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Research article

Persistent T cell proliferation and MDSCs expansion precede incomplete CD4$^+$ T cell recovery in people with acute HIV-1 infection with early ART

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ABSTRACT

HIV-1 infection causes T cell dysfunction that cannot be fully restored by anti-retroviral therapy (ART). Myeloid-derived suppressor cells (MDSCs) expand and suppress T cell function during viral infection. In this study, we evaluated the dynamics of phenotypes and function of T cells and MDSCs and the effects of their interaction on CD4$^+$ T cell reconstitution in people with acute HIV-1 infection (PWAH) with early ART. Flow cytometry was used to detect the phenotypic dynamics and function of T cells and MDSCs at pre-ART, 4, 24, 48, and 96 weeks of ART. We observed that T cells were hyper-activated and hyper-proliferative in PWAH at pre-ART. Early ART normalized T cell activation but not their proliferation. T cell proliferation, enriched in PD-1$^+$ T cells, was persisted and negatively associated with CD4$^+$ T-cell counts after ART. Moreover, M-MDSCs frequency was increased and positively correlated with T cell proliferation after 96 weeks of ART. M-MDSCs persisted and inhibited T cell proliferation ex vivo, which could be partially reversed by PD-L1 blockade. Further, we found higher frequencies of proliferative CD4$^+$ T cells and M-MDSCs in PWAH with lower CD4$^+$ T cell numbers (<500 cells/μL) compared to PWAH with higher CD4$^+$ T cell numbers (>600 cells/μL) after 96 weeks of ART. Our findings indicate that persistent T cell proliferation, MDSCs expansion, and their interaction may affect CD4$^+$ T-cell recovery in PWAH with early ART.

1. Introduction

T cells represent critical factors in controlling HIV-1 infection [1]. HIV-1 infection induces dramatic T cell activation, proliferation,

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and exhaustion, leading to progressive T cell dysfunction. The dysfunctional T cells are associated with disease progression and incomplete immune reconstitution [2–5]. Anti-retroviral therapy (ART) rapidly suppresses HIV-1 replication and checks HIV-1 infection. ART initiated in the chronic phase of HIV-1 infection attenuates but does not fully abrogate T cell activation. Early ART during acute HIV-1 infection quickly reduces the levels of soluble immune activation markers, but the rate of decline of T cell activation is slower [6–10]. However, T cell functional alteration in people with acute HIV-1 infection (PWAH) with early ART and its effect on immune reconstitution is not well-established.

Myeloid-derived suppressor cells (MDSCs) are a heterogenic population of myeloid cells generated in various pathological conditions. Cancer, chronic viral infection, and inflammation promote the accumulation of MDSCs [11,12]. MDSCs can be monocytic (M-MDSCs) and polymorphonuclear (PMN-MDSC), and be characterized by inhibiting immune response, especially T cell immune response [12]. The immunosuppression of MDSCs is mediated by several molecules, including inducible nitric oxide synthase (iNOS), arginase 1 (ARG1), reactive oxygen species (ROS), and signal transduction activator 3 (STAT3) [13]. Moreover, immune regulatory molecule, like PD-L1, involves in the inhibitory process of MDSCs. PD-L1 expression on MDSCs is up-regulated, and PD-L1 blockade enhances T cell immune response in tumors [14–16].

PMN-MDSCs expand during primary HIV-1 infection, and the increase in PMN-MDSCs is regardless of clinical stage and ART [17, 18]. PMN-MDSCs expansion persists even after an early ART, affecting the recovery of $CD4^+$ T cells [19]. During HIV-1 infection, MDSCs inhibit T cell function by triggering ARG1, promoting Treg cells differentiation and accumulation, and inhibiting $CD4^+$ T cell proliferation [20–23]. HIV-1 infection increases the frequencies of M-MDSCs in the chronic stage [24–29], but the effect during acute infection is controversial.

In the current study, we conducted a longitudinal study in PWAH with early ART to clarify the effect of early ART on the immune response of T cells and MDSCs, and to reveal the relationships between these immunological changes and $CD4^+$ T cells recovery. We discovered that early ART could normalize T cell activation and exhaustion but not their proliferation. Abnormal T cell proliferation, dominant in PD-1^+ T cells, persisted and was negatively associated with $CD4^+$ T cell counts after ART. Moreover, M-MDSCs expanded, persisted and inhibited T cell proliferation ex vivo, which could be partially reversed by PD-L1 blockade. Therefore, our data reveal that the persistence of aberrant T cell proliferation and M-MDSCs expansion may impact the immune reconstitution in PWAH with early ART.

2. Materials and methods

2.1. Subjects

In the current retrospective study, people with acute HIV-1 infection (PWAH, n = 69) were enrolled from the same clinical cohort as our published study [30]. Acute HIV-1 infection is defined as detectable HIV-1 RNA in the plasma and negative or indeterminate anti-HIV-1 antibody [31,32]. The inclusion criteria were as follows: patients above 18 years and had not received ART before recruitment; the exclusion criteria were subjects with opportunistic infections and co-infections (with hepatitis B virus, hepatitis C virus). ART was immediately initiated once the patients were identified as AHI. The patients were then followed-up at weeks 4, 24, 48, and 96. Subsequently, based on the numbers of $CD4^+$ T cells at 96 weeks after ART, we divided the subjects into two groups: immunological responders (IRs: $CD4^+$ T cells count >600 cells/μL, with an undetectable plasma viral load) and immunological non-responders (INRs: $CD4^+$ T cells count <500 cells/μL, with an undetectable plasma viral load) [33,34]. Age and sex-matched HIV-1-negative men were simultaneously enrolled as healthy controls (HCs, n = 21).

The study was approved by the Beijing Youan Hospital Research Ethics Committee (No. 2020-147, approved date: 2020-12-15; No. 2021-371, approved date: 2021-12-31). Written informed consent was obtained from all participants following the Declaration of Helsinki. 10 mL venous blood were drawn at each visit, and PBMCs were isolated and frozen in liquid nitrogen.

2.2. Determination of absolute $CD4^+$ T cell count

Whole blood $CD4^+$ T-cell absolute counts were determined with a TruCount tube with multicolor antibodies ($CD3^+$, $CD45^+$, $CD4^+$, and $CD8^+$ T cells) using Multiset software (BD FACS Canto II, BD Biosciences, New Jersey, USA).

2.3. Plasma HIV-1 RNA

Plasma HIV-1 viral load was quantified using the automated Real-time PCR M2000 system (Abbott Molecular Inc., Des Plaines, IL, USA), with a 40 copies/mL detection limit.

2.4. Antibodies

Pacific blue-conjugated anti-human CD14 (63D3) monoclonal antibody (mAb), Phycoerythrin (PE)-conjugated anti-human CD15 (HI98) mAb, phycoerythrin-cyanine 7 (PE-cy7)-conjugated anti-human PD-L1 (29E.2A3) mAb, Brilliant violent (BV) 510-conjugated anti-human CD33 (WM53) mAb, Peridinin-Chlorophyll-Protein Complex (Percp)-conjugated anti-human HLA-DR (L243) mAb, Fluorescein isothiocyanate (FITC)-conjugated anti-human CD11b (ICRF44) mAb, allophycocyanin-cyanine 7 (APC-cy7) -conjugated anti-human CD4 (RPA-T4) mAb, PE-cy7-conjugated anti-human CD3 (UCHT1) mAb, PE-conjugated anti-human CD38 (HIT2), APC-conjugated HLA-DR (L243) mAb, BV510-conjugated anti-human CD3 (OKT3) mAb, FITC-conjugated anti-human CD4 (RPA-T4)

mAb, BV421-conjugated anti-human PD-1 (NAT105) mAb, and PE-cy7-conjugated anti-human Ki67 (Ki-67) mAb. All the fluorescent antibodies and isotype controls were purchased from Biolegend (San Diego, CA, USA).

2.5. Flow cytometry

Cryopreserved PBMCs were thawed and washed with PBS with 1% bovine serum albumin (BSA). Cells were labeled with LIVE/DEAD fixable viability stain 510 (BD Biosciences, SanJose, CA, USA) and then surface labeled with different fluorescent mAbs and incubated for 20 min at room temperature (RT) in dark. Then, cells were washed, fixed, and detected on a flow cytometer (BD FACS Canto II, BD Biosciences). M-MDSC's phenotype was defined as $CD33^{+}HLA\text{-}DR^{-/low}CD11b^{+}CD14^{+}CD15^{-}$, and PMN-MDSCs's phenotype was characterized as $CD33^{+}HLA\text{-}DR^{-/low}CD11b^{+}CD14^{-}CD15^{+}$. Data analysis was performed using FlowJo software (Version 10; TreeStar., Ashland, OR, USA).

2.6. Cell proliferation

After surface staining, cells were fixed and permeabilized (Invitrogen, USA) for 30 min at 4°C in the refrigerator. Then, cells were washed, labeled with anti-human Ki67 mAbs and incubated at 4°C for 30 min in darkness. Cells were washed, fixed, and analyzed on a flow cytometer.

2.7. Cell separation

Commercial microbeads (Mitenyi Biotec, Germany) were used to separate T cells and M-MDSCs. For M-MDSCs sorting, CD3, HLA-DR, CD33, and CD14 microbeads were gradually used according to the protocol [35]. The purity of T cells and M-MDSCs was all above 90%.

2.8. Co-culture of M-MDSCs and T cells

Purified T cells, used as target cells, were resuspended at 2×10^6 cells/mL in R10 with IL-2 (200 IU/mL). The Cell suspension was seeded into the 96-wells plate (100 μL/well) and activated by Phytohaemagglutinin (PHA, 2.5 μg/mL). Effector M-MDSCs were added at E:T = 1:4, with PD-L1 blocking antibody or control antibody (Sino Biological). Cells were incubated for 20 h at 37°C with 5% CO_2. Then, cells were harvested, and T cell proliferation was detected by flow cytometry.

2.9. Statistical analysis

Data were presented as the median and interquartile range (IQR). SPSS (21.0) and GraphPad Prism 8.3.0 were used for statistical analysis and creating figures. Differences of the parameters between HCs and PWAH, or IRs and INRs were performed by Mann-Whitney *U* test. The Kruskal-Wallis test assessed differences in parameters among HCs and PWAH at different follow-ups. The Friedman test was performed to evaluate parameters' differences among PWAH at different follow-ups. Correlations were analyzed by

Table 1
Basic characteristics of all the participants.

Characteristics	HCs	PWAH	*P* values
Cases	21	69	
Sex, Male (%)	21 (100%)	69 (100%)	0.99
Age (y)	28 (25–46)	31 (18–54)	0.38
Infection time (day)	NA	68 (36.5–102)	
Fiebig stage	NA		
I		7 (10.1%)	
II		3 (4.4%)	
V–VI		59 (85.5%)	
CD4 T-cell counts (cells/μL)			
at baseline	NA	386 (174–1033)	
at 24W	NA	534 (160 - 1280)[a]	
at 48W	NA	556 (232 - 1912)[a]	
at 96W	NA	653 (234 - 1342)[a,b]	
HIV RNA (log_{10} copies/mL)			
at baseline	NA	4.5 (2.6–7.0)	
after ART	NA	TND	
Drugs	NA		
TDF+3TC+EFV		65 (94%)	
TDF+3TC+LPV/r		4 (6%)	

Data were depicted as median and IQR (interquartile range).*P* values were calculated by Mann-Whitney *U* test. a, compared with baseline, $P < 0.001$; b, compared with 24W, $P < 0.05$; NA, not applicable; TND, test not detected. TDF, Tenofovir; 3TC, Lamivudine; EFV, Efavirenz; LPV/r, Lopinavir/ritonavir.

using the non-parametric Spearman's rank correlation test. All tests were two-tailed, and *P* values less than 0.05 were considered statistically significant.

3. Results

3.1. Characteristics of the participants

PWAH (n = 69) and HCs (n = 21) were enrolled, and the clinical information was described in Table 1. The sex and age of PWAH and HCs were matched ($P > 0.05$). The estimated infection time of PWAH was 68 (IQR 36.5 - 102) days. At baseline, the median $CD4^+$ T-cell count was 386 (IQR 174 - 1033), and median plasma HIV-1 RNA was 4.5 $\log_{10}$ copies/mL (IQR 2.6 -7.0). After 96 weeks of ART, the median $CD4^+$ T-cell count was 653 (IQR 234 - 1342), and median HIV-1 RNA <40 copies/mL (range: not detected - < 40 copies/mL). Compared with INRs, IRs had higher $CD4^+$ T-cell count at baseline and 24, 48 and 96 weeks after ART (Table S1).

3.2. Early ART caused a faster normalization of $CD8^+$ T cell activation

Immune activation is a key feature of HIV-1 infection and plays an essential role in HIV-1 pathogenesis, disease progression and incomplete immune recovery [36]. As shown in Fig. 1, the frequencies of $CD38^+HLA\text{-}DR^+CD4^+$ T cells and $CD38^+HLA\text{-}DR^+CD8^+$ T cells were significantly higher in PWAH at pre-ART than that in HCs. Although early ART significantly reduced and normalized the

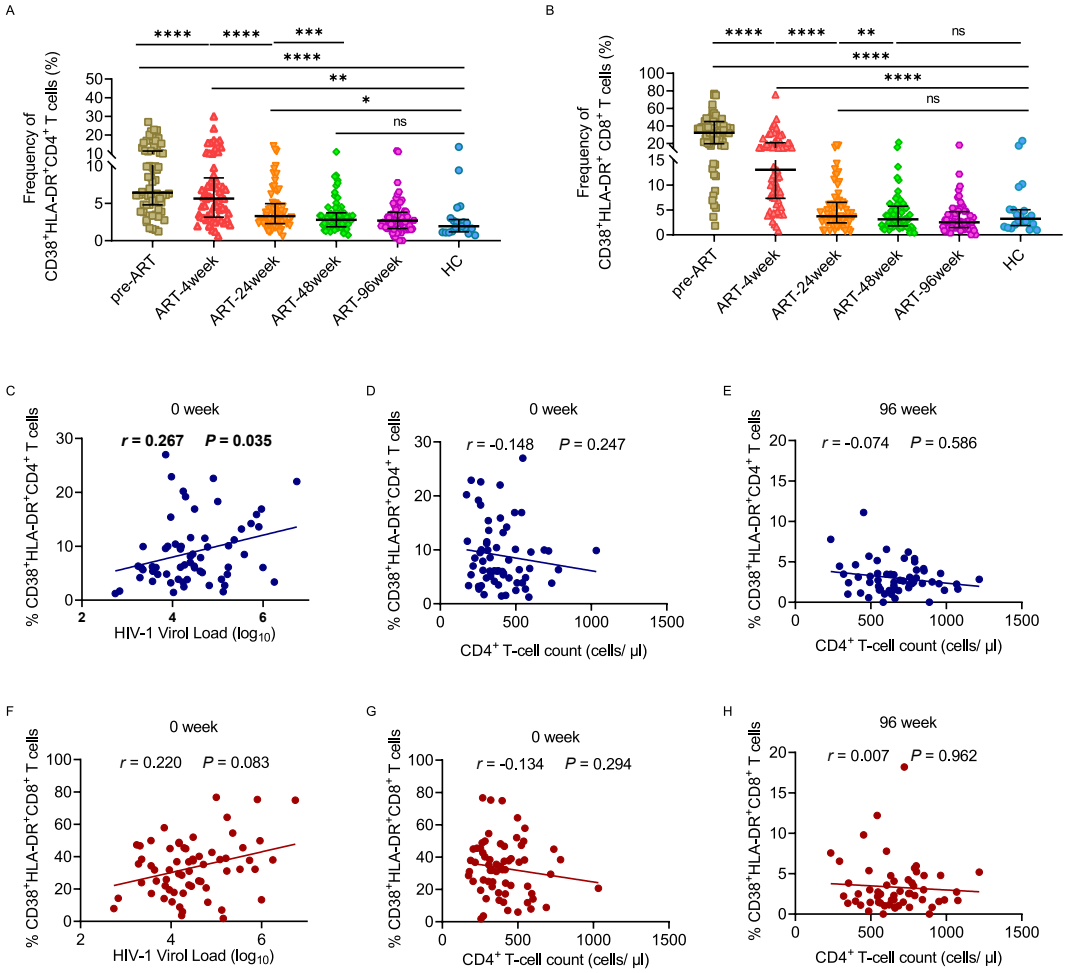

Fig. 1. Early ART normalized T cell activation in PWAH. Comparisons of the frequencies of $CD38^+HLA\text{-}DR^+CD4^+$ T cells (A) and $CD38^+HLA\text{-}DR^+CD8^+$ T cells (B) among HCs (fx1) and PWAH at pre-ART (fx2), 4W (fx3), 24W (fx4), 48W (fx5) and 96W (fx6) after ART. The frequencies of $CD38^+HLA\text{-}DR^+CD4^+$ T cells were positively associated with plasma HIV-1 viral load (C), but not $CD4^+$ T cell counts at baseline (D) or 96W after ART-treated (E) in PWAH. There was no correlation between the frequencies of $CD38^+HLA\text{-}DR^+CD8^+$ T cells and plasma HIV-1 viral load (F), or $CD4^+$ T cell counts at baseline (G) or 96W after ART-treated (H) in PWAH. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. HCs, healthy controls; PWAH, people with acute HIV infection.

frequencies of CD38$^+$HLA-DR$^+$CD4$^+$ T cells and CD38$^+$HLA-DR$^+$CD8$^+$ T cells, the decrease in the frequencies of CD38$^+$HLA-DR$^+$CD8$^+$ T cells (week 24) was faster than the frequencies of CD38$^+$HLA-DR$^+$CD4$^+$ T cells (week 48) (Fig. 1A and B). Moreover, the frequencies of CD38$^+$HLA-DR$^+$CD4$^+$ T cells were positively associated with HIV-1 RNA at pre-ART ($r = 0.267$, $P = 0.035$), but no associations were found between these cells and CD4$^+$ T-cell counts at pre-ART and week 96 (Fig. 1C–E). Furthermore, we did not observe any correlations between the percentages of CD38$^+$HLA-DR$^+$CD8$^+$ T cells and HIV-1 RNA or CD4$^+$ T-cell counts at pre-ART and week 96 (Fig. 1F–H). Additionally, there were no significant differences in the frequencies of CD38$^+$HLA-DR$^+$CD4$^+$ T cells and CD38$^+$HLA-DR$^+$CD8$^+$ T cells between IRs and INRs (Figs. S1A and B).

3.3. PD-1 expression on CD4$^+$ T cells after early ART impacts CD4$^+$ T cell recovery in PWAH

PD-1-expressing T cells, named exhausted T cells, are increased in chronic HIV-1-infected individuals and can be reduced by ART in the first year [37]. Herein, we found that the frequencies of PD-1$^+$CD4$^+$ T cells and PD-1$^+$CD8$^+$ T cells were significantly increased in PWAH compared with HCs. Early ART rapidly reduced the frequencies of PD-1$^+$CD4$^+$ T cells and PD-1$^+$CD8$^+$ T cells, reaching the similar levels as HCs within 24 weeks (Fig. 2A and B). This result suggests that early ART effectively reduce T cell exhaustion. Moreover, the frequencies of PD-1$^+$CD4$^+$ T cells were negatively associated with CD4$^+$ T-cell counts at week 0 ($r = -0.337$, $P = 0.007$) and week 96 ($r = -0.393$, $P = 0.002$), but no association of these cells with HIV-1 RNA was observed (Fig. 2C–E). Furthermore, we found an inverse association between the frequencies of PD-1$^+$CD8$^+$ T cells and CD4$^+$ T-cell counts at week 0 ($r = -0.299$, $P = 0.017$) but not CD4$^+$ T-cell counts at week 96 or HIV-1 RNA at week 0 (Fig. 2F–H). In addition, we observed that the frequencies of PD-1$^+$CD4$^+$ T cells rather than the frequencies of PD-1$^+$CD8$^+$ T cells were significantly increased in INRs compared with IRs (Fig. S1C, D). Therefore, the levels of PD-1 expression on CD4$^+$ T cells after ART may involve in incomplete CD4$^+$ T-cell recovery.

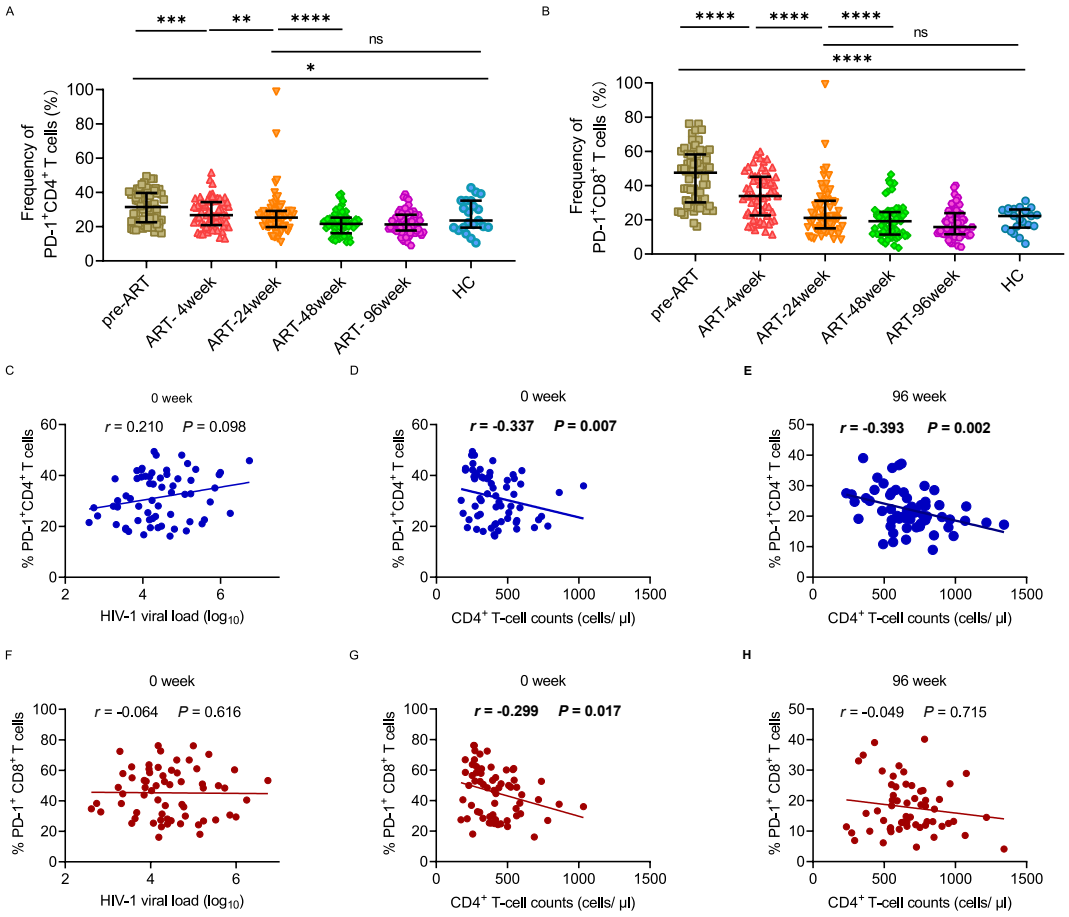

Fig. 2. Residual PD-1 expression impacts CD4$^+$ T cells recovery in PWAH after early ART. Comparison of the frequencies of CD4$^+$PD-1$^+$ T cells (A) and PD-1$^+$ CD8$^+$ T cells (B) among HCs (fx1) and PWAH at pre-ART (fx2), 4W (fx3), 24W (fx4), 48W (fx5) and 96W (fx6) after ART. The frequencies of PD-1$^+$CD4$^+$ T cells were negatively associated with CD4$^+$ T cell counts at baseline (D) and 96W after ART-treated (E), but no correlation between the frequencies of CD4$^+$PD-1$^+$ T cells and plasma HIV-1 viral load (C) was found in PWAH. There was no correlation between the frequencies of PD-1$^+$ CD8$^+$ T cells and plasma HIV-1 viral load (F). The frequencies of PD-1$^+$CD8$^+$ T cells were negatively associated with CD4$^+$ T cell counts at baseline (G) but not 96W after ART-treated (H) in PWAH. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. HCs, healthy controls; PWAH, people with acute HIV-1 infection.

3.4. T cell proliferation persisted and was associated with incomplete CD4$^+$ T-cell recovery in PWAH with early ART

PD-1 is an essential immune checkpoint that inhibits T cell activation and immune response [38]. However, the effect of PD-1 expression on T cell proliferation is still controversial. As shown in Fig. 3A, cells were gated on CD3$^+$CD4$^+$ and CD3$^+$CD8$^+$ T cells, then PD-1 and Ki67 expression were analyzed. The frequencies of Ki67$^+$CD4$^+$ T cells and Ki67$^+$CD8$^+$ T cells were significantly increased in PWAH compared with HCs (Fig. 3A–C). To our surprise, we observed that PD-1$^+$ T cells have higher levels of Ki67 expression than PD-1$^-$ T cells in both IRs and INRs at baseline, but not at 96 weeks of ART and in HCs (Fig. S1 I–K). Moreover, the frequencies of Ki67$^+$CD4$^+$ T cells and Ki67$^+$CD8$^+$ T cells were positively associated with the frequencies of PD-1$^+$CD4$^+$ T cells and PD-1$^+$CD8$^+$ T cells respectively (Fig. S2). The frequencies of Ki67$^+$PD-1$^+$CD4$^+$ T cells and Ki67$^+$PD-1$^+$CD8$^+$ T cells were significantly increased in PWAH compared with HCs (Fig. 3D and E). Although early ART significantly reduced the frequencies of Ki67$^+$ T cells and Ki67$^+$PD-1$^+$ T cells, these cells were still much higher in PWAH after 96 weeks of ART than in HCs (Fig. 3B–E).

Moreover, compared with IRs, the frequencies of Ki67$^+$CD4$^+$ T cells and Ki67$^+$PD-1$^+$CD4$^+$ T cells were much higher in INRs (Figs. S1E and 1G). In addition, we observed that the frequencies of Ki67$^+$CD4$^+$ T cells and Ki67$^+$PD-1$^+$CD4$^+$ T cells were negatively correlated with CD4$^+$ T cell counts at week 0 or 96 (Fig. 3F–H). However, we didn't find significant difference in the frequencies of Ki67$^+$CD8$^+$ T cells and Ki67$^+$PD-1$^+$CD8$^+$ T cells between IRs and INRs (Figs. S1F and 1H). The frequencies of Ki67$^+$CD8$^+$ T cells and Ki67$^+$PD-1$^+$CD8$^+$ T cells were negatively correlated with CD4$^+$ T cell counts at week 0, but not at week 96 (Fig. 3I–K). Therefore, our results indicate that T cell proliferation was increased and persisted in PWAH who even experienced long-time ART and that T cell

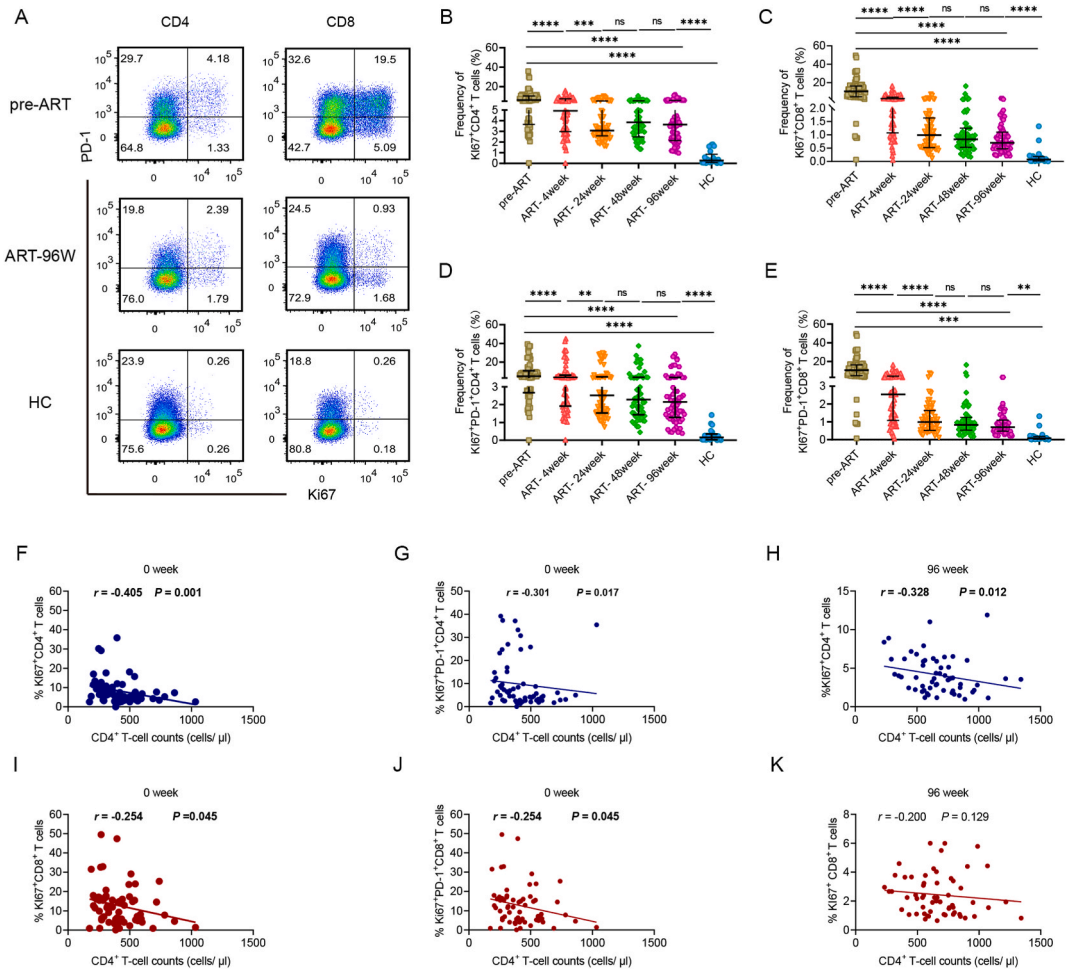

Fig. 3. T cell proliferation persisted after ART and was negatively associated with CD4$^+$ T cell counts in PWAH. (A) Typical flow chart for PD-1 and ki67 expression in CD4$^+$ or CD8$^+$ T cells from HCs and PWAH at baseline and 96W after ART treatment. Comparisons of the frequencies of Ki67$^+$CD4$^+$ T cells (B), Ki67$^+$CD8$^+$ T cells (C), Ki67$^+$PD-1$^+$CD4$^+$ T cells (D) and Ki67$^+$PD-1$^+$CD8$^+$ T cells (E) among HCs (fx1) and PWAH at pre-ART (fx2), 4W (fx3), 24W (fx4), 48W (fx5) and 96W (fx6) after early ART. F–H, Correlations of the frequencies of Ki67$^+$CD4$^+$ T cells or Ki67$^+$PD-1$^+$CD4$^+$ T cells with CD4$^+$ T cell counts at week 0 and 96 in PWAH. I–K, Correlations of the frequencies of Ki67$^+$CD8$^+$ T cells or Ki67$^+$PD-1$^+$CD8$^+$ T cells with CD4$^+$ T cell counts at week 0 and 96 in PWAH. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. HCs, healthy controls; PWAH, people with acute HIV-1 infection.

proliferation may associate with HIV-1 disease progress and the refractory $CD4^+$ T cell recovery.

3.5. M-MDSCs expanded in PWAH after early ART

To distinguish the MDSCs from monocytes or neutrophils, several markers were used to identify them [13,39]. Herein, we characterized M-MDSCs as $CD33^+HLA\text{-}DR^{-/low}CD11b^+CD14^+CD15^-$, PMN-MDSCs as $CD33^+HLA\text{-}DR^{-/low}CD11b^+CD14^-CD15^+$ (Fig. 4A). Compared with HCs, the frequencies of M-MDSCs in PWAH were not statistically changed at pre-ART, but they were significantly increased after more than 48 weeks of ART (Fig. 4B). The frequencies of M-MDSCs were negatively correlated with HIV-1 RNA at pre-ART ($r = -0.286$, $P = 0.018$), but no correlations between the frequencies of M-MDSCs and $CD4^+$ T-cell counts at both pre-ART and week 96 were observed (Fig. 4C–E). However, to PMN-MDSCs, the frequencies decreased in PWAH, and early ART failed to increase these cells. The frequencies of PMN-MDSCs correlated with neither HIV-1 RNA nor $CD4^+$ T-cell counts (Fig. S3). Moreover, we observed a positive association between the frequencies of M-MDSCs and the frequencies of $Ki67^+PD\text{-}1^+CD4^+$ T cells ($r = 0.322$, $P = 0.011$) (Fig. 4F). Furthermore, we observed that the frequencies of M-MDSCs in INRs were significant higher compared with IRs and HCs (Fig. S1 L). Therefore, our results suggest that M-MDSCs expansion after early ART may associate with abnormal $CD4^+$ T cell proliferation in PWAH.

3.6. M-MDSCs inhibited T cell proliferation via PD-1/PD-L1 interaction

MDSCs inhibited T cell immune response through the PD-1/PD-L1 pathway [14]. PD-L1 expression on M-MDSCs were analyzed (Fig. 4A), and we found a significant increase of PD-L1 expression on M-MDSCs in PWAH at pre-ART compared with HCs. Early ART reduced PD-L1 expression on M-MDSCs since week 24, and there were no statistically significant differences in PD-L1 expression on M-MDSCs after 48 and 96 weeks of ART compared to HCs (Fig. 5A). However, we found that the frequencies of $PD\text{-}L1^+$M-MDSCs were negatively associated with the frequencies of $Ki67^+PD\text{-}1^+CD4^+$ T cells in PWAH at weeks 48 and 96 ($r = -0.370$, $P = 0.003$; $r = -0.384$, $P = 0.002$), but not at week 0 (Fig. 5B–D). Therefore, we speculate that M-MDSCs, expanded after early ART, may inhibit T cell proliferation through the PD-L1 pathway. Subsequently, we co-cultured M-MDSCs and T cells ex vivo to detect the inhibitory effect of M-MDSCs on T cells. As expected, M-MDSCs inhibited both $CD4^+$T cell and $CD8^+$ T cell proliferation, and PD-L1 blockade cloud partially restore T cell proliferation (Fig. 5E and F). Our findings demonstrate that M-MDSCs suppress T cell proliferation may through PD-1/PD-L1 pathway in PWAH undergone ART.

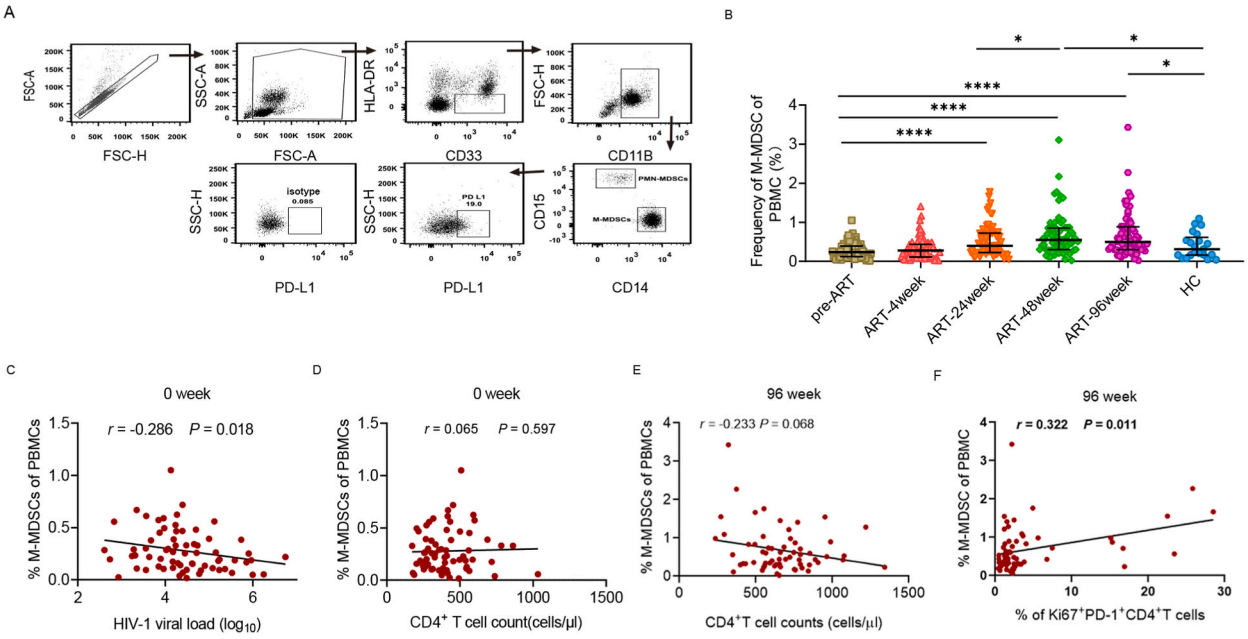

Fig. 4. M-MDSCs increased in PWAH after early ART treatment. (A) Gating strategy for M-MDSCs ($HLA\text{-}DR^{-/low}CD33^+CD11b^+CD14^+CD15^-$) and PMN-MDSCs ($HLA\text{-}DR^{-/low}CD33^+CD11b^+CD14^-CD15^+$). Comparisons of the frequencies of M-MDSCs (B) among HCs (fx1) and PWAH at pre-ART (fx2), 4W (fx3), 24W (fx4), 48W (fx5) and 96W (fx6) after early ART. The frequencies of M-MDSCs were negatively associated with HIV-1 viral load (C) but not $CD4^+$ T cell counts at baseline (D) and week 96 (E). The frequencies of M-MDSCs were positively associated with $Ki67^+PD\text{-}1^+CD4^+$ T cells (F) after 96 weeks of ART. $^*P < 0.05$, $^{****}P < 0.0001$. HCs, healthy controls; PWAH, people with acute HIV-1 infection.

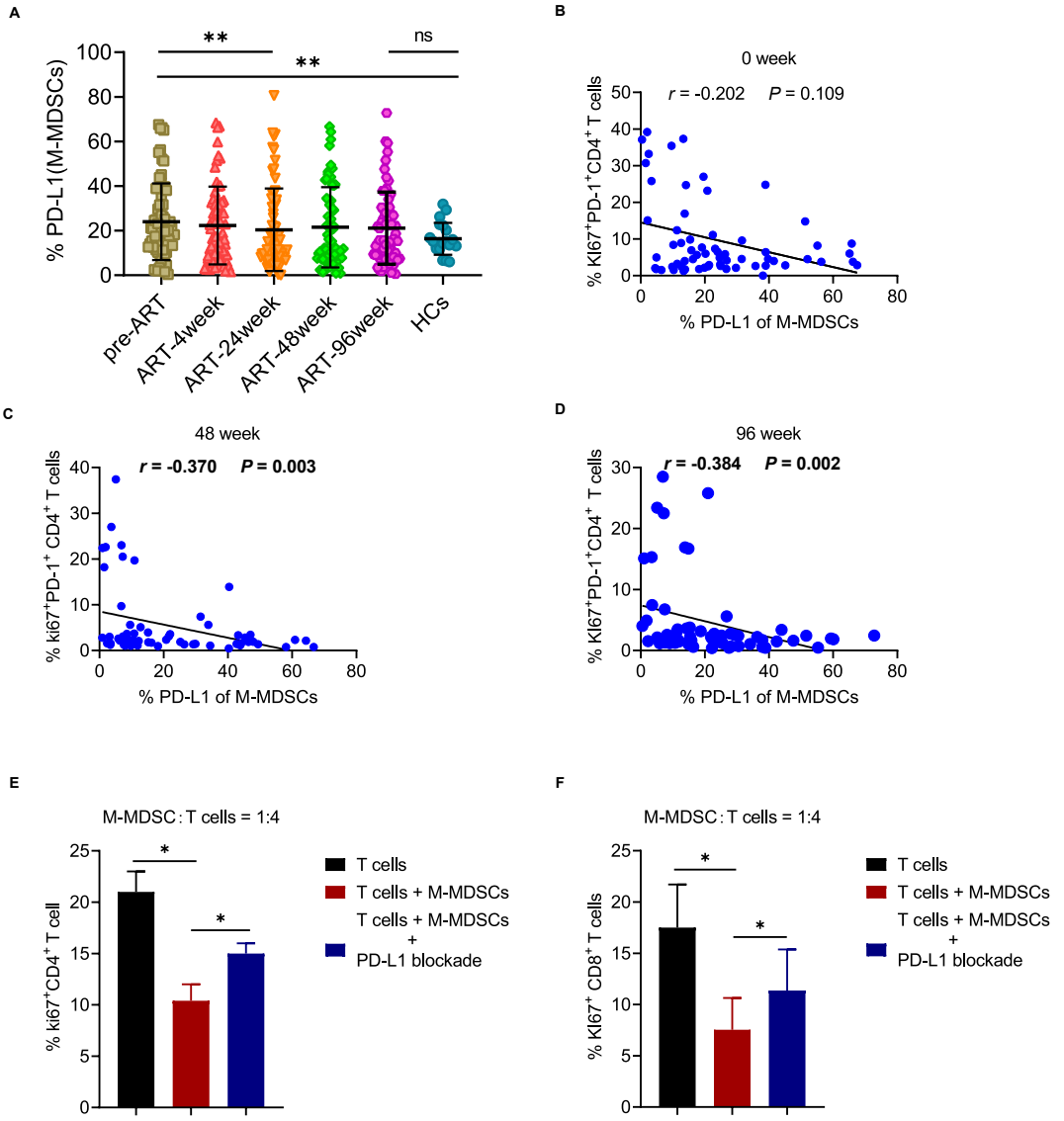

Fig. 5. M-MDSCs inhibited T cell proliferation may via PD-1/PD-L1 pathway. Gated on M-MDSCs, PD-L1 expression were measured by flow cytometry. (A) Comparisons of PD-L1 expression on M-MDSCs among HCs (fx1) and PWAH at pre-ART (fx2), 4W (fx3), 24W (fx4), 48W (fx5) and 96W (fx6) after early ART. The frequencies of PD-L1^{+}M-MDSCs were negatively associated with the frequencies of Ki67^{+}PD-1^{+}CD4^{+} T cells after 48W and 96W treatment (C–D), but not at baseline (B). M-MDSCs inhibited both CD4^{+} T cell and CD8^{+} T cell proliferation ex vivo, and which could be partially reversed by PD-L1 antibody blockade (E–F). *$P < 0.05$, **$P < 0.01$. HCs, healthy controls; PWAH, people with acute HIV-1 infection.

4. Discussion

Immune activation is crucial for HIV-1 disease progression and immune reconstitution after ART. In this study, we conducted a longitudinal study in a clinical cohort of individuals who initiated ART once diagnosed with acute HIV-1 infection to elucidate the dynamics of T cell phenotypes and function. Consistent with the previous study [40], T cells were hyper-activated and positively associated with viral load, which suggested that HIV-1 infection itself serves as the primary trigger for T cell immune activation and disease progression. Early ART could normalize T cell activation, but the rate of decline of CD4^{+} T cell activation was slower compared with CD8^{+} T cells (Fig. 1). Therefore, CD4^{+} T cell activation persists for a longer time even after very early ART. However, there was no apparent association between T cell activation and CD4^{+} T cell counts after early ART. Moreover, no significant differences in CD4^{+} T and CD8^{+} T cell activation were observed between IRs and INRs. Thus, T cell activation may not be the main reason for blunted CD4^{+} T cell recovery in PWAH with early ART.

During chronic HIV-1 infection, PD-1 was up-regulated on HIV-1-specific CD8^{+} T cells and associated with disease progression [37, 38,41]. Similarly, PD-1 expression on T cells was increased and associated with CD4^{+} T-cell decrease in acute HIV-1 infection (Fig. 2).

Moreover, early ART was more effective in normalizing PD-1 expression on T cells than ART initiated at the chronic stage of HIV-1 infection (delayed ART) since early ART normalized PD-1 expression in 24 weeks, while delayed ART normalized PD-1 expression in the first year [37]. Although PD-1 expression on $CD4^+$ T cells was normalized, the frequencies of $PD\text{-}1^+CD4^+$ T cells in INRs were significantly higher compared with IRs. Besides, the frequencies of $PD\text{-}1^+CD4^+$ T cells were negatively associated with $CD4^+$ T cell counts after 96 weeks of ART. These results indicate that residual PD-1 expression on $CD4^+$ T cells after ART may affect the $CD4^+$ T cell recovery. Previous studies have demonstrated that PD-1 expression was abundant on memory $CD4^+$ T cells and was associated with HIV-1 persistence in latently infected $CD4^+$ T cells after ART [42,43]. Furthermore, HIV-1-infected cells (p24-producing cells), detected in both untreated and treated HIV-1-infected patients, preferentially displayed memory phenotype and expressed PD-1 and Ki67 [44]. Therefore, we speculate that $PD\text{-}1^+$ $CD4^+$ T cells exist as a population with memory phenotype and may highly proliferate after ART.

As we expected, Ki67 expression was dominant in $PD\text{-}1^+$ T cells, and the frequencies of $Ki67^+$ T cells were positively associated with $PD\text{-}1^+$ T cells (Fig. S2). The frequencies of $Ki67^+$ T cells and $Ki67^+PD\text{-}1^+$ T cells were persisted and failed to be normalized by early ART in PWAH (Fig. 3). Compared with IRs, the frequencies of $Ki67^+CD4^+$ T cells and $Ki67^+PD\text{-}1^+CD4^+$ T cells were significantly increased in INRs. Moreover, the frequencies of $Ki67^+CD4^+$ T cells were negatively associated with $CD4^+$ T cell counts at week 96 after ART. Consistent with our results, Saidakova et al. demonstrated that INRs have higher frequencies of $Ki67^+PD\text{-}1^+CD4^+$ T cells, and the frequencies of $Ki67^+CD4^+$ T cells were inversely associated with $CD4^+$ T cell counts in patients who experienced long-time ART (>7 years) [45]. Besides, Li et al. recently reported that dysfunctional T cells were a heterogeneous population that expressed PD-1 and regulatory molecules (CSF-1, ZBED2), had the highest clone expansion, and were highly proliferated and dynamically differentiated [46]. Overall, to our knowledge, it is the first study to demonstrate that HIV-1 infection induces persistent T cell proliferation even after early ART, promoting T cell dysfunction and affecting the recovery of $CD4^+$ T cells. However, in this study, we haven't considered PD-1 expression on $CD4^+$ T cell subsets (such as Tfh cells and Tregs) and the status of T cell differentiation. Therefore, PD-1 expression and its exact effects on T cell proliferation in HIV-1 infection need further investigation in the future.

MDSCs expansion during HIV-1 infection has been elaborated [18–20]. However, the reports of the effect of ART on MDSC frequency are contrasting. In contrast to these studies, we found a kinetic increase in the frequencies of M-MDSCs, but the frequencies of PMN-MDSCs were decreased in PWAH after early ART (Fig. 4). These differences in MDSCs frequency may ascribe to the different samples used in studies, that we used frozen PBMCs, but the other studies used fresh PBMCs; and may depend on the subjects' race, which was Asian in our study and Caucasian in others.

Moreover, we observed a significant increase in the frequencies of M-MDSCs in INRs compared with IRs. Pathological immune activation and prolonged presence of inflammatory cytokines that related to incomplete immune reconstitution promotes the emergence of MDSCs [5,12]. IL-6, an essential mediator of M-MDSCs expansion, remains at higher levels in HIV-1-infected individuals with virological suppression after ART [24,47]. In addition, HIV-1 itself and HIVgp120 protein directly or indirectly induced M-MDSCs expansion [28]. However, we didn't observe a correlation between M-MDSCs expansion and T cell activation (data not shown). Instead, we found that M-MDSCs expansion was positively associated with the frequencies of $Ki67^+PD\text{-}1^+CD4^+$ T cells after 96 weeks of ART. However, the causal relationship between aberrant T cell proliferation and M-MDSCs expansion was unclear, and which may cause host immunodiscordance in PWAH with early ART.

M-MDSCs and PMN-MDSCs share common features in inhibiting T cell immune response, but the molecules that mediate immune suppression are diverse. Different from PMN-MDSCs, M-MDSCs preferentially use the expression of immune regulatory molecules such as PD-L1 [13,48]. We found higher frequencies of $PD\text{-}L1^+$M-MDSCs in PHAWs at pre-ART compared with HCs, and which were negatively associated with the frequencies of $Ki67^+PD\text{-}1^+CD4^+$ T cells in PWAH after 48 and 96 weeks of ART. Also, M-MDSCs inhibited T cell proliferation ex vivo and could be partially reversed by PD-L1 blockade (Fig. 5). Therefore, M-MDSCs inhibited abnormal T cell proliferation may through PD-1/PD-L1 interaction. It was revealed that individuals with poor immune recovery have a higher frequency of $PD\text{-}L1^+$MDSCs than individuals who responded well to ART [49]. Microbial translocation and inflammatory cytokines, the critical triggers for incomplete immune reconstitution, could induce PD-L1 expression ex vivo or *in vitro* in HIV-1-infected individuals [49,50]. However, we didn't find a significant difference in the frequencies of $PD\text{-}L1^+$MDSCs between IRs and INRs after early ART (data not shown). MDSCs are heterogeneous, the exact effects of M-MDSCs expansion and their phenotypical alteration after ART on T cell function in HIV-1 infection and immune recovery are contradictory [39]. Hence, new technologies, such as single-cell RNA sequence and single-cell proteomics, should be used to identify the precise phenotypes and explore the novel function of MDSCs.

5. Conclusions

In summary, early ART completely normalized the abnormal T cell activation but not proliferation. Persistent T cell proliferation and M-MDSCs expansion might predict poor $CD4^+$ T cell recovery in individuals with ART. Our findings provide new insights and directions for exploring the role and mechanisms of MDSCs in immune recovery, which need to be addressed in-depth in further studies. However, our study still has limitations. Firstly, we used frozen rather than fresh samples to conduct the experiments, which may cause the differences and experimental deviation from others; Secondly, we did not enroll PWAH who initiated ART later, which may differ from the subjects with early ART. Thirdly, we enrolled only male participants in our study. Our findings should be compared and confirmed in PWAH with delayed ART, or in female participants.

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Author contribution statement

Zhen Li; Hao Wu; Xiaojie Huang: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.
Rui Wang; Xiaofan Lu; Zhiying Liu; Yang Zhang: Performed the experiments.
Zhen Li; Ping Yan; Xin Zhang; Wei Jiang: Analyzed and interpreted the data; Wrote the paper.
Lin Yuan; Bin Su; Tong Zhang: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e15590.

References

[1] S.A. Migueles, et al., HIV-specific CD8+ T cell proliferation is coupled to perforin expression and is maintained in nonprogressors, Nat. Immunol. 3 (11) (2002) 1061–1068, https://doi.org/10.1038/ni845.
[2] M.N. Wykes, S.R. Lewin, Immune checkpoint blockade in infectious diseases, Nat. Rev. Immunol. 18 (2) (2018) 91–104, https://doi.org/10.1038/nri.2017.112.
[3] S. Piconi, et al., Immune activation, apoptosis, and Treg activity are associated with persistently reduced CD4+ T-cell counts during antiretroviral therapy, AIDS 24 (13) (2010) 1991–2000, https://doi.org/10.1097/QAD.0b013e32833c93ce.
[4] C.L. Day, et al., PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression, Nature 443 (7109) (2006) 350–354, https://doi.org/10.1038/nature05115.
[5] X. Yang, et al., Incomplete immune reconstitution in HIV/AIDS patients on antiretroviral therapy: challenges of immunological non-responders, J. Leukoc. Biol. 107 (4) (2020) 597–612, https://doi.org/10.1002/JLB.4MR1019-189R.
[6] J. Hellmuth, et al., Very early initiation of antiretroviral therapy during acute HIV infection is associated with normalized levels of immune activation markers in cerebrospinal fluid but not in plasma, J. Infect. Dis. 220 (12) (2019) 1885–1891, https://doi.org/10.1093/infdis/jiz030.
[7] Y. Bekele, et al., Mass cytometry identifies distinct CD4+ T cell clusters distinguishing HIV-1-infected patients according to antiretroviral therapy initiation, JCI Insight 4 (3) (2019), e125442, https://doi.org/10.1172/jci.insight.125442.
[8] B.J. Macatangay, et al., Brief report: changes in levels of inflammation after antiretroviral treatment during early HIV infection in AIDS clinical trials group study A5217, J. Acquir. Immune Defic. Syndr. 75 (1) (2017) 137–141, https://doi.org/10.1097/QAI.0000000000001320.
[9] S.J. Krebs, J. Ananworanich, Immune activation during acute HIV infection and the impact of early antiretroviral therapy, Curr. Opin. HIV AIDS 11 (2) (2016) 163–172, https://doi.org/10.1097/COH.0000000000000228.
[10] T.A. Crowell, et al., Initiation of antiretroviral therapy before detection of colonic infiltration by HIV reduces viral reservoirs, inflammation and immune activation, J. Int. AIDS Soc. 19 (1) (2016), 21163, https://doi.org/10.7448/IAS.19.1.21163.
[11] L. Cassetta, et al., Differential expansion of circulating human MDSC subsets in patients with cancer, infection and inflammation, J. Immunother. Cancer 8 (2) (2020), e001223, https://doi.org/10.1136/jitc-2020-001223.
[12] F. Veglia, M. Perego, D. Gabrilovich, Myeloid-derived suppressor cells coming of age, Nat. Immunol. 19 (2) (2018) 108–119, https://doi.org/10.1038/s41590-017-0022-x.
[13] F. Veglia, E. Sanseviero, D.I. Gabrilovich, Myeloid-derived suppressor cells in the era of increasing myeloid cell diversity, Nat. Rev. Immunol. 21 (8) (2021) 485–498, https://doi.org/10.1038/s41577-020-00490-y.
[14] K. Nakamura, M.J. Smyth, Myeloid immunosuppression and immune checkpoints in the tumor microenvironment, Cell. Mol. Immunol. 17 (1) (2020) 1–12, https://doi.org/10.1038/s41423-019-0306-1.
[15] A. Hou, et al., Targeting myeloid-derived suppressor cell, a promising strategy to overcome resistance to immune checkpoint inhibitors, Front. Immunol. 11 (2020) 783, https://doi.org/10.3389/fimmu.2020.00783.
[16] M.Z. Noman, et al., PD-L1 is a novel direct target of HIF-1alpha, and its blockade under hypoxia enhanced MDSC-mediated T cell activation, J. Exp. Med. 211 (5) (2014) 781–790, https://doi.org/10.1084/jem.20131916.
[17] N.L. Bowers, et al., Immune suppression by neutrophils in HIV-1 infection: role of PD-L1/PD-1 pathway, PLoS Pathog. 10 (3) (2014), e1003993, https://doi.org/10.1371/journal.ppat.1003993.

[18] T. Vollbrecht, et al., Chronic progressive HIV-1 infection is associated with elevated levels of myeloid-derived suppressor cells, AIDS 26 (12) (2012) F31–F37, https://doi.org/10.1097/QAD.0b013e328354b43f.
[19] C. Agrati, et al., Myeloid derived suppressor cells expansion persists after early ART and may affect CD4 T cell recovery, Front. Immunol. 10 (2019) 1886, https://doi.org/10.3389/fimmu.2019.01886.
[20] N. Tumino, et al., Granulocytic myeloid-derived suppressor cells increased in early phases of primary HIV infection depending on TRAIL plasma level, J. Acquir. Immune Defic. Syndr. 74 (5) (2017) 575–582, https://doi.org/10.1097/QAI.0000000000001283.
[21] L. Wang, et al., Expansion of myeloid-derived suppressor cells promotes differentiation of regulatory T cells in HIV-1+ individuals, AIDS 30 (10) (2016) 1521–1531, https://doi.org/10.1097/QAD.0000000000001083.
[22] N. Tumino, et al., In HIV-positive patients, myeloid-derived suppressor cells induce T-cell anergy by suppressing CD3zeta expression through ELF-1 inhibition, AIDS 29 (18) (2015) 2397–2407, https://doi.org/10.1097/QAD.0000000000000871.
[23] Z.N. Zhang, et al., Myeloid-derived suppressor cells associated with disease progression in primary HIV infection: PD-L1 blockade attenuates inhibition, J. Acquir. Immune Defic. Syndr. 76 (2) (2017) 200–208, https://doi.org/10.1097/QAI.0000000000001471.
[24] R. Singh, et al., Residual immune activation in HIV-Infected individuals expands monocytic-myeloid derived suppressor cells, Cell. Immunol. 362 (2021), 104304, https://doi.org/10.1016/j.cellimm.2021.104304.
[25] J. Zhang, et al., Long noncoding RNA HOTAIRM1 promotes myeloid-derived suppressor cell expansion and suppressive functions through up-regulating HOXA1 expression during latent HIV infection, AIDS 34 (15) (2020) 2211–2221, https://doi.org/10.1097/QAD.0000000000002700.
[26] M. Marquez-Coello, et al., Antiretroviral therapy partially improves the abnormalities of dendritic cells and lymphoid and myeloid regulatory populations in recently infected HIV patients, Sci. Rep. 9 (1) (2019), 11654, https://doi.org/10.1038/s41598-019-48185-2.
[27] A.H. Lei, et al., Clinical significance of myeloid-derived suppressor cells in human immunodeficiency virus-1/hepatitis C virus-coinfected patients, Scand. J. Immunol. 83 (6) (2016) 438–444, https://doi.org/10.1111/sji.12429.
[28] A. Garg, S.A. Spector, HIV type 1 gp120-induced expansion of myeloid derived suppressor cells is dependent on interleukin 6 and suppresses immunity, J. Infect. Dis. 209 (3) (2014) 441–451, https://doi.org/10.1093/infdis/jit469.
[29] A. Qin, et al., Expansion of monocytic myeloid-derived suppressor cells dampens T cell function in HIV-1-seropositive individuals, J. Virol. 87 (3) (2013) 1477–1490, https://doi.org/10.1128/JVI.01759-12.
[30] A. Song, et al., Effects of early and delayed antiretroviral therapy on plasma anti-CD4 autoreactive IgG and its association with CD4(+) T-cell recovery in acute HIV-infected individuals, Front. Pharmacol. 11 (2020) 449, https://doi.org/10.3389/fphar.2020.00449.
[31] A.J. McMichael, et al., The immune response during acute HIV-1 infection: clues for vaccine development, Nat. Rev. Immunol. 10 (1) (2010) 11–23, https://doi.org/10.1038/nri2674.
[32] E.W. Fiebig, et al., Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection, AIDS 17 (13) (2003) 1871–1879, https://doi.org/10.1097/00002030-200309050-00005.
[33] B. Stiksrud, et al., Activated dendritic cells and monocytes in HIV immunological nonresponders: HIV-induced interferon-inducible protein-10 correlates with low future CD4+ recovery, AIDS 33 (7) (2019) 1117–1129, https://doi.org/10.1097/QAD.0000000000002173.
[34] G.R. Kaufmann, et al., Characteristics, determinants, and clinical relevance of CD4 T cell recovery to <500 cells/microL in HIV type 1-infected individuals receiving potent antiretroviral therapy, Clin. Infect. Dis. 41 (3) (2005) 361–372, https://doi.org/10.1086/431484.
[35] L.A. Kotze, V.N. Leukes, N. Du Plessis, Isolation and functional characterization of myeloid-derived suppressor cells in infections under high containment, Methods Mol. Biol. 2236 (2021) 129–156, https://doi.org/10.1007/978-1-0716-1060-2_12.
[36] M. Younas, et al., Immune activation in the course of HIV-1 infection: causes, phenotypes and persistence under therapy, HIV Med. 17 (2) (2016) 89–105, https://doi.org/10.1111/hiv.12310.
[37] B.J.C. Macatangay, et al., T cells with high PD-1 expression are associated with lower HIV-specific immune responses despite long-term antiretroviral therapy, AIDS 34 (1) (2020) 15–24, https://doi.org/10.1097/QAD.0000000000002406.
[38] F. Porichis, D.E. Kaufmann, Role of PD-1 in HIV pathogenesis and as target for therapy, Curr. HIV AIDS Rep. 9 (1) (2012) 81–90, https://doi.org/10.1007/s11904-011-0106-4.
[39] M. Ademe, Paradoxes in the phenotype, frequency and roles of myeloid-derived suppressor cells during HIV infection, HIV AIDS (Auckl) 12 (2020) 151–156, https://doi.org/10.2147/HIV.S248642.
[40] H.H.S. de Paula, et al., Reduction of inflammation and T cell activation after 6 months of cART initiation during acute, but not in early chronic HIV-1 infection, Retrovirology 15 (1) (2018) 76, https://doi.org/10.1186/s12977-018-0458-6.
[41] L.R. Cockerham, et al., Programmed death-1 expression on CD4(+) and CD8(+) T cells in treated and untreated HIV disease, AIDS 28 (12) (2014) 1749–1758, https://doi.org/10.1097/QAD.0000000000000314.
[42] Y. Ghiglione, et al., PD-1 expression in HIV-specific CD8+ T cells before antiretroviral therapy is associated with HIV persistence, J. Acquir. Immune Defic. Syndr. 80 (1) (2019) 1–6, https://doi.org/10.1097/QAI.0000000000001887.
[43] R. Fromentin, et al., PD-1 blockade potentiates HIV latency reversal ex vivo in CD4(+) T cells from ART-suppressed individuals, Nat. Commun. 10 (1) (2019) 814, https://doi.org/10.1038/s41467-019-08798-7.
[44] M. Pardons, et al., Single-cell characterization and quantification of translation-competent viral reservoirs in treated and untreated HIV infection, PLoS Pathog. 15 (2) (2019), e1007619, https://doi.org/10.1371/journal.ppat.1007619.
[45] E.V. Saidakova, et al., Lymphopenia-induced proliferation of CD4 T-cells is associated with CD4 T-lymphocyte exhaustion in treated HIV-infected patients, Indian J. Med. Res. 147 (4) (2018) 376–383, https://doi.org/10.4103/ijmr.IJMR_1801_15.
[46] H. Li, et al., Dysfunctional CD8 T cells form a proliferative, dynamically regulated compartment within human melanoma, Cell 176 (4) (2019), https://doi.org/10.1016/j.cell.2018.11.043, 775-789.e718.
[47] A. Yadav, et al., Evidence for persistent monocyte and immune dysregulation after prolonged viral suppression despite normalization of monocyte subsets, sCD14 and sCD163 in HIV-infected individuals, Pathog. Immun. 4 (2) (2019) 324–362, https://doi.org/10.20411/pai.v4i2.336.
[48] D.I. Gabrilovich, Myeloid-derived suppressor cells, Cancer Immunol. Res. 5 (1) (2017) 3–8, https://doi.org/10.1158/2326-6066.CIR-16-0297.
[49] I. Rosado-Sanchez, et al., Increased frequencies of myeloid-derived suppressor cells precede immunodiscordance in HIV-infected subjects, Front. Immunol. 11 (2020), 581307, https://doi.org/10.3389/fimmu.2020.581307.
[50] A. Leon-Flores, et al., Increased levels of soluble co-stimulatory molecule PD-L1 (B7-H1) in the plasma of viraemic HIV-1(+) individuals, Immunol. Lett. 203 (2018) 70–79, https://doi.org/10.1016/j.imlet.2018.09.007.