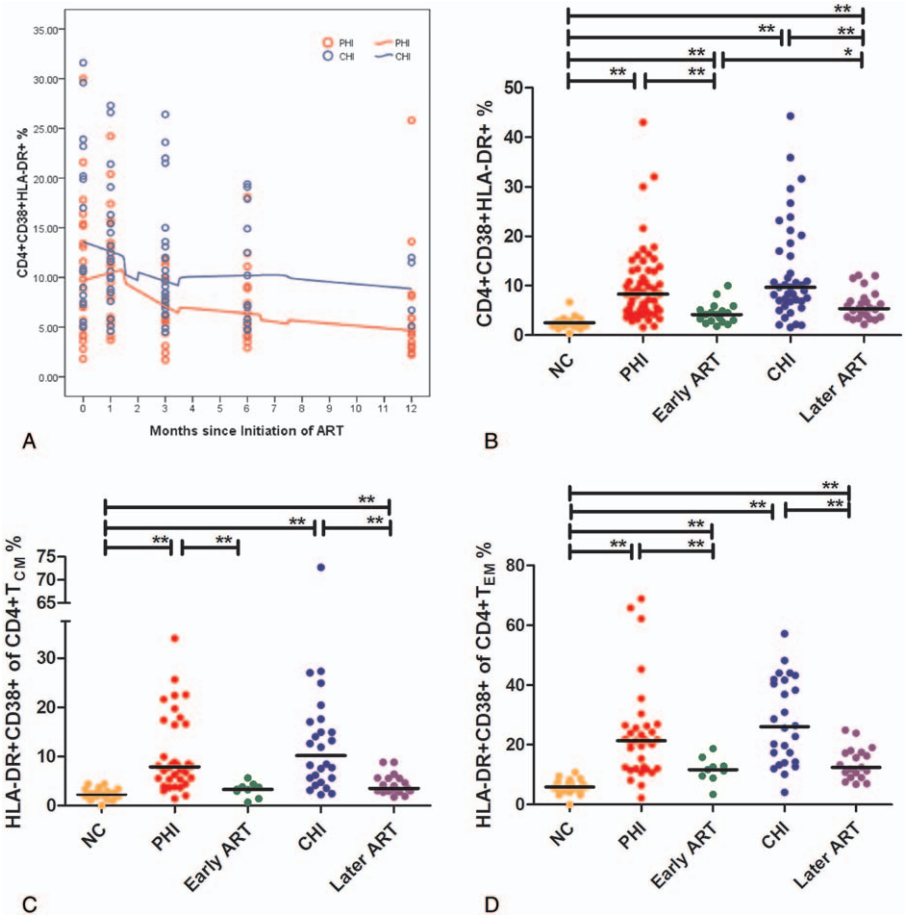

Figure 3. CD4⁺T-cell activation status with early antiretroviral therapy (ART) or later ART. Activation of CD4⁺T cell was evaluated by HLA-DR and CD38. (A) Trajectory of activation of CD4⁺T cell in patients with early ART (red, n=24) or later ART (blue, n=25). We evaluated the activation level at baseline (0), 3, 6, 12 months since initiation of ART. (B) Activation status was determined in total CD4⁺T cells (normal controls [NC], n=21; primary HIV infection (PHI), n=55; early ART, n=18; chronic HIV infection (CHI), n=40; Later ART, n=25). The level of activation was evaluated in CD4⁺ central memory T cell (C) and CD4⁺ effector memory T cell (D) (NC, n=21; PHI, n=34; early ART, n=9; CHI, n=26; Later ART, n=19). Horizontal lines represent mean values. $^{*}P<.05$, $^{**}P<.01$.

early ART than in those receiving later ART. This indicated that early ART was more efficient than later ART in promoting the recovery of the CD4⁺T-cell counts. PHI was regarded as "restorative time window," that is, a time when the immune system could be strategically poised for recovery.[12,35,36] Therefore, it represents a good opportunity for initiating ART to improve the chances that the immune system can optimally be restored.

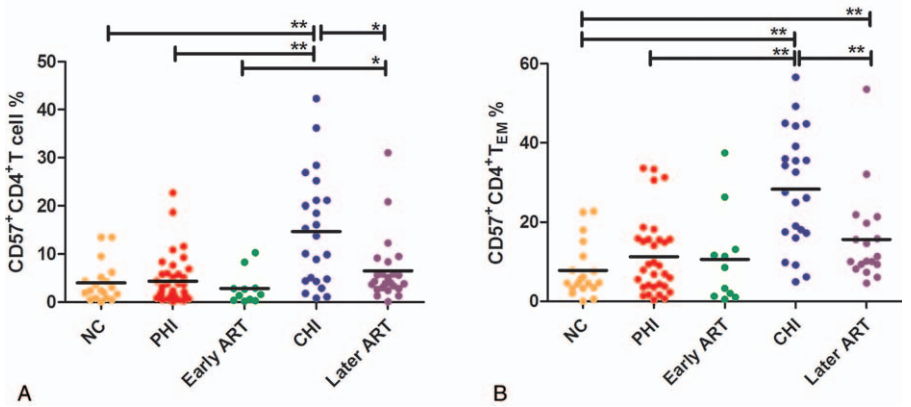

Figure 4. CD4⁺T-cell senescence status in patients receiving either early antiretroviral therapy (ART) or later ART. CD57 was used to characterize senescent status of the CD4⁺T cells. Percentages of CD57 were determined in CD4⁺T cells (A) (normal controls [NC], n=19; primary HIV infection [PHI], n=39; Early ART, n=11; chronic HIV infection (CHI), n=23; Later ART, n=24) and CD4⁺ effector memory T cell (B) (NC, n=19; PHI, n=32; Early ART, n=11; CHI, n=23; Later ART, n=18). Horizontal lines represent mean values. $^{*}P<.05$, $^{**}P<.01$.

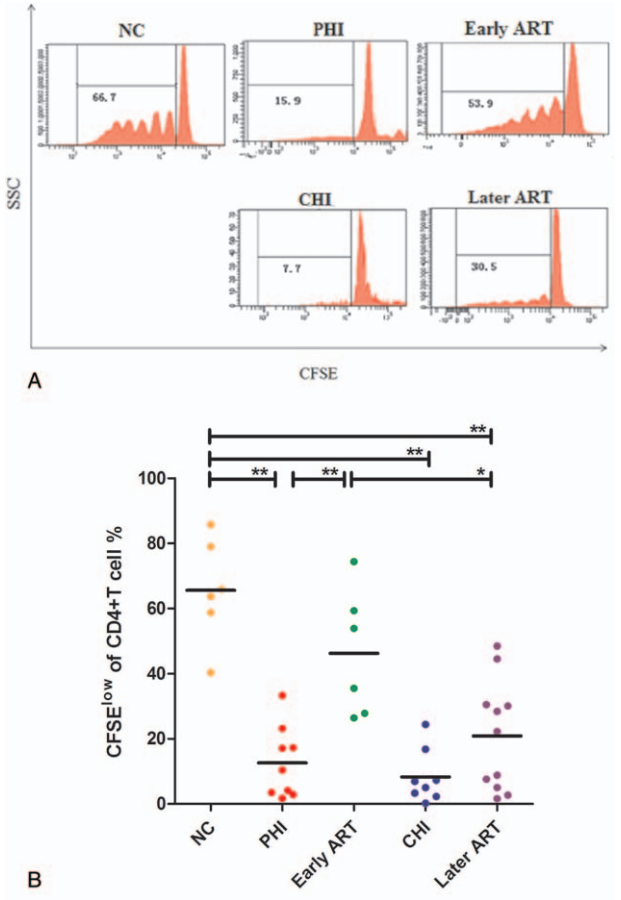

Figure 5. Proliferation capacity of CD4$^+$T cells. Carboxyfluorescein diacetate succinimidyl ester (CFSE) was used to determine the proliferation capacity of CD4$^+$T cells. (A) Representative flow cytometric plots showed the proliferative capacity of CD4$^+$T cells in normal controls (NC), primary HIV infection (PHI), early antiretroviral therapy (ART), chronic HIV infection (CHI), and later ART. Peripheral blood mononuclear cells stained with CFSE were incubated with anti-CD3 and anti-CD28 for 5 days. The CFSE^{low} gate was used to determine proliferative CD4$^+$T cells. (B) The percentages of proliferative CD4$^+$T cells in 5 groups. (NC, n=6; PHI, n=9; Early ART, n=6; CHI, n=8; Later ART, n=11). Horizontal lines represent mean values. $^*P<.05$, $^{**}P<.01$.

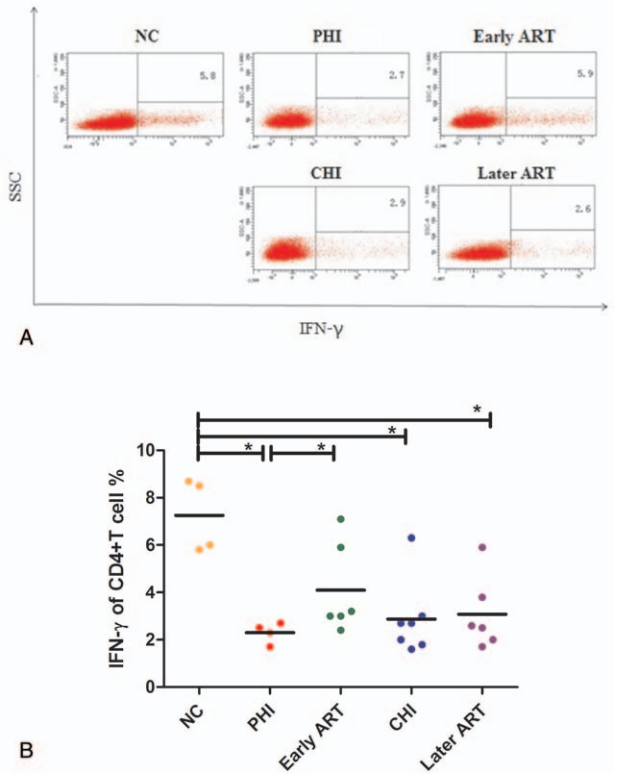

Figure 6. Cytokine secretion of CD4$^+$T cell. (A) Representative flow cytometric plots illustration the interferon-γ (IFN-γ) secretion in CD4$^+$T cells between normal controls (NC), primary HIV infection (PHI), early antiretroviral therapy (ART), chronic HIV infection (CHI) and Later ART. (B) The percentages of IFN-γ secretion in CD4$^+$T cells in 5 groups (NC, n=4; PHI, n=4; Early ART, n=6; CHI, n=7; Later ART, n=6). Horizontal lines represent mean values. $^*P<.05$, $^{**}P<.01$.

Part of the reason for the recovery of CD4$^+$T-cell counts may be due to thymic emigrants function. The thymus represents the major site of the production and generation of CD4$^+$T cells, and thus its function affects the number and constitution of CD4$^+$T cells during HIV infection.[5] For example, decreased thymic function might result in the failure to restore circulating CD4$^+$T cells after the control of HIV replication with ART. Our data showed that HIV-infected patients with early ART had better thymic emigrant function than those with later ART. Nonetheless, the early ART patients still exhibited lower thymic emigrant function than NC, and in fact, there was no difference in this property between primary/CHI with or without therapy. We conclude that thymic emigrant function becomes damaged during the primary stage of HIV infection, and once damaged, it is difficult to be restored. Therefore, ideally HIV-infected individuals should be identified and encouraged to initiate ART as soon as possible, in order to avoid the destruction of thymic function.

HIV infection evoked a generalized activation of the immune system, and a higher level of T-cell activation has been associated with a reduced recovery of the CD4$^+$T-cell counts, increased mortality, and cardiovascular diseases.[16,21–23,37–40] Abnormal activation can increase HIV replication promote cell apoptosis, accelerate cell senescence, and cellular exhaustion, and it has also been associated with the HIV reservoir size.[41–43] We observed that although the activation of CD4$^+$T cells was less intensive in individuals with early ART than in those receiving later ART, it still remained elevated when compared with NC. It is consistent with previous studies that is, early ART may help reduce the level of T-cell activation.[13,21] We further tested the activation of CD4$^+$T-cell subsets; it was observed that the CD4$^+$T$_{CM}$ activation level was lower in early ART compared with later ART. As it is known that CD4$^+$T$_{CM}$ is an important component of the viral reservoir,[35] 1 could speculate that the lower degree of activation may have decreased the HIV reservoir size by impairing HIV-1 DNA replication. We also found higher frequency and activation of CD4$^+$T$_{EM}$ in patients with later ART. A possible reason for those elevation could be attributed to IRS, which usually takes place in chronic HIV-infected individuals with CD4$^+$T-cell lymphopenia before ART initiation. After the restoration of CD4$^+$T cells, bacterial and viral infections may result in the expansion of the effector memory T cells and lead to remarkable T-cell activation.

Immune activation and chronic inflammatory are thought to drive T-cell senescence.[2,44,45] The expression of CD57 is frequently used as a biological marker of T-cell senescence.[46,47] Previous studies revealed that the senescence of CD8$^+$T cell can be reversed with early ART initiation.[16,48] We observed less

senescence of CD4+T cell in early ART compared with later ART; furthermore, the data showed that there was no significant difference in the level of senescence of the CD4+T cells between primary infection with/without ART and NC. Our data also indicated that the percentage of senescent CD4+T cells was higher in patients with CHI, became decreased in later ART, but still remained higher in comparison with early ART. In summary, although later ART can reduce CD4+T-cell senescence, early ART is more efficacious in this respect.

In addition to the improvement of CD4+T-cell counts and phenotypes, a recovery of immune functions is also very important. Our results revealed the impaired proliferation capacity and reduced IFN-γ secretion of CD4+T cells in HIV-infected patients; early ART restored these CD4+T-cell functions, but if the ART was provided later, then there was only a partial recovery of the functions of CD4+T cells, that is, it still remained lower than that of NC. The restored proliferative capacity is related to the recovery of CD4+T-cell counts and IFN-γ secretion play an important role as it possesses antiviral, antitumor, and antimicrobial properties. We conclude that HIV-infected individuals should receive ART during the primary phase of the infection to promote the recovery of immune functions.

Our study has some limitations. First, initial CD4+T-cell counts were not matched between early ART and later ART, because it was difficult to find higher initial CD4+T-cell counts in later ART. Second, because of the limitation of blood collection at 1 time, we examined a relatively small number of cases in the experiments assessing CD4+T-cell functions. Third, our study was not a randomized clinical trial, the subjects decided for themselves whether and when to initiate ART.

5. Conclusion

Our data demonstrated that early ART confers not only quantitative but also qualitative benefits for CD4+T-cell reconstitution. The greater CD4+T counts, faster rate of CD4+T-cell recovery, lower immune activation and immune cell senescence, better proliferative capacity and IFN-γ secretion of CD4+T cells and restored thymic emigrants function were all associated with early ART and it was superior to later ART. Therefore, initiation of early ART could significantly promote immune functions of CD4+T cells.

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