Multiple factors involved in T cell depletion in AIDS patients with immune non-responder to cART

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Research

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Abstract

Background: The replication of HIV-1 can be effectively controlled by combination antiretroviral therapy (cART) at present. However, in approximately 20% of AIDS patients with undetectable viral load under effective cART, the amount of CD4+ T cells continues to be lower than normal, showing poor immune reconstitution, which is called immune non-responder (INR). The mechanism of immune reconstitution in AIDS patients is still unclear, and thus far there is no effective improvement strategy.

Aim: To investigate what factors involved in T lymphocyte depletion in INR patients.

Methods: A retrospective analysis of a cohort from Suzhou Infectious Disease Hospital was performed. From October 2015 to April 2019, 29 AIDS patients, whose CD4+ T cells were less than 100/ul before treatment, were included in this study. Laboratory results were obtained from the medical records, including basic clinical information, and the results of Flow cytometer detection for CD4 T cells and ELISA for T cell-related cytokines.

Results: According to the number of CD4+ T cells after 24 months of treatment, the 29 patients were divided into two groups: 15 patients with CD4+ T cells <200/ul were INR, and 14 patients with CD4+ T cells >200/ul were immune responder (IR). When Compared with IR patients, the total number of red blood cells and white blood cells, especially T lymphocytes in INR patients, was significantly lower, while programmed cell death protein 1 (PD-1) on CD4+T and CD8+ T lymphocyte surface were significantly higher in INR group, which implies that the probability of T cell apoptosis was greatly increased ($p<0.05$). ELISA results showed that the overall production of T cell-related cytokines were lower in INR group, but the amount of cytokine secreted from each T cell was higher in INR group than that in IR group due to the significant decrease of T cell in this group. Furthermore, the proportion and the killing efficiency of CD56dim subgroup of NK cells were significantly higher in INR group.

Conclusion: Multiple factors are involved in the immune reconstitution in AIDS patients, including myelosuppression, T cell destruction enhanced through PD-1 pathway, over-activation of T lymphocyte, and higher killing efficiency of CD56dim subgroup of NK cells.

Background

Approximately 20% AIDS patients are unable to achieve good immune reconstitution although virus replication is well controlled by combination antiretroviral therapy (cART). Immune non-responder (INR) state usually occurs in patients who start treatment late, and whose CD4 + T cell numbers still stay at a lower level, i.e.[1]. The definition of INR is that the virus replication is controlled by cART for 1 year, but the CD4 + T cell count is still less than 200/ul by at least two years after the treatment [2]. On the other hand, the immune responder (IR) is defined when CD4 + T cell count is greater than 200/ul after the treatment.

It has been reported that HIV-1 infection affects bone marrow function [3], while the immune system returns to normal in 60%-80% AIDS patients after effective cART[4], indicating that HIV-1 infection not

only induces myelosuppression but also increases lymphocyte destruction. T cells are produced by bone marrow hematopoietic stem cells and enter the peripheral blood after thymic maturation. In the meantime, the programmed cell death protein 1 (PD-1) is one of the inhibitory receptors associated with lymphocyte destruction [5], which can be expressed on the surface of T lymphocytes, and lymphocytes that overexpress PD-1 can trigger their own apoptosis resulting in a reduction in their number [6].

T cell activation-associated cytokines (IL-4, IL-6, IL-10, IL-17 and IFN- γ) secreted in various stages of T cell reflect the activation state of T cells, and cytokine disorders can be observed during HIV infection [7], especially IFN- γ , one of the most important T cell activation markers [8–10]. Studies have showed that lymphocytes with excessive cytokine secretion can also induce activation induced cell death (AICD) [11].

NK cells are mainly differentiated from hematopoietic stem cells. According to the distribution of CD56 molecules, they are divided into CD56^{bright} with more cytokine secretion function and CD56^{dim} with more cells killing function [12]. When stimulated by certain cytokines (such as IL-15), CD56^{bright} NK cells can differentiate into CD56^{dim} NK cells, and the latter contain Perforin and Granzyme B intracellular for the killing function and accounts for 80%-90% of the total number of NK cells [13, 14]. Activated NK cells can effectively clear virus-infected cells, including other virus-associated lymphocytes [15].

In this study, we found that poor immune reconstitution in INR patients is due to reduced production resulted from the myelosuppression, and increased destruction of lymphocyte, which is caused by the over expression of PD-1 on cell surface, over-activation of T cell and enhanced killing activity of NK cells. Our finding suggested that multiple factors are involved in the immune reconstitution in AIDS patients.

Methods

From October 2015 to April 2019, 29 AIDS patients who had less than 100/ul CD4 + T cells before cART treatment and consistently completed same cART regimen for 24 months were enrolled for the study. Informed consent was signed for all patients and testing involved the project has been certified by the relevant ethics committee. The antiviral treatment regimen used was tenofovir/zidovudine + lamivudine + efavirenz/nevirapine. After cART, their plasma viral load was below the detection limit (< 50 copies/ml). According to the number of CD4 + T cells after 24 months of treatment, 15 patients with CD4 + T cells < 200/ul were classified as INR; 14 patients with CD4 + T cells > 200/ul were IR. The basic data of from the both groups were collected including gender, age, route of transmission, time of treatment, and the baseline of CD4 + T cell count.

Polychromatic flow cytometry was used to detect peripheral blood mononuclear cells (PBMCs) from each participant. In brief, cells were stained using fluorochrome-conjugated monoclonal antibodies (BD Biosciences, San Jose, California, USA; BioLegend, San Diego, California, USA) for T-cell subset (CD3/CD4, CD3/CD8) associated with PD-1, NK cell subset (CD56/CD16) with CD107A/Perforin/Granzyme B (markers of kill capability) [16]. All cells were fixed in 1% formaldehyde, detected within 2 h by an LSRFortessa flow cytometer (BD Biosciences), and analyzed using BD FACSDiva

software v7.0. Futher analysis of flow cytometry data was performed using FlowJo software (FlowJo, LLC, Ashland, Oregon, USA).

Quantification of plasma HIV RNA was performed using the Roche COBAS AmpliPrep/COBAS® TaqMan® HIV-1 Test according to the manufacture’s instructions[17]. The detection range of this method was 50 – 1 × 10⁷ copies/ml.

Serum concentrations of cytokines IL-2, IL-4, IL-6, IL-10, IL-17 and IFN-γ in patients were measured by corresponding ELISA kits (R&D Systems, Minneapolis, MN, USA), and the amount of cytokine secreted from a single T cell was calculated as follows: the amount of cytokine secretion per T cell equals to systemic serum cytokine concentration divided by the T cell number.

Statistical analyses were performed using SPSS v19 (IBM, New York, USA) and figures were generated using Prism v6 (GraphPad Software, California, USA). Comparisons between INR and IR were performed using t-test.

Results

29 enrolled AIDS patients were all male, and the infection routes were all through homosexual transmission. In 15 patients in INR group and 14 patients in IR group before cART treatment, the median ages were 49 years and 41 years, respectively, and the mean CD4 + T cell counts were 64.69/ul and 88.60/ul, respectively (Table 1).

Table 1
comparison of the general information

Item	IR group(n = 14)	INR group(n = 15)
Gender		
Male	14	15
Female	0	0
Age (Median)	41(29–64)	49(27–61)
The route of transmission	Homosexual	Homosexual
Median duration of HAART	2.9(2.5–3.6)	3.1(2.7–3.6)
Baseline level of CD4 + T cell (Mean ± SEM)	88.60 ± 14.19	64.69 ± 8.65
IR: immune responder INR: immune non-responder;		

Bone marrow suppression was associated with high expression level of PD-1 on T cell surface in INR patients

Blood routine test results showed that the counts of red blood cell, white blood cell, total lymphocyte, CD4 + and CD8 + T cell subsets were lower in INR group than those in IR group (Fig. 1.a-g) ($p < 0.05$), suggesting that the hematopoietic capacity of bone marrow was suppressed in INR group. In the meantime, the levels of PD-1 on CD4 + T and CD8 + T cell surface were significantly increased in INR group (Fig. 2.a-b) ($p < 0.05$). These results suggested that T cell depletion resulted not only from bone marrow suppression but also from cell destruction.

Excessive cytokine secretion of single T lymphocytes reflects over-activation of T cells in INR patients

In order to determine the activity of T lymphocytes, we further tested and compared the cytokines secreted from T lymphocytes in INR and IR patients. The concentrations of the detected cytokines, i.e. IL-2, IL-4, IL-10, IL-17 and IFN- γ , are all significantly lower in INR group than in IR group (Fig. 3.a). However, the amount of most cytokines, i.e. IL-4, IL-10, IL-17 and IFN- γ (except IL-2), secreted from a single T cell is significantly higher in INR patient than in IR patients (Fig. 3.a). This is due to the significantly lower T cell number in INR than in IR group as showed in Fig. 1.e-f. The increased cytokine secretion in single cell reflects the over-activation of T lymphocytes, and might further induce the occurrence of AICD in T lymphocytes.

The proportion and the killing ability of CD56^{dim} NK cells significantly increased in INR patient

NK cells play a key role in innate immunity and immune surveillance, and regulate immune responses, as well as interact with T cells through a complex network of [18]. Our flow cytometry analysis showed that there is no significant difference in the percentage of NK cells in peripheral blood between two group patients (Fig. 4.a) ($p < 0.05$). However, the proportion of CD56^{dim} subgroup of NK cells was significantly higher in INR group than that in IR group (Fig. 4.b) ($p < 0.05$). We further tested the killing capability of CD56^{dim} NK cells by determining the expression level of CD107a, Granzyme B and Perforin in these cells. The data showed that these killing factors were surprisingly increased in the INR group when compared with the IR group (Fig. 4.c-e, $p < 0.05$), these results suggest that the increased number and function of CD56^{dim} NK cells might be a factor responsible for the depletion of T lymphocytes in the INR patients.

Discussion

During HIV-1 infection, CD4 + T cells are largely destroyed. Therefore, maintaining sufficient CD4 + T cell level may depend primarily on the ability of lymphocyte production in the host. It was reported that CD34 + hematopoietic progenitor cells in the blood of HIV-infected patients were significantly destroyed and unable to maintain sufficient lymphocyte count in AIDS patients [10], and the disease progression is associated with exhaustion of lymphopoiesis in spite of suppression of viral replication [19]. All of these suggest that the progression of AIDS is associated with sustained damage to the lymphoid system,

individual genetic differences and latent infections [20]. In this study, we found that bone marrow suppression and PD-1 overexpression on the surface of T lymphocytes were more severe in INR group, which are likely to be responsible for T lymphocyte depletion.

At present, it is believed that the cause of abnormal activation of the immune system in AIDS patients is related to the persistent low-level viral replication from HIV latent reservoir, ectopic parasitization of intestinal flora, and other viral infections [21]. Cytokine production disorders are one of the manifestations of abnormal activation of the immune system. Studies have found that the level of IL-7 in peripheral blood of HIV-infected patients is elevated, and is negatively correlated with CD4 + T-lymphocyte count, but the level of IL-7 receptor is decreased [22]. Cytokines IL-2, IL-4, IL-10, IL-17 and IFN- γ are closely related to the activation, proliferation and function of T lymphocytes [23–25]. Our study found that those cytokine concentrations in patients with INR were much lower than that in the IR group. The overall low-level concentration of T cell-related cytokines in INR is insufficient to stimulate the bone marrow hematopoiesis. However, when we converted it to single T cell level, we were surprised to find that the INR patients had higher cytokine production than the IR patients. This suggests that T lymphocytes might be over-activated and cause AICD and finally lead to the depletion of T lymphocytes. Other studies have reported that IL-2 is of limited help for HIV treatment [26]. We have also found that there is no significant difference in IL-2 secretion at single T cell level in the two groups (Fig. 3.b), further proved that there is no significant correlation between IL-2 and AIDS development.

NK cells play an important role in regulating T cells by directly lysing activated T cells. For example, during LCMV infection, selective deletion of NK cells can restore the number of activated CD8 + T cells, thereby promote virus clearance [27]. NK cells can also directly remove CD4 + T cells through perforin pathway, which causes CD8 + T cells to fail to exert antiviral effects [28]. Our present study found that the average proportion and the killing function of CD56^{dim} NK cells in the INR group were higher than that in the IR group. We speculated that even through systemic therapy, virus components from HIV-1 latent infection continue to stimulate NK cells, resulting in an increased proportion of CD56^{dim} NK cell subsets and enhanced killing capability, which might destroy more T lymphocytes and finally lead to the depletion of CD4 + T lymphocytes.

In summary, the poor immune reconstitution in AIDS patients might be resulted from multiple factors, including bone marrow suppression, high expression of PD-1 on T cell surface, over-activation of T and NK cells. The detailed variation of immune system in AIDS patients are being further investigated in our ongoing long-term cohort studies. The results of this study imply that the activity of NK cells and bone marrow suppression might be an important auxiliary indicator to judge the outcome of immune reconstitution, and provide a clue for the design of immune reconstitution improvement strategies.

Conclusion

The poor immune reconstitution in INR patients is likely caused by multiple factors, including myelosuppression, T cell destruction enhanced through PD-1 pathway, over-activation of T lymphocyte

resulting in higher production of various cytokines, and the increased killing efficiency of CD56dim NK cells. These findings provide a theoretical basis for designing novel strategies to improve the immune reconstitution.

Declarations

Abbreviations

cART: combination antiretroviral therapy; INR: immune non-responder; IR: immune responder; PD-1: Programmed cell death protein 1; AICD: activation induced cell death.

Author disclosure statement

The authors have no conflicts of interest to declare.

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Authors' contributions

FQ and SH-principle investigator, data management and statistical analysis, writing manuscript; YZ and YW JL and JQ-data collection and analysis; YG and XS-manuscript writing, BS and CZ-proposal writing and manuscript writing;

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Availability of data and materials

The data that support the findings of this study are available from the study team but restrictions apply to availability of these data and so not publicly available. Data are however available from corresponding author upon reasonable request.

Ethical approval and consent to participate

Approval was obtained from the Fifth People's Hospital of Suzhou, China (Approval number: 2018003)

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Lisco A, Wong CS, Lage SL, Levy I, Brophy J, Lennox J, *et al.* Identification of rare HIV-1-infected patients with extreme CD4+ T cell decline despite ART-mediated viral suppression. *JCI Insight* 2019,**4**.
2. Ross E, Tanser F, Pei P, Newell ML, Losina E, Thiebaut R, *et al.* The impact of the 2013 WHO antiretroviral therapy guidelines on the feasibility of HIV population prevention trials. *HIV Clin Trials* 2014,**15**:185-198.
3. Carter CC, Onafuwa-Nuga A, McNamara LA, Riddell Jt, Bixby D, Savona MR, *et al.* HIV-1 infects multipotent progenitor cells causing cell death and establishing latent cellular reservoirs. *Nat Med* 2010,**16**:446-451.
4. Ayele T, Jarso H, Mamo G. Immunological outcomes of Tenofovir versus Zidovudine-based regimens among people living with HIV/AIDS: a two years retrospective cohort study. *AIDS Res Ther* 2017,**14**:5.
5. Odorizzi PM, Pauken KE, Paley MA, Sharpe A, Wherry EJ. Genetic absence of PD-1 promotes accumulation of terminally differentiated exhausted CD8+ T cells. *J Exp Med* 2015,**212**:1125-1137.
6. Rallon N, Garcia M, Garcia-Samaniego J, Cabello A, Alvarez B, Restrepo C, *et al.* Expression of PD-1 and Tim-3 markers of T-cell exhaustion is associated with CD4 dynamics during the course of untreated and treated HIV infection. *PLoS One* 2018,**13**:e0193829.
7. Giuliani E, Vassena L, Di Cesare S, Malagnino V, Desimio MG, Andreoni M, *et al.* NK cells of HIV-1-infected patients with poor CD4(+) T-cell reconstitution despite suppressive HAART show reduced IFN-gamma production and high frequency of autoreactive CD56(bright) cells. *Immunol Lett* 2017,**190**:185-193.
8. Cencioni MT, Santini S, Ruocco G, Borsellino G, De Bardi M, Grasso MG, *et al.* FAS-ligand regulates differential activation-induced cell death of human T-helper 1 and 17 cells in healthy donors and multiple sclerosis patients. *Cell Death Dis* 2015,**6**:e1785.
9. Riou C, Dumont AR, Yassine-Diab B, Haddad EK, Sekaly RP. IL-4 influences the differentiation and the susceptibility to activation-induced cell death of human naive CD8+ T cells. *Int Immunol* 2006,**18**:827-835.

10. Ayroldi E, Zollo O, Cannarile L, F DA, Grohmann U, Delfino DV, *et al.* Interleukin-6 (IL-6) prevents activation-induced cell death: IL-2-independent inhibition of Fas/fasL expression and cell death. *Blood* 1998,**92**:4212-4219.
11. Kunkele A, Johnson AJ, Rolczynski LS, Chang CA, Hoglund V, Kelly-Spratt KS, *et al.* Functional Tuning of CARs Reveals Signaling Threshold above Which CD8+ CTL Antitumor Potency Is Attenuated due to Cell Fas-FasL-Dependent AICD. *Cancer Immunol Res* 2015,**3**:368-379.
12. Bayigga L, Nabatanzi R, Sekiziyivu PN, Mayanja-Kizza H, Kamya MR, Kambugu A, *et al.* High CD56++CD16- natural killer (NK) cells among suboptimal immune responders after four years of suppressive antiretroviral therapy in an African adult HIV treatment cohort. *BMC Immunol* 2014,**15**:2.
13. Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. *Trends Immunol* 2001,**22**:633-640.
14. Chan A, Hong DL, Atzberger A, Kollnberger S, Filer AD, Buckley CD, *et al.* CD56bright human NK cells differentiate into CD56dim cells: role of contact with peripheral fibroblasts. *J Immunol* 2007,**179**:89-94.
15. Huang WC, Easom NJ, Tang XZ, Gill US, Singh H, Robertson F, *et al.* T Cells Infiltrating Diseased Liver Express Ligands for the NKG2D Stress Surveillance System. *J Immunol* 2017,**198**:1172-1182.
16. Zhang X, Lu X, Moog C, Yuan L, Liu Z, Li Z, *et al.* KIR3DL1-Negative CD8 T Cells and KIR3DL1-Negative Natural Killer Cells Contribute to the Advantageous Control of Early Human Immunodeficiency Virus Type 1 Infection in HLA-B Bw4 Homozygous Individuals. *Front Immunol* 2018,**9**:1855.
17. Damond F, Avettand-Fenoel V, Collin G, Roquebert B, Plantier JC, Ganon A, *et al.* Evaluation of an upgraded version of the Roche Cobas AmpliPrep/Cobas TaqMan HIV-1 test for HIV-1 load quantification. *J Clin Microbiol* 2010,**48**:1413-1416.
18. Desimio MG, Covino DA, Doria M. Potential of the NKG2D/NKG2DL Axis in NK Cell-Mediated Clearance of the HIV-1 Reservoir. *Int J Mol Sci* 2019,**20**.
19. Sauce D, Larsen M, Fastenackels S, Pauchard M, Ait-Mohand H, Schneider L, *et al.* HIV disease progression despite suppression of viral replication is associated with exhaustion of lymphopoiesis. *Blood* 2011,**117**:5142-5151.
20. Mestre G, Garcia F, Martinez E, Milinkovic A, Lopez A, Leon A, *et al.* Short Communication: Natural killer cells and expression of KIR receptors in chronic HIV type 1-infected patients after different strategies of structured therapy interruption. *AIDS Res Hum Retroviruses* 2008,**24**:1485-1495.
21. Jin CZ, Zhao Y, Zhang FJ, Yao HP, Wu LJ, Zhao HX, *et al.* Different plasma levels of interleukins and chemokines: comparison between children and adults with AIDS in China. *Chin Med J (Engl)* 2009,**122**:530-535.
22. Waggoner SN, Cornberg M, Selin LK, Welsh RM. Natural killer cells act as rheostats modulating antiviral T cells. *Nature* 2011,**481**:394-398.
23. Shaw DM, Merien F, Braakhuis A, Dulson D. T-cells and their cytokine production: The anti-inflammatory and immunosuppressive effects of strenuous exercise. *Cytokine* 2018,**104**:136-142.

24. Huang W, August A. The signaling symphony: T cell receptor tunes cytokine-mediated T cell differentiation. *J Leukoc Biol* 2015,**97**:477-485.
25. Matsuzaki G, Umemura M. Interleukin-17 family cytokines in protective immunity against infections: role of hematopoietic cell-derived and non-hematopoietic cell-derived interleukin-17s. *Microbiol Immunol* 2018,**62**:1-13.
26. Sellier P, Lafuente-Lafuente C, Bergmann JF. Interleukin-2 therapy in patients with HIV infection. *N Engl J Med* 2010,**362**:270-271; author reply 271.
27. Karwacz K, Bricogne C, MacDonald D, Arce F, Bennett CL, Collins M, *et al*. PD-L1 co-stimulation contributes to ligand-induced T cell receptor down-modulation on CD8+ T cells. *EMBO Mol Med* 2011,**3**:581-592.
28. Soderquest K, Walzer T, Zafirova B, Klavinskis LS, Polic B, Vivier E, *et al*. Cutting edge: CD8+ T cell priming in the absence of NK cells leads to enhanced memory responses. *J Immunol* 2011,**186**:3304-3308.

Figures

Figure 1

INR patients show strong myelosuppression. Detection of peripheral blood clles in INR and IR AIDS patients. The count of a) red blood cell, b) white blood cell, c) blood platelet, d) lymphocytes, e) CD4+ T cells and f) CD8+ T cells were detected with FACS.

Figure 2

Enhanced expression of PD-1 on T cells in INR patients. Detection of PD-1 molecule expression in T lymphocytes of peripheral blood by flow cytometry. a) Percentage of PD-1+ cell in CD4+ T cell; b) Percentage of PD-1+ cell in CD8+ T cell.

Figure 3

Secretion of T cell-associated cytokines in AIDS patients. Detection of cytokines associated with T lymphocytes in peripheral blood: a) cytokine production per T cell; b) Systemic serum concentration of cytokines *: $p < 0.05$

Figure 4

The proportion and the killing capability of CD56^{dim} subset of NK cells in INR patients. Detection of the number of NK cell and CD56 subset using flow cytometry, and assessment of killing capability of CD56^{dim} NK cell by detecting the related cellular molecules. a) the percentage of NK cells in lymphocyte, b) the percentage of CD56^{dim} cell in NK cell, c) CD107a⁺ cell in CD56^{dim} cell, d) Granzyme B⁺ cell in CD56^{dim} cell, e) Perforin⁺ cell in CD56^{dim} cell.