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CASE REPORT

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Short- and long-term effects of antiretroviral therapy on peripheral regulatory CD4+/CD25hi/CD127low T lymphocytes in people living with HIV/AIDS

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ABSTRACT

The effect of antiretroviral therapy (ART) on CD4+/CD25hi/CD127low T lymphocyte changes in people living with HIV/AIDS (PLWHA) is still a matter of debate. From October 2015 to December 2019, peripheral blood from 70 cases of PLWHA were collected for the detection of CD4+/CD25hi/CD127low T lymphocytes by flow cytometry. Statistical analysis was performed to detect changes of CD4+/CD25hi/CD127low T lymphocytes in patients with different duration of ART and different treatment effects. We found that the number of CD4+/CD25hi/CD127low T lymphocytes in ART-naive PLWHA were lower than those in healthy volunteers (10.3±6.0 cells/uL vs 31.7±8.0 cells/uL, $P < 0.05$). CD4+/CD25hi/CD127low T lymphocyte counts increased to 17.8±4.0 cells/uL 6 months post-ART and 25.0±11.9 cells/uL 9 months post-ART, respectively ($P < 0.05$). There was no significant difference in CD4+/CD25hi/CD127low T lymphocyte counts between PLWHA who reached a complete immune reconstruction after ART and healthy volunteers. The growth of CD4+/CD25hi/CD127low T lymphocyte counts in patients who had baseline CD4 > 200 cells/uL was greater than those who had baseline CD4 ≤ 200 cells/uL (12.6±4.6 cells/uL vs 5.6±5.0 cells/uL, $P = 0.027$). CD4+/CD25hi/CD127low T lymphocyte counts were positively correlated with CD4+ T lymphocyte counts ($r = 0.923$, $P < 0.001$) and CD4+/CD8+ ratio ($r = 0.741$, $P < 0.001$), but were negatively correlated with HIV-VL ($r = –0.648$, $P = 0.000$). In conclusion, the results of the present study showed that changes in CD4+/CD25hi/CD127low T lymphocyte counts can be used to assess the effect of ART in PLWHA.

KEYWORDS: HIV. AIDS. CD4+/CD25hi/CD127low T lymphocyte. ART.

INTRODUCTION

The epidemic of Acquired immunodeficiency syndrome (AIDS) caused by the human immunodeficiency virus (HIV) infection is a serious global public health issue. HIV infection causes depletion of CD4+ T lymphocytes, leading to immune deficiency and a series of clinical manifestations of opportunistic infections and even death[1]. Combined antiretroviral therapy (cART) has been proven to be an effective way to recover CD4+ T lymphocytes and improve the life expectancy of people living with HIV/AIDS (PLWHA).

Regulatory T (Treg) cells are a subset of T lymphocytes with immunosuppressive actions that play an important role in the regulation of autoimmunity and immunity[2]. Tregs are crucial to prevent immune dysregulation, and the role of Tregs in disease progression among people living with HIV/AIDS (PLWHA) is complex[3-8]. However,

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little is known about the correlation between Tregs changes trends and the therapeutic effect after cART.

As Tregs constitutively express CD25 at high levels and also express low levels of IL-7 receptor a-chain (CD127)[9], $CD4^+/CD25^{hi}/CD127^{low}$ were selected as the markers of Tregs in this study. The short- or long-term effects of cART on $CD4^+/CD25^{hi}/CD127^{low}$ Tregs and the correlations between $CD4^+/CD25^{hi}/CD127^{low}$ Tregs and therapeutic effect after cART were analyzed.

MATERIALS AND METHODS

Study population

This was an observational study including 70 cases of PLWHA and 30 healthy volunteers recruited in the Zhongnan Hospital of Wuhan University, from October 2015 to December 2019.

The recruitment criteria for HIV/AIDS participants were as follows: (1) confirmed HIV infection; (2) ART-naive; (3) no previous opportunistic infection or the opportunistic infection was effectively controlled; and (4) adherence to a regular monitoring in Zhongnan Hospital of Wuhan University.

All healthy volunteers were negative for anti-HIV antibodies. After completing a questionnaire including the present health status and previous medical conditions, patients and controls underwent a physical examination. Necessarily absent medical conditions were: malignant tumor, cardiovascular and cerebrovascular diseases, chronic liver or kidney diseases and uncontrolled infectious diseases or uncontrolled life-threatening diseases.

This study was approved by the Institutional Ethics Committee of the Zhongnan Hospital of Wuhan University. All patients signed the informed consent form.

Study protocol

Peripheral blood from the participants were collected for detection of T lymphocytes, including $CD4^+/CD25^{hi}/CD127^{low}$ regulatory T lymphocytes, $CD3^+$ T lymphocyte, $CD4^+$ T lymphocyte and $CD8^+$ T lymphocyte, through flow cytometry and HIV RNA viral load through polymerase chain reaction (PCR).

Before ART was initiated, the level of $CD4^+/CD25^{hi}/CD127^{low}$ regulatory T lymphocytes in PLWHA was compared with those of healthy volunteers. In addition, the correlation between $CD4^+/CD25^{hi}/CD127^{low}$ regulatory T lymphocytes and $CD4^+$ T lymphocyte or CD4/CD8 ratio or HIV viral load were analyzed.

The level of $CD4^+/CD25^{hi}/CD127^{low}$ regulatory T lymphocytes was monitored 6 months, 9 months and 5 years post-ART. The results obtained 6 and 9 months post-ART were considered as short-term changes, while the 5 years post-ART results were considered as the long-term changes.

Flow cytometry analysis

K3 EDTA vials were used to collect whole blood samples from the patients. According to manufacturer's protocol, 20 μL of mouse anti-human CD4-FITC, CD8-PE and CD3-PerCP antibodies were added to the absolute counter tube. Then, 50 μL of whole blood were added followed by staining for 15 min at room temperature, in the dark. After staining, 450 μL of 1 X hemolysin were added and incubated for 10 min at room temperature, in the dark. The absolute count of $CD3^+$ T lymphocyte, $CD4^+$ T lymphocyte and $CD8^+$ T lymphocyte were determined by flow cytometry using FACSCalibur (Becton Dickinson and Company, New York, USA). Likewise, 20 μL of mouse anti-human CD4-FITC, 20 μL of anti-CD25-PE, 5 μL of anti-CD127-PreCP-Cy5.5 and 50 μL of whole blood were added into the absolute counter tube and mixed. After the staining and lysing process mentioned above, the $CD3^+$ T lymphocyte, $CD4^+$ T lymphocyte, $CD8^+$ T lymphocyte and $CD4^+/CD25^{hi}/CD127^{low}$ T lymphocyte absolute count were automatically calculated using Calibrite microspheres, the BD FACSComp™ 2.0 software and the TreeStar Flow-Jo software version 8.8.7 (Becton Dickinson and Company, New York, USA) after acquiring more than 100,000 lymphocyte signals by Flow analyzer. Figure 1 shows a=the representative plot of flow cytometry gating.

Other laboratory measurements

HIV viral load was determined using the NucliSens Easy Q HIV-1 version 2.0 kit (bioMérieux, Lyon, France), with a limit of detection of 20 copies/mL. HBsAg, anti-HCV and specific syphilis antibody were tested with a third-generation enzyme immunoassay (EIA) (Shanghai Kehua Biology Company, China).

Statistical analysis

The SPSS statistical software version 23.0 (IBM, Armonk, USA) was used to analyze the data. Frequency rates and percentages were used to describe categorical variables, and χ^2 analysis was conducted to examine the categorical variables. The continuous data comparisons were expressed as mean ± standard deviation (SD). Paired testing was used to compare the difference when there was a follow-up. When the data were not normally distributed,

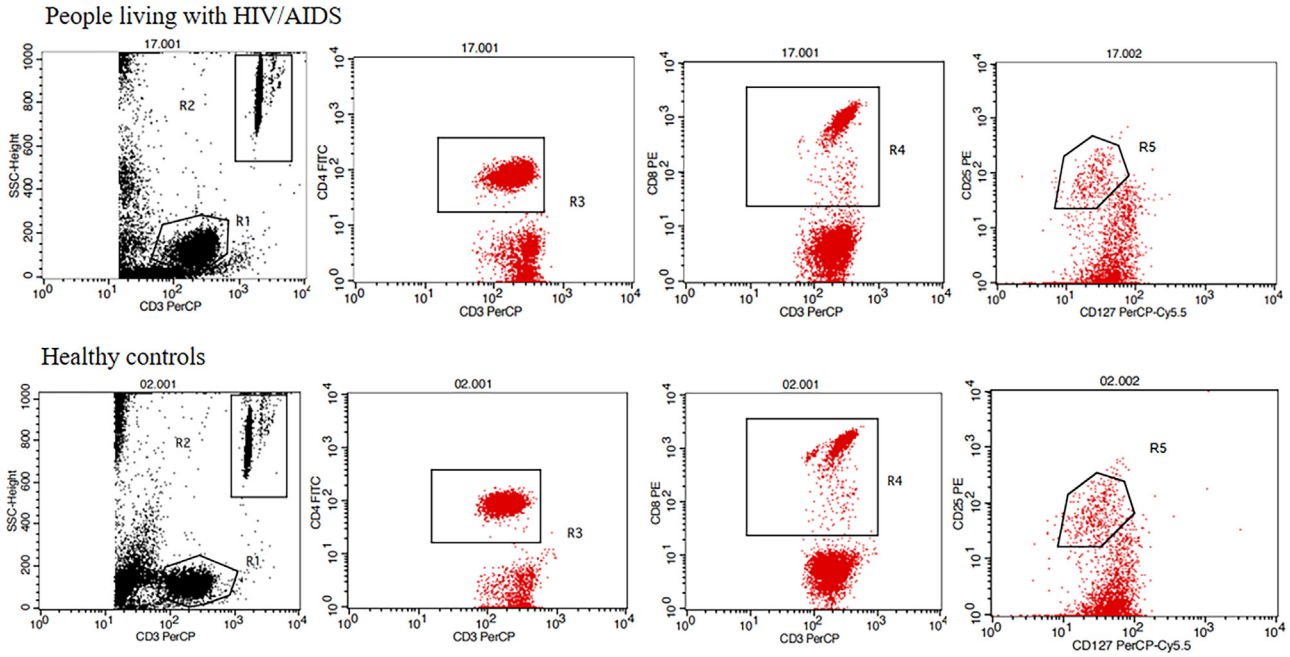

Figure 1 - Representative plot to show the flow cytometry gating (R1: CD3+T lymphocyte; R3: CD4+ T lymphocyte; R4: CD8+ T lymphocyte; R5: CD4+/CD25hi/CD127low T lymphocyte).

non-parametric statistics were used. When analyzing the correlation between CD4+/CD25hi/CD127low regulatory T lymphocytes and CD4+ T lymphocyte counts or HIV viral load, the Spearman's correlation test was used. A *P* value < 0.05 was considered as the level of significance.

RESULTS

Baseline characteristics

Seventy cases of ART-naive people living with HIV/AIDS and 30 cases of healthy blood donors were analyzed in this study. The mean age was comparable in the two groups (31.7±5.6 vs 30.2±9.8 years, P = 0.388). While the proportions of HBV and HCV coinfections were not statistical different, the syphilis coinfection rate was higher in the ART-naïve group in comparison with healthy blood donors (*P*= 0.006). The result was shown in Table 1.

Effect of HIV infection on CD4+/CD25hi/CD127low T lymphocyte counts

The results of lymphocyte subsets were compared between ART-naive PLWHA and healthy blood donors

Table 1 - Effect of HIV infection on CD4+/CD25hi/CD127low T lymphocyte counts.

Variable	ART-naive PLWHA (n=70)	Healthy controls (n=30)	*Test*	*P*
Age in years, mean (SD)	31.7±(5.6)	30.2±(9.8)	0.869	0.388
CD4+/CD25hi/CD127low T	10.3±6.0	31.7±8.0	54.415	0.000
CD4+/CD25hi/CD127low T/CD3(%)	0.9±0.5	2.4±0.8	35.727	0.000
CD3+ T lymphocyte counts (cells/uL)	1,017.2±376.2	1,377.4±310.5	10.928	0.001
CD4+ T lymphocyte counts (cells/uL)	205.7±111.9	685.9±172.6	64.695	0.000
CD8+ T lymphocyte counts (cells/uL)	722.8±315.9	551.1±186.4	5.537	0.015
CD4/CD8	0.3±0.2	1.3±0.4	68.742	0.000
HBV coinfection	7 (10.0)	2 (6.7)	0.285	0.594
HCV coinfection	0 (0)	0 (0)	---	---
Syphilis coinfection	15 (21.4)	0 (0)	7.563	0.006*

PLWHA = People living with HIV/AIDS.

(Table 1). In comparison with health blood donors, the counts of $CD4^+/CD25^{hi}/CD127^{low}$ T lymphocyte and the proportion of $CD4^+/CD25^{hi}/CD127^{low}$ T lymphocyte/CD3 in ART-naive PLWHA were lower (10.29±5.99 vs 31.70±8.02, $P<0.001$; 0.94±0.45 vs 2.44±0.75, $P<0.001$). In ART-naive PLWHA, $CD3^+$, $CD4^+$ T lymphocyte counts and CD4/CD8 ratio were lower compared to health blood donors. However, $CD8^+$ T lymphocyte counts in ART-naive PLWHA were higher compared to health blood donors (722.75±315.86 vs 551.07±186.38, $P= 0.015$). Syphilis coinfection was more common in PLWHA than in healthy blood donors ($P= 0.006$).

Changes of lymphocyte subsets after short-term ART

ART-naive PLWHA in this study were followed-up after the initiation of ART and their lymphocyte subsets counts were monitored (Table 2). Compared to the baseline levels before ART, the $CD4^+/CD25^{hi}/CD127^{low}$ T lymphocyte counts increased from 10.3±6.0 cells/uL to 17.8±4.0 cells/uL 6 months post-ART, and further increased to 25.0±12.0 cells/uL 9 months post-ART ($P= 0.026$). The proportion of $CD4^+/CD25^{hi}/CD127^{low}$ T lymphocyte/CD3 increased from 0.9±0.5 cells/uL to 1.2±0.3 cells/uL 6 months post-ART, and further increased to 1.7±0.6 cells/uL 9 months post-ART ($P= 0.029$). After 6 months and 9 months of ART, $CD4^+$ T lymphocyte counts ($P= 0.002$) and CD4/CD8 ratio ($P= 0.026$) gradually increased with extension of ART duration. However, as for $CD3^+$ and $CD8^+$ T lymphocyte counts, there was no statistical significant difference between groups with different ART duration.

Short- and long-term changes of $CD4^+/CD25^{hi}/CD127^{low}$ T lymphocytes after ART

In ART-naive, 9 months post-ART, 5 years post-ART and in healthy blood donors, the $CD4^+/CD25^{hi}/CD127^{low}$ T lymphocyte counts were 10.3±6.0, 25.0±11.9, 31.4±9.2 and 31.7±8.0 cells/uL, respectively; and the proportion of $CD4^+/CD25^{hi}/CD127^{low}$ T lymphocyte/CD3 were 0.9±0.5, 1.7±0.6, 2.5±0.7 and 2.4±0.8, respectively. The statistical analyses are shown in Figure 2. It showed that $CD4^+/CD25^{hi}/CD127^{low}$ T lymphocyte counts and the proportion of $CD4^+/CD25^{hi}/CD127^{low}$ T lymphocyte/CD3 were significantly increased after ART for the evaluation performed 9 months and 5 years post-ART. Nevertheless, the $CD4^+/CD25^{hi}/CD127^{low}$ T lymphocyte counts and the proportion of $CD4^+/CD25^{hi}/CD127^{low}$ T lymphocyte/CD3 in the 9 months post-ART group were still lower than those found in healthy blood donors. Excitingly, as for $CD4^+/CD25^{hi}/CD127^{low}$ T lymphocyte counts and the proportion of $CD4^+/CD25^{hi}/CD127^{low}$ T lymphocyte/CD3, there was no statistical difference between the 5 years post-ART group and healthy blood donors.

The effect of baseline $CD4^+$ T lymphocyte levels of ART-naive patients on the growth in counts of $CD4^+/CD25^{hi}/CD127^{low}$ T lymphocytes

The baseline $CD4^+$ T lymphocyte counts were divided into CD4 ≤ 200 cells/uL group and CD4 > 200 cells/uL group (Table 3). The results showed that patients in CD4 > 200 cells/uL group had higher growth counts of $CD4^+/CD25^{hi}/CD127^{low}$ T lymphocytes ($P = 0.027$) and $CD4^+$ T lymphocyte counts ($P < 0.001$). But $CD8^+$ T lymphocyte counts decreased in CD4 > 200 cells/uL group after ART ($P < 0.001$).

The correlation trend between $CD4^+/CD25^{hi}/CD127^{low}$ T lymphocyte counts and HIV specific detection indicators

In ART-naive PLWHA, $CD4^+/CD25^{hi}/CD127^{low}$ T lymphocyte counts were significantly positively correlated with $CD4^+$ T lymphocyte counts (Figure 3A) and CD4/CD8 ratio (Figure 3B). The correlation index were 0.923 ($P < 0.001$) and 0.741 ($P < 0.001$), respectively. On the contrary, $CD4^+/CD25^{hi}/CD127^{low}$ T lymphocyte

Table 2 - Changes of lymphocyte subsets after short-term ART.

Variable	ART-naive PLWHA	6 months post-ART	9 months post-ART	*Test*	*P*
$CD4^+/CD25^{hi}/CD127^{low}$ T (cells/uL)	10.3±6.0	17.8±4.0	25.0±11.9	7.078	0.026
$CD4^+/CD25^{hi}/CD127^{low}$ T/CD3 (%)	0.9±0.5	1.2±0.3	1.7±0.6	6.720	0.029
$CD3^+$ T lymphocyte counts (cells/uL)	1,017.2±376.2	1,442.3±370.1	1,570.3±601.7	0.736	0.455
$CD4^+$ T lymphocyte counts (cells/uL)	205.7±111.9	301.3±82.3	384.3±108.8	20.786	0.002
$CD8^+$ T lymphocyte counts (cells/uL)	722.8±315.9	1,066.8±366.3	1,112.0±516.9	0.116	0.892
CD4/CD8	0.3±0.2	0.3±0.1	0.4±0.2	7.183	0.026

*PLWHA = People living with HIV/AIDS.

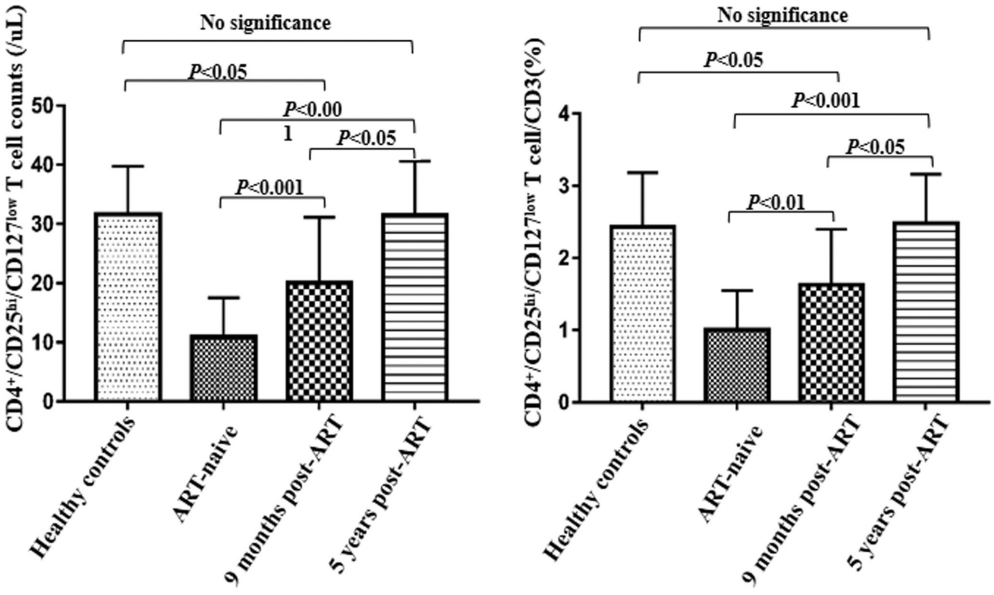

Figure 2 - Short- and long-term changes of CD4+/CD25hi/CD127low T lymphocyte after ART (all samples of peripheral blood were detected by the same technician on the same flow cytometer within 24 h after collection).

Table 3 - Changes in growth counts of CD4+/CD25hi/CD127low T lymphocytes by different baseline CD4+ T lymphocyte levels.

Variable	ART-naive		9 months post-ART		Growth values		*Test*	*P*
	Baseline CD4 ≤ 200 cells/uL	Baseline CD4 > 200 cells/uL	Baseline CD4 ≤ 200 cells/uL	Baseline CD4 > 200 cells/uL	Baseline CD4 ≤ 200 cells/uL	Baseline CD4 > 200 cells/uL		
CD4+/CD25hi/CD127low T lymphocyte counts (/μL)	5.8±4.6	16.0±3.5	11.4±6.1	28.6±7.6	5.6±5.0	12.6±4.6	3.305	0.027
CD4+ T lymphocyte counts (/μL)	119.0±64.6	258.2±45.9	217.2±125.0	442.4±144.0	98.2±80.1	184.2±142.1	10.772	0.000
CD8+ T lymphocyte counts (/μL)	540.4±123.0	963.2±359.5	612.0±423.1	952.4±481.2	71.6±32.0	-10.8±25.1	7.178	0.000

counts and CD4+ T lymphocyte counts were significantly negatively correlated with HIV viral load (Figures 3C and 3D). The correlation index were 0.785 ($P < 0.001$) and 0.712 ($P < 0.001$), respectively.

Correlation between growth of CD4+/CD25hi/CD127low T lymphocytes value and opportunist agents' reactivation

At the 9-month time point post-ART, the average growth of CD4+/CD25hi/CD127low T lymphocytes value was calculated. Seventy cases of PLWHA were divided into group A [growth in CD4+/CD25hi/CD127low T lymphocytes count > Average Value (N=33)] and group B [growth in CD4+/CD25hi/CD127low T lymphocytes count < Average Value (N=37)] according to the growth level in CD4+/CD25hi/CD127low T lymphocytes counts. The type of opportunist agents' reactivation in group A was as follows: 6 cases of tuberculosis, 3 cases of pneumocystis pneumonia, 2 cases of cryptococcosis, 2 cases of cytomegalovirus, 1 case of *Mycobacterium avium* complex infection and 1 case of toxoplasmosis. The type of opportunist agents' reactivation in group B was as follows: 4 cases of tuberculosis, 1 cases of pneumocystis pneumonia, 2 cases of cryptococcosis and 1 case of *Mycobacterium avium* complex infection. Overall, there were 15 (45.5%) cases of PLWHA that had opportunist agents' reactivation in group A and 8 (21.6%) in group B. The difference in the incidence of opportunist agent reactivation between group A and group B was statistically significant ($X^2 = 4.491$, $P = 0.034$).

DISCUSSION

At present, the role of regulatory T lymphocyte (Tregs) in people living with HIV/AIDS is still under debate[10,11]. Some authors believed that it is beneficial as Tregs can suppress the activation of naïve T-cells, while other believe that this role is detrimental because HIV-specific responses can be weakened by Tregs, therefore contributing

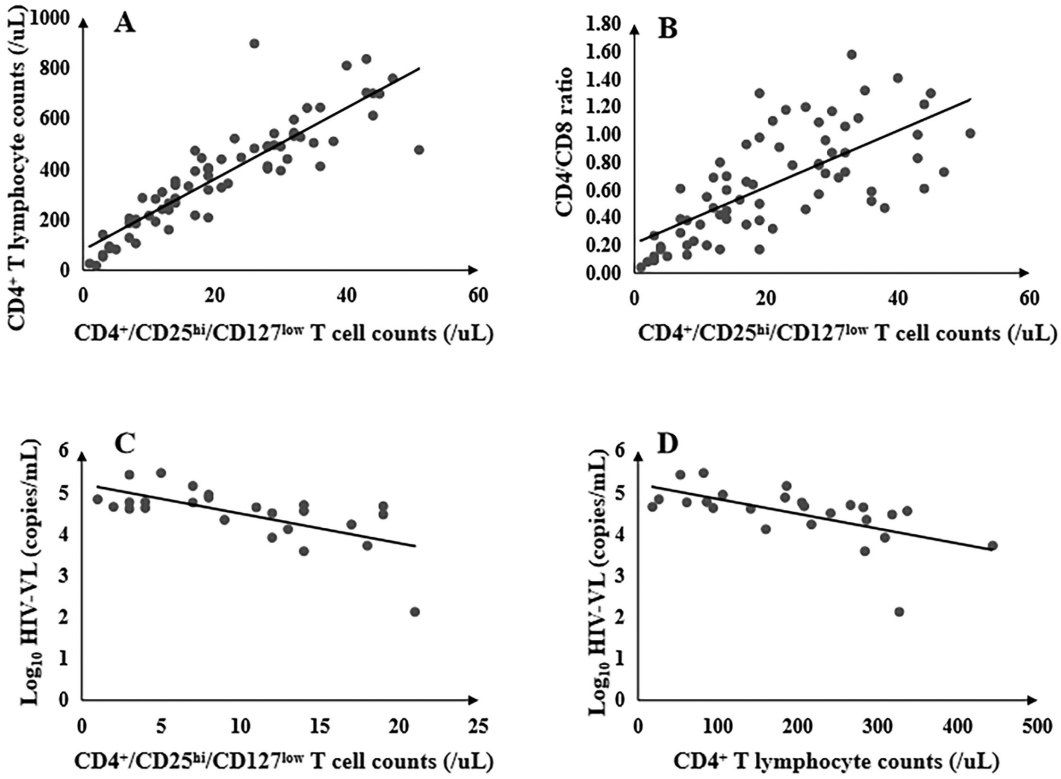

Figure 3 - The correlation trend between $CD4^+/CD25^{hi}/CD127^{low}$ T lymphocyte counts and HIV-related specific test indicators in ART-naive people living with HIV/AIDS (A and B: In ART-naive people living with HIV/AIDS, $CD4^+$ T lymphocyte, CD4/CD8 ratio and $CD4^+/CD25^{hi}/CD127^{low}$ T lymphocyte were detected from same blood samples; C and D: In ART-naive people living with HIV/AIDS, HIV viral loads were done at the same time point as $CD4^+/CD25^{hi}/CD127^{low}$ T lymphocyte and $CD4^+$ T lymphocyte).

to viral persistence. In previous reports, it was found that HIV-specific immune responses could be suppressed by regular Tregs[12,13], but the role of $CD4^+/CD25^{hi}/CD127^{low}$ T lymphocytes in PLWHA is still unclear. This study aimed to clarify the short- or long-term effects of ART on $CD4^+/CD25^{hi}/CD127^{low}$ T lymphocytes, which could further improve medical care and treatment of PLWHA.

A progressive depletion of $CD4^+$ lymphocytes, either by destruction or decreased production and dysfunctional virus-specific T-cell responses, is characteristic of PLWHA[14,15]. Detection of changing trends in $CD4^+$ T lymphocyte and $CD4^+/CD8^+$ ratio can be used to estimate the immune function damage and the disease progression in PLWHA, providing an important basis for clinical staging, treatment timing, treatment efficacy and prognosis of AIDS. In this study, it was found that $CD4^+/CD25^{hi}/CD127^{low}$ T lymphocyte counts were closely correlated with $CD4^+$ T lymphocyte, $CD4^+/CD8^+$ ratio and HIV viral load, suggesting that $CD4^+/CD25^{hi}/CD127^{low}$ T lymphocytes can be used as a complementary immunological indicator to evaluate the dynamic change of disease in PLWHA.

$CD4^+/CD25^{hi}/CD127^{low}$ T lymphocytes could be able to play an important role in establishing and maintaining autoimmune tolerance and immune homeostasis. Meanwhile, they are also the target cells of HIV infection, and $CD4^+/CD25^{hi}/CD127^{low}$ T lymphocytes can significantly decrease after the onset of HIV infection[16,17]. The data in this study confirmed this conclusion.

People living with HIV/AIDS receiving ART can greatly benefit from these results in clinical practice. For the vast majority of patients, plasma HIV viral load can be reduced below the detection limit and $CD4^+$ T-lymphocyte or CD4/CD8 ratio can decrease over time, even though there is a small number of immunological non-responders[18,19]. In this study, by comparing $CD4^+/CD25^{hi}/CD127^{low}$ T lymphocyte counts before ART, 6 months and 9 months post-ART, it was found that $CD4^+/CD25^{hi}/CD127^{low}$ T lymphocyte counts and the proportion of $CD4^+/CD25^{hi}/CD127^{low}$ T lymphocyte/CD3 steadily increased, and the two indexes could reach the level of healthy people after 5 years of ART, suggesting that long-term ART duration was helpful for $CD4^+/CD25^{hi}/CD127^{low}$ T lymphocytes to achieve a complete immune reconstruction. A similar study found T-regs transiently increased and then normalized to values similar to the normal[20], while the values of $CD4^+/CD25^{hi}/CD127^{low}$ T lymphocytes in this study steadily increased towards the value found in normal participants.

In addition to the ART duration, baseline CD4+ T lymphocyte counts was found to be an important factor affecting CD4+/CD25hi/CD127low T lymphocytes growth. Therefore, initiation of ART early in PLWHA is helpful not only for achieving CD4+ T lymphocyte reconstruction as far as possible[21], but also for the CD4+/CD25hi/CD127low T lymphocytes reconstruction. We believe that maximizing the recovery of CD4+/CD25hi/CD127low T lymphocytes is another reason to advocate in favor of the early initiation of ART.

We recognize that our study has limitations. Given the nature of a single center study, we were not able to avoid the selection bias of objects of the observation. In addition, our sample size was small and larger studies are needed. Finally, the function of CD4+/CD25hi/CD127low T lymphocyte has not been analyzed in this study, and will be further explored and improved in the future. However, in this study, we analyzed the growth of CD4+/CD25hi/CD127low T lymphocyte counts after ART, and we found that PLWHA in the group with greater growth of CD4+/CD25hi/CD127low T lymphocytes in a short time was more likely to have opportunist agents' reactivation. This phenomenon could indirectly suggest that CD4+/CD25hi/CD127low T lymphocytes could play a regulating role in maintaining the immune response in PLWHA.

CONCLUSION

In summary, CD4+/CD25hi/CD127low T lymphocyte deficiency was common in PLWHA and ART can promote CD4+/CD25hi/CD127low T lymphocyte reconstruction. In the short-term after ART initiation, CD4+/CD25hi/CD127low T lymphocyte counts gradually increased, and it can further increase to the same level of healthy people after a long-term use of ART. Starting ART when baseline CD4+ T lymphocyte counts are high can better improve the degree of immune function reconstruction of CD4+/CD25hi/CD127low T lymphocytes, which was another strong reason to recommend the start of ART as early as possible in PLWHA.

AUTHORS' CONTRIBUTIONS

YX conceptualized the study design; RH and TC recruited the patients, collected specimens, collected demographic and clinical data; RH, YZ and YY did the laboratory tests; RY and YX interpreted the results; RY wrote the initial drafts of the manuscript; TC, RY and YX revised the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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REFERENCES

1. Cohen MS, Chen YQ, McCauley M, Gamble T, Hosseinipour MC, Kumarasamy N, et al. Prevention of HIV-1 infection with early antiretroviral therapy. N Engl J Med. 2011;365:493-505.
2. Qiu J, Che G, Liu F, Sha X, Ju S, Ma H, et al. The detection and clinical significance of peripheral regulatory CD4+ CD25hi CD127low T cells in patients with non-small cell lung cancer. Clin Transl Oncol. 2019;21:1343-7.
3. Montes M, Lewis DE, Sanchez C, Castilla DL, Graviss EA, Seas C, et al. Foxp3+ regulatory T cells in antiretroviral-naive HIV patients. AIDS. 2006;20:1669-71.
4. Matavele Chissumba R, Namalango E, Maphossa V, Macicame I, Bhatt N, Polyak C, et al. Helios + Regulatory T cell frequencies are correlated with control of viral replication and recovery of absolute CD4 T cells counts in early HIV-1 infection. BMC Immunol. 2017;18:50.
5. Presicce P, Orsborn K, King E, Pratt J, Fichtenbaum CJ, Chougnet CA. Frequency of circulating regulatory T cells increases during chronic HIV infection and is largely controlled by highly active antiretroviral therapy. PloS One. 2011;6:e28118.
6. Moreno-Fernandez ME, Presicce P, Chougnet CA. Homeostasis and function of regulatory T cells in HIV/SIV infection. J Virol. 2012;86:10262-9.
7. Wang WH, Ming L, Wang Y, Kan QC, Zhang XY. High frequency of regulatory T cells among HIV type 1-infected men who have sex with men correlates with disease progression. Chin Med J (Engl). 2013;126:2054-61.
8. Sakaguchi S, Miyara M, Costantino CM, Hafler DA. FOXP3+ regulatory T cells in the human immune system. Nat Rev Immunol. 2010;10:490-500.
9. Chougnet CA, Shearer GM. Regulatory T cells (Treg) HIV/AIDS: summary of the September 7-8, 2006 workshop. AIDS Res Hum Retroviruses. 2007;23:945-52.
10. Chevalier MF, Weiss L. The split personality of regulatory T cells in HIV infection. Blood. 2013;121:29-37.

11. Simonetta F, Bourgeois C. CD4+FOXP3+ regulatory T-cell subsets inhuman immunodeficiency virus infection. Front Immunol. 2013;4:215.
12. Kared H, Lelièvre JD, Donkova-Petrini V, Aouba A, Melica G, Balbo M, et al. HIV-specific regulatory T cells are associated with higher CD4 cell counts in primary infection. AIDS. 2008;22:2451-60.
13. Petitjean G, Chevalier MF, Tibaoui F, Didier C, Manea ME, Liovat AS, et al. Level of double negative T cells, which produce TGF-β and IL-10, predicts CD8 T-cell activation in primary HIV-1 infection. AIDS. 2012;26:139-48.
14. Deeks SG, Kitchen CM, Liu L, Guo H, Gascon R, Narváez AB, et al. Immune activation set point during early HIV infection predicts subsequent CD4+ T lymphocyte changes independent of viral load. Blood. 2004;104:942-7.
15. Sodora DL, Silvestri G. Immune activation and AIDS pathogenesis. AIDS. 2008;22:439-46.
16. Hasenkrug KJ, Chougnet CA, Dittmer U. Regulatory T cells in retroviral infections. PLoS Pathog. 2018;14:e1006776.
17. Ji J, Cloyd MW. HIV-1 binding to CD4 on CD4+CD25+ regulatory T cells enhances their suppressive function and induces them to home to, and accumulate in, peripheral and mucosal lymphoid tissues: an additional mechanism of immunosuppression. Int Immunol. 2009;21:283-94.
18. Yang X, Su B, Zhang X, Liu Y, Wu H, Zhang T. Incomplete immune reconstitution in HIV/AIDS patients on antiretroviral therapy: challenges of immunological non-responders. J Leukoc Biol. 2020;107:597-612.
19. Soriano V. Update of the DHHS Antiretroviral treatment guidelines. AIDS Rev. 2014;16:117-8.
20. Montes M, Sanchez C, Lewis DE, Graviss EA, Seas C, Gotuzzo E, et al. Normalization of FoxP3^{+} regulatory T lymphocyte in response to effective antiretroviral therapy. J Infect Dis. 2011;203:496-9.
21. World Health Organization. Consolidated guidelines on general HIV care and the use ofantiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach. Geneva: WHO; 2013.