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Over-activation and dysfunction of platelet-NK cell aggregates in HIV-infected individuals

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Abstract

Background Human immunodeficiency virus type 1 (HIV-1) infection is associated with over-activation, which contributes to disease progression. Platelet-leukocyte aggregates play a critical role in HIV-1 infection. However, research on the characteristics of platelet-natural killer (NK) cell aggregates in HIV-infected individuals still has certain limitations.

Methods Platelet-NK cell aggregates in the peripheral blood of participants were detected by flow cytometry and confirmed by imaging flow cytometry. Platelet activation was evaluated by CD62P expression. The expression of various activating and inhibitory receptors, markers of apoptosis, lipid droplets, interferon-gamma (IFN-γ), Granzyme B, and Perforin in platelet-NK cell aggregates were assessed. The signaling lymphocyte activating molecule (SLAM) family receptors in both platelets and NK cells and the levels of phosphorylation signals in NK cells were respectively measured through flow cytometry.

Results In this study, we observed an increase in platelet-NK cell aggregates that were negatively correlated with CD4 count, a prognostic marker for HIV-1 disease progression. Furthermore, platelet activation was inversely associated with both HIV-1 disease progression and the platelet-NK cell aggregates. However, antiretroviral therapy (ART) couldn't restore the levels of these aggregates or platelet activation. Compared to platelet-free NK cells, platelet-NK cell aggregates exhibited over-activation (CD69) and exhaustion phenotypes (CD39, LAG-3, PD-1), increased levels of apoptosis (Annexin V and CD95) and lipid droplets (Bodipy 493/503 and LipidTOX). Furthermore, NK cells' cytokine secretion (IFN-γ) and cytotoxic function (Granzyme B and Perforin) within the aggregates were declined. Screening results of SLAM receptors in NK cells and platelets suggested that platelets may transmit signals to NK cells via SLAMF5. Moreover, elevated levels of p-Fyn, p-PLC-γ2, p-SHP-1, and p-SHP-2 denoted disturbances in the downstream signals of the SLAM family within platelet-NK cell aggregates.

Conclusion Our study indicates that platelet-NK cell aggregates exhibit characteristics of over-activation and dysfunction during HIV-1 infection. Hyperactivated platelets and the formation of platelet-NK cell aggregates contribute to the HIV-1 disease progression and the inflammation of the immune system. These findings may implicate potential targets of overactivated platelets for HIV-1 disease progression.

Keywords Platelet, NK cell, Platelet-NK cell aggregates, HIV-1, SLAMF5

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Introduction

Although antiretroviral therapy (ART) effectively controls human immunodeficiency virus (HIV) replication, a residual chronic immune activation persists throughout the disease [1, 2]. Platelets, which have important functions in thrombosis and modulation of immune responses, are also over-activated during HIV-1 infection [3]. Activated platelets, which could mediate inflammatory and immune responses [4–6], interact with leukocytes via P-selectin (CD62P) binding to P-selectin glycoprotein ligand-1 (PSGL-1) [3].

Various platelet-leukocyte aggregates have been reported in HIV-1 infection. Platelet binding modified the characteristics of permissiveness for T cells to HIV-1 infection [7]. Platelet-T cell aggregates exhibited higher levels of HIV co-receptors (CCR5 and CXCR4) and were positively correlated with markers of immune activation (plasma sCD163 and sCD14) during HIV-1 infection. Furthermore, these aggregates demonstrated increased activation levels, indicated by CD38 and HLA-DR co-expression, when compared to platelet-free T cells. Additionally, these aggregates displayed elevated levels of caspase-1 and caspase-3, alongside reduced levels of Bcl-2, implying a tendency towards cell death and a potential role in T cell loss during HIV-1 infection [7, 8]. Increased levels of platelet-derived sCD40L contribute to HIV-associated neuroinflammation by inducing platelet-monocyte aggregates (PMA) and facilitating infiltration through the blood–brain barrier [9, 10]. Moreover, a heightened immune activation status was correlated with increased PMA [11]. Meanwhile, the formation of PMA led to monocyte activation and was associated with the secretion of pro-inflammatory cytokines, as well as the upregulation of adhesion molecules and tissue factor expression [4, 12, 13]. While various platelet-leukocyte aggregates exhibit distinct characteristics, comprehensive research on platelet-natural killer (NK) cell aggregates in the context of HIV-1 infection remains incomplete.

It also remains unclear whether platelets transmit signals to NK cells and which specific molecules are involved. The signaling lymphocyte activating molecule (SLAM) family is a group of hematopoietic cell-specific receptors and plays a crucial role in cellular adhesion, immune recognition, NK cell function, and platelet activation [14, 15]. Shaobo Wang analyzed the impact of HIV infection on immune cell exhaustion through single-cell RNA sequencing (scRNA-seq) [16]. They identified a subpopulation of exhausted $CD8^+$ T cells (CD8-Tex), with SH2D1A (encoding SAP) specifically upregulated. In addition, it was found that HIV infection induced the upregulation of SLAMF5 and SLAMF7 in NK cells [16]. Given that SAP is the downstream signal of SLAM receptor family proteins, and that SLAMF5 and SLAMF7 are members of the SLAM family, further studies are necessary to elucidate the role of SLAM family proteins in the interaction between platelets and NK cells.

In this study, we demonstrated an increased proportion of platelet-NK cell aggregates in HIV-infected individuals, which was negatively correlated with CD4 count, an indicator of disease progression. Additionally, platelet activation was negatively associated with both HIV-1 disease progression and the formation of platelet-NK cell aggregates. Notably, ART failed to reduce the levels of platelet-NK cell aggregates or platelet activation. Compared to platelet-free NK cells, platelet-NK cell aggregates possessed over-activated (CD69) and exhausted phenotypes (CD39, LAG-3, PD-1), along with higher levels of apoptosis (Annexin V and CD95) and lipid droplets (Bodipy 493/503 and LipidTOX). Furthermore, cytokine secretion (IFN-γ) and cytotoxic function (Granzyme B and Perforin) of NK cells among the aggregates were diminished, implying functional exhaustion of NK cells among these aggregates. Screening revealed that both NK cells and platelets could express SLAMF5, indicating that platelets may transmit signals to NK cells via SLAMF5. Subsequently, we assessed the downstream phosphorylation signals of the SLAM family and found that platelet-NK cell aggregates exhibited elevated levels of both activating-related signals (p-Fyn and p-PLC-γ2) and inhibitory-related signals (p-SHP-1 and p-SHP-2), denoting disturbances in the downstream phosphorylation levels of the SLAM family within these aggregates.

Methods

Participants

A cohort of participants was recruited from the Beijing Ditan Hospital, including 81 treatment-naïve patients (TNs), 32 ART-experienced individuals (ARTs), and 9 gender and age-matched healthy donors (HDs). TNs were defined as being diagnosed with HIV-1 infection and mostly received ART within one week after their diagnosis. ARTs, who had received antiretroviral therapy for more than 4 years with viral suppression (HIV-RNA < 50 copies/ml), included 14 immunological responders (IRs, with CD4 count ≥ 350 cells/μl) and 18 non-immunological responders (INRs, with CD4 count < 350 cells/μl). Exclusion criteria were pregnancy, individuals below 18 years old, lack of good adherence, active hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, severe hepatic or renal impairment, opportunistic infections, and other concomitant diseases such as autoimmune disease, malignancy, and cardiovascular diseases. The baseline characteristics of the participants are listed in Table 1.

Table 1 Characteristics of cohort

Parameters N or Median (IQR)	HDs	TNs			ARTs	IRs	INRs
		CD4 ≥ 350	200 ≤ CD4 < 350	CD4 < 200			
Gender (Male/Female)	8/1	26/4	22/1	28/0	28/4	13/1	15/3
Age (years)	37 (27–45)	33 (25–38)	35 (29–40)	38 (29–38)	39 (36–54)	39 (36–52)	39 (36–57)
CD4 count(cells/μl)	–	514 (435–564)	290 (274–320)	68 (15–114)	327 (179–450)	476 (432–793)	250 (118–300)
CD4/CD8 ratio	–	0.43 (0.3–0.49)	0.34 (0.2–0.43)	0.13 (0.04–0.18)	0.55 (0.33–0.76)	0.74 (0.56–1.01)	0.39 (0.25–0.65)
Virus load (log copies/mL)		4.51 (4.38–5.03)	4.43 (4.05–4.75)	5.29 (4.81–5.79)	< LDL	< LDL	< LDL

N number of individuals, *IQR* interquartile range, *HDs* healthy controls, *TNs* treatment-naïve HIV-1-infected individuals, *IRs* immunologic responders, *INRs* immune non-responders, *LDL* lower detection limit

Isolation of peripheral blood mononuclear cells (PBMCs) and platelets

Peripheral blood samples were collected into acid-citrate dextrose tubes using standard Ficoll-Paque gradient centrifugation according to the instructions of the manufacturer (Amersham Pharmacia Biotech, Sweden).

Citrated blood, plus prostaglandin E1 (PGE1), was centrifuged for 15 min at 150 g and the upper layer was harvested as platelet-rich plasma (PRP). PRP was washed twice with CGS buffer (adding PGE1), with centrifuging for 10 min at 700 g. Platelets resuspended in CGS buffer.

Plasma HIV-1 viral load and CD4 count

The HIV-1-RNA levels in plasma were quantified by a Standard Amplicor HIV Monitor assay, version 1.5 (Roche Diagnostics, Indianapolis, IN, USA), with a limit of detection of 50 copies/ml. The CD4 count was measured by a standard flow cytometry technique with a TruCOUNT tube in routinely equipped laboratories (BD Biosciences, San Jose, CA, USA).

Immunofluorescence staining and flow cytometry analysis

PBMCs from participants were incubated with directly conjugated antibodies for 30 min at 4 °C. Antibodies used included anti-human CD3-BUV737; CD14-BV650; CD16-PE-CY7; CD57-BV421; CD62P-APC; CD69-PE-CF594; CD95-PE-CY7; CD158b-BUV737; PD-1-BV711; LAG-3-APC-H7; SLAMF1-BV421; SLAMF2-BV421; 7-AAD-Percp (BD Biosciences, San Diego, CA, USA), CD19-BV650; CD39-BV605; CD41-BV510; CD56-BV510; KLRG-1-APC-Fire750; NKG2 A-APC-Fire750; NKG2 C-PE; NKG2D-PE; NKp30-PE-CF594; NKp46-AF700; SLAMF4-PE-CF594; SLAMF5-PE; SLAMF7-APC; Annexin V-FITC (BioLegend, San Diego, CA, USA), SLAMF3-APC; SLAMF6-PE; TIGIT-PE-CY7 (Ebioscience, San Diego, CA, USA), along with the corresponding isotype controls. For intracellular staining of Ki67-FITC, Perforin-PE-D594, Granzyme B-AF700 (BD Biosciences, San Diego, CA, USA), cells were fixed and permeabilized using Transcription Factor Buffer Set (BD Biosciences, San Diego, CA, USA) according to the manufacturer's recommendations. A fixable viability dye eFluor 506 (Ebioscience, San Diego, CA, USA) was used to assess cell viability. For intracellular lipid droplet assays, incubated for 30 min at 37 °C with Bodipy 493/503 or LipidTOX (both Ebioscience, San Diego, CA, USA) after surface staining. Data were acquired with the LSR Fortessa flow cytometer (BD Biosciences) and analyzed with FlowJo software version 10.5 (Tree Star, Ashland, OR, USA).

Imaging flow cytometry

Cells were stained with antibodies at 4 °C for 30 min. Platelet-NK cell aggregate formation and SLAMF5 expression were acquired at 60 ×magnification on an Amnis ImageStream^X Mk II and analyzed using IDEAS 6.2 software (both Merck Millipore, Billerica, MA, USA).

Determination of cytokine production

PBMCs were cultured with IL-12 (PeproTech, London, UK), IL-15(PeproTech, London, UK), and IL-18(MBL, Nagoya, Japan) for 4 h, in the presence of Golgiplug (BD Biosciences, San Diego, CA, USA), according to the manufacturer's instructions. After surface-staining, the cells were intracellularly stained with IFN-γ-AF700 (eBioscience, San Diego CA, USA), using a Cytofix/Cytoperm and Perm/Wash kit from BD Biosciences. Data were collected by flow cytometry.

Measurement of phosphorylation

Fixation, permeabilization, and staining were carried out according to the manufacturer's phosflow protocol (BD Biosciences, San Diego, CA, USA). The cells were fixed with pre-warmed BD Phosflow™ Fix Buffer I (BD Biosciences, San Diego, CA, USA) for 10 min at 37 °C, then permeabilized with BD Phosflow™ Perm Buffer III (BD Biosciences, San Diego, CA, USA) on ice for at least 10 min, and then stained with antibodies. Antibodies included anti-human p-Fyn-AF647 (Signalway Antibody,

College Park, MD, USA), p-PLC-γ1-PE (BioLegend, San Diego, CA, USA), and p-SHP-1-APC (Ebioscience, San Diego, CA, USA); p- PLC-γ2-AF488; p-SHP-2-PE (BD Biosciences, San Diego, CA, USA). Data were measured by LSR Fortessa flow cytometer (BD Biosciences).

Statistical analysis

The data are expressed as the mean ± standard deviation (SD). All data were analyzed using GraphPad Prism 7 (GraphPad Software, La Jolla, CA, USA). The normality of each variable was assessed using the Kolmogorov–Smirnov test. For two normally distributed data, the comparison of variables was performed using unpaired or paired two-tailed Student's t-tests. A one-way ANOVA or repeated-measures ANOVA followed by Tukey's multiple comparisons test was employed to compare more than two independent samples. When the data were not normally distributed, comparisons were analyzed with the Mann–Whitney U test or Wilcoxon matched-pairs signed rank test for unpaired and paired data, respectively. For comparing more than two independent samples, Kruskal-Walli's test followed by Dunn's multiple comparisons test was applied. Correlation coefficients were calculated for nonparametric distributions using Spearman's correlation test. The Chi-square test was utilized to compare categorical variables. $P < 0.05$ was considered statistically significant.

Results

The increased platelet-NK cell aggregates were associated with HIV-1 disease progression

CD41 was a specific marker expressed on platelets in peripheral blood. Flow cytometry showed the population of $CD41^+$ NK cells (platelet-NK cell aggregates) and $CD41^-$ NK cells (platelet-free NK cells) (gating strategy in Additional file: Fig. S1). In comparison to healthy donors (HDs), a significant increase of platelet-NK cell aggregates was observed in treatment-naive individuals (TNs), particularly in severely ill individuals with a CD4 count of less than 200 cells/μl (Fig. 1A, B). Moreover, the rates of these aggregates in TNs showed a negative correlation with CD4 count ($r = -0.4253$, $p < 0.0001$) and the CD4/CD8 ratio ($r = -0.2466$, $p = 0.0264$) (Fig. 1C, D. However, no significant correlation was identified between the rates of platelet-NK cell aggregates and virus load ($r = 0.1014$, $p = 0.3710$) or platelet count ($r = 0.0967$, $p = 0.3904$) (Fig. 1E, F). Additionally, the expression of CD69, an activation marker, was higher in platelet-NK cell aggregates than in platelet-free NK cells (Fig. 1G), suggesting that NK cells in aggregates were more activated. Furthermore, CD62P served as a marker of platelet activation. To assess the status of platelet activation in these aggregates, we measured the proportion of $CD62P^+$ cells among the platelet-NK cell aggregates by flow cytometry, finding that approximately 59.6% of the aggregates consisted of activated platelet-NK cell aggregates (Fig. 1H). Furthermore, we utilized immunofluorescence imaging to confirm the presence of platelet-NK cell aggregates in TNs (Fig. 1I). According to the expression of CD56 and CD16, NK cells are generally divided into three subsets: $CD56^{bright}CD16^-$, $CD56^{dim}CD16^+$, and $CD56^{neg}CD16^+$. The data revealed that there was no significant difference in the distribution of platelet-NK cell aggregates among the three NK cell subsets. Additionally, within both $CD41^-$ NK cells and $CD41^+$ NK cells, no differences were observed in the distribution of the three NK cell subsets, suggesting that the formation of aggregates occurs without bias towards any specific subgroup (Fig. 1J). Conclusively, these data implied that the formation of platelet-NK cell aggregates, which were associated with HIV-1 disease progression, may promote the activation of platelets and NK cells.

Hyperactivation of platelets contributed to HIV-1 disease progression and the formation of platelet-NK cell aggregates

Subsequently, we measured the levels of platelet activation by flow cytometry. Whether in terms of percentage or the mean fluorescence intensity (MFI) of CD62P in platelets, TNs displayed markedly higher expression of CD62P in platelets compared to HDs (Fig. 2A). To further explore potential factors affecting platelet activation in TNs, we investigated the relationship between CD62P expression in platelets and factors that related to disease progression, including CD4 count, virus load, and platelet count. The expression of CD62P in platelets was inversely correlated to CD4 count (%: $r = -0.4080$, $P = 0.0347$; MFI: $r = -0.5092$, $P = 0.0067$) (Fig. 2B). A positive correlation trend was observed between CD62P expression in platelets and viral load in TNs, but with no statistical significance (%: $r = 0.4444$, $P = 0.1975$; MFI: $r = 0.3458$, $P = 0.1887$) (Fig. 2C). There was no correlation between platelet activation and platelet count (%: $r = 0.1318$, $P = 0.5123$; MFI: $r = 0.0049$, $P = 0.9807$) (Fig. 2D). Additionally, a negative correlation was presented between platelet activation and NK cell count (%: $r = -0.3838$, $P = 0.0481$; MFI: $r = -0.5029$, $P = 0.0075$) (Fig. 2E). Notably, a significant positive correlation was identified between platelet activation and the formation of platelet-NK cell aggregates in TNs (%: $r = 0.4903$, $P = 0.0094$; MFI: $r = 0.3874$, $P = 0.0459$), signifying that platelet activation facilitated the formation of these aggregates (Fig. 2F). Overall, these data signified that platelet activation was associated with HIV-1 disease progression and promoted the formation of platelet-NK cell aggregates.

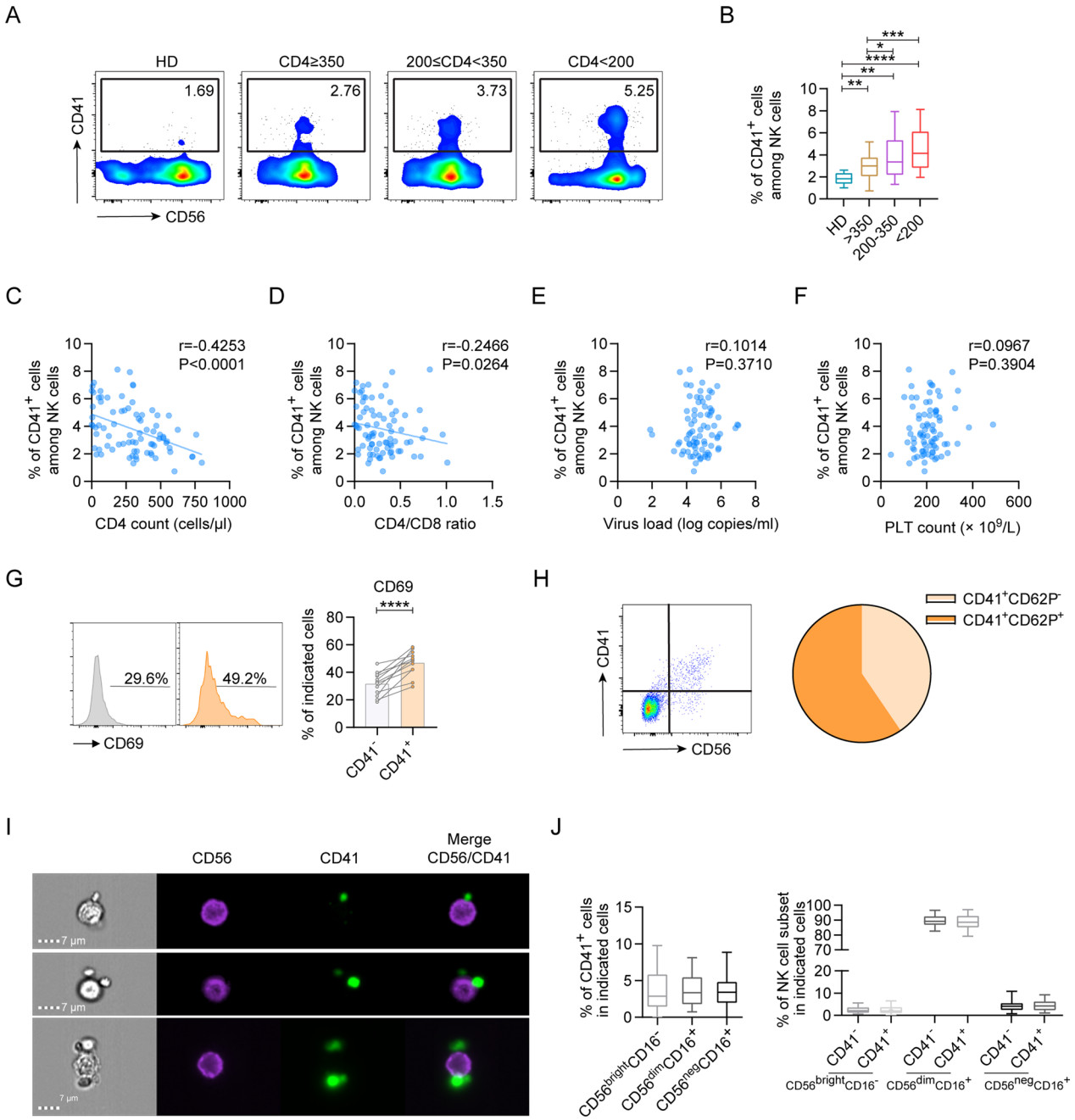

Fig. 1 The increased platelet-NK cell aggregates were associated with HIV-1 disease progression. **A**, **B** Representative flow data (**A**) and box plots (**B**) of the rates of platelet-NK cell aggregates gated on NK cells from different groups (n = 9–81 for each group). **C**–**F** Correlations between the rates of platelet-NK cell aggregates among NK cells and CD4 count (**C**), CD4/CD8 ratio (**D**), virus load (**E**) and platelet count (**F**) in TNs (n = 81). **G** Representative histograms (left) and plots (right) displayed the percentages of $CD69^+$ cells in platelet-free NK cells vs. platelet-NK cell aggregates (n = 12). **H** Representative flow data (left) and pie chart (right) showed the expression of CD62P in platelets. Data were shown as the median. **I** Representative image of platelet-NK cell aggregates obtained from imaging flow cytometry. NK cells (purple) and platelets (green) **J** Box plots for rates of platelet-NK cell aggregates among NK cell subsets (left) and the distribution of the three NK cell subsets in both $CD41^-$NK cells and $CD41^+$NK cells (right) (n = 32). P values were obtained by the Kruskal–Wallis test followed by Dunn's multiple comparisons test or a paired two-tailed Student's t-test. Spearman's non-parametric test was used to test for correlations. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001

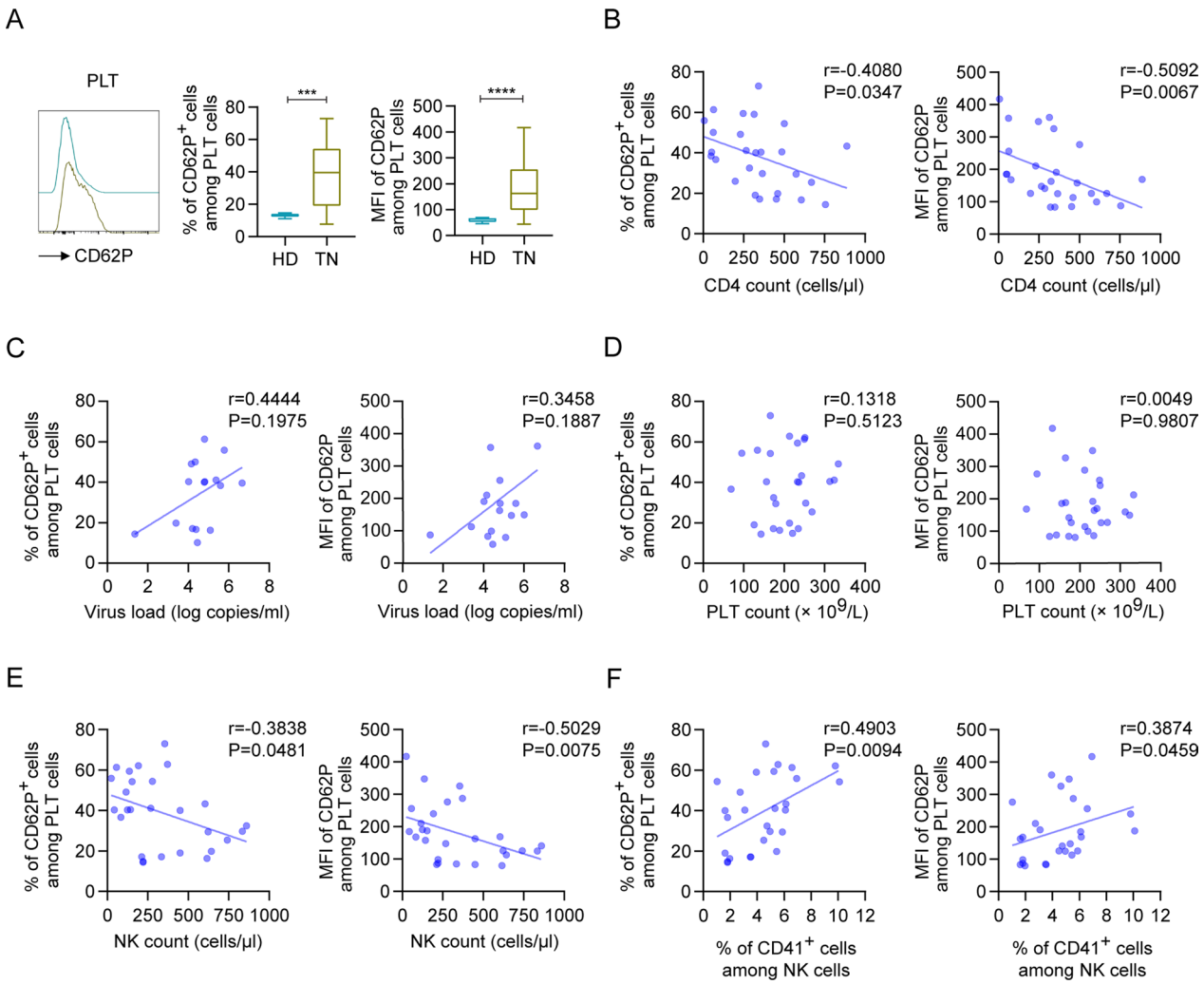

Fig. 2 Hyperactivation of platelets contributed to HIV-1 disease progression and the formation of platelet-NK cell aggregates. **A** Representative histograms (left) and box plots displayed the percentages of $CD62P^+$ cells (middle) and the MFI of CD62P (right) in platelets from HDs (n = 9) and TNs (n = 27). **B**–**F** Correlations between the percentages of $CD62P^+$ cells (left) and the MFI of CD62P (right) among platelets and CD4 count (**B**), virus load (**C**), PLT count (**D**), NK count (**E**), the rates of platelet-NK cell aggregates (**F**) from TNs (**B**, **D**–**F**: n = 27; **C**: n = 17). P values were obtained by the Kruskal–Wallis test followed by Dunn's multiple comparisons test. Spearman's non-parametric test was used to test for correlations. ***P < 0.001, ****P < 0.0001

INRs exhibited higher levels of platelet activation and platelet-NK cell aggregates than IRs

Furthermore, we evaluated the impact of antiretroviral therapy (ART) on platelet activation and the formation of platelet-NK cell aggregates. There was a trend indicating that immunological responders (IRs) displayed lower levels of CD62P compared to TNs. Immunological non-responders (INRs) exhibited a higher expression of CD62P in platelets than IRs (Fig. 3A). Additionally, the levels of platelet-NK cell aggregates in IRs were lower than those in TNs. INRs showed increased rates of platelet-NK cell aggregates in comparison to IRs (Fig. 3B). Apparently, the rates of platelet-NK cell aggregates in ARTs were negatively relevant to CD4 count (r = −0.6487, P < 0.0001) and the CD4/CD8 ratio (r = −0.4714, P = 0.0065) (Fig. 3C, D). Regardless of IRs or INRs, the expression of CD69 in platelet-NK cell aggregates was higher than in platelet-free NK cells (Fig. 3E). Collectively, these data illustrated that increased platelet activation and frequencies of platelet-NK cell aggregates in ARTs were associated with poor immune reconstitution.

Platelet-NK cell aggregates were characterized by exhaustion and functional impairment

Next, we described the phenotypic and functional characteristics of platelet-NK cell aggregates. Platelet-NK

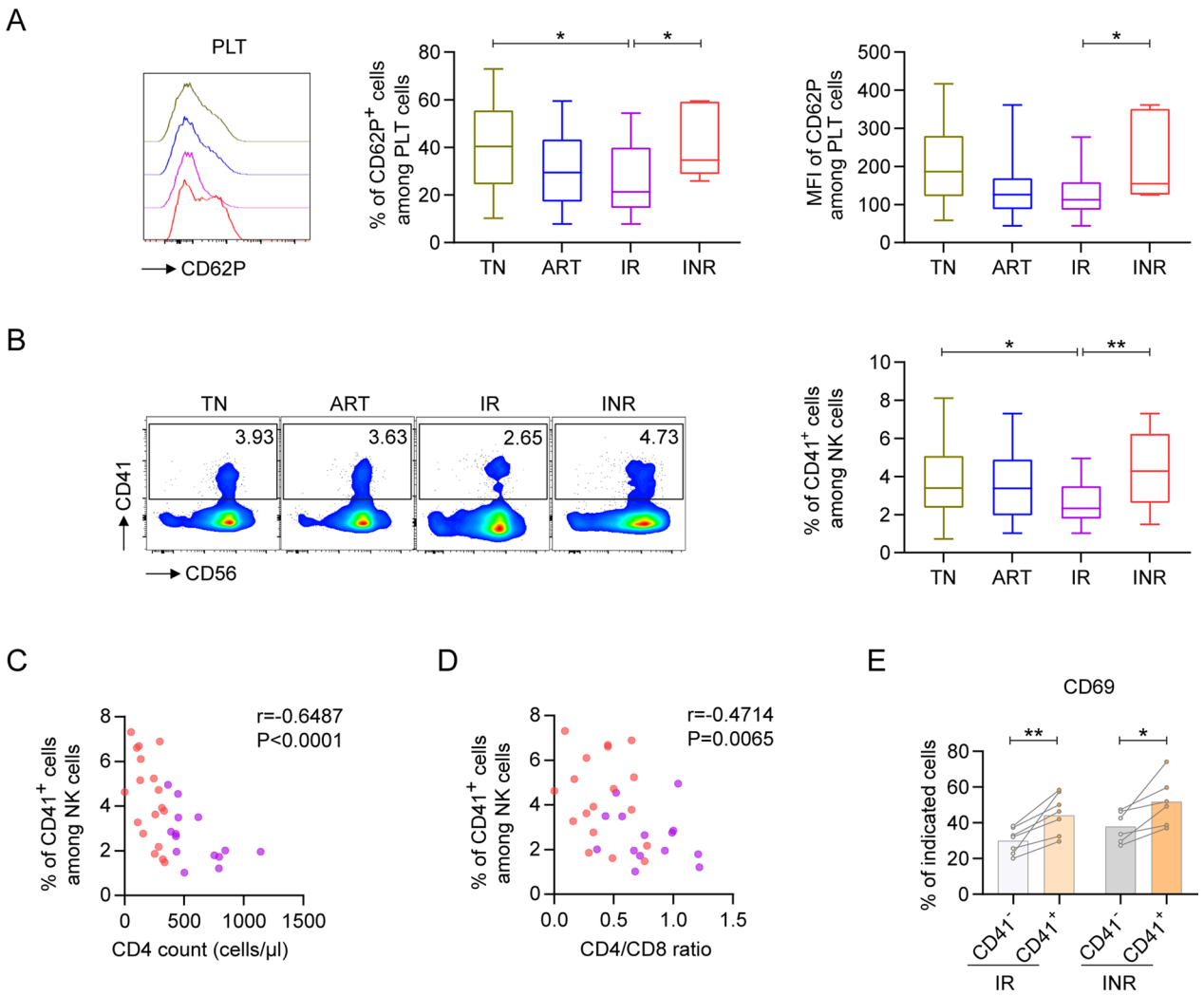

Fig. 3 INRs exhibited higher levels of platelet activation and platelet-NK cell aggregates than IRs. **A** Representative histograms (left) and box plots displayed the percentages of $CD62P^+$ cells (middle) and the MFI of CD62P (right) in platelets from different groups (n = 6–20 each group). **B** Representative flow data (left) and box plots (right) displayed the rates of platelet-NK cell aggregates from different groups (n = 14–81 each group). **C**, **D** Correlations between the rates of platelet-NK cell aggregates and CD4 count (**E**) or CD4/CD8 ratio (**F**) from IRs (n = 14) and INRs (n = 18). **E** Plots displayed the percentages of $CD69^+$ cells among platelet-free NK cells vs. platelet-NK cell aggregates (n = 6). P values were obtained by the Kruskal–Wallis test followed by Dunn's multiple comparisons test or paired t-test. Spearman's non-parametric test was used for correlation analysis. *P < 0.05, **P < 0.01

cell aggregates showed elevated expression of CD69, decreased expression of activating receptors (NKG2D and NKp46), and increased expression of inhibitory receptors (CD39, LAG-3, and PD-1) (Fig. 4A). Apoptosis was evaluated by Annexin V and CD95 expression. Compared to platelet-free NK cells, the percentages of Annexin V^+ cells and the MFI of CD95 in platelet-NK cell aggregates were significantly increased (Fig. 4B, C). To evaluate lipid metabolism within these aggregates, Bodipy 493/503 or LipidTOX were incubated for 30 min with cells after surface staining to measure the levels of intracellular lipid droplets. These aggregates showed increased MFI of Bodipy 493/503 and LipidTOX, denoting higher levels of intracellular lipid droplets than platelet-free NK cells (Fig. 4D, E). Next, we investigated the effect of platelets on NK cell function. Platelet-NK cell aggregates displayed reduced expression of interferon-gamma (IFN-γ), Granzyme B, and Perforin compared to platelet-free NK cells, while no significant difference was observed in Ki67 (Fig. 4F–I). Overall, these data emphasized that the platelet-NK cell aggregates were exhausted and dysfunctional, likely reflecting a state of hyperactivation following multiple cell-to-cell interactions.

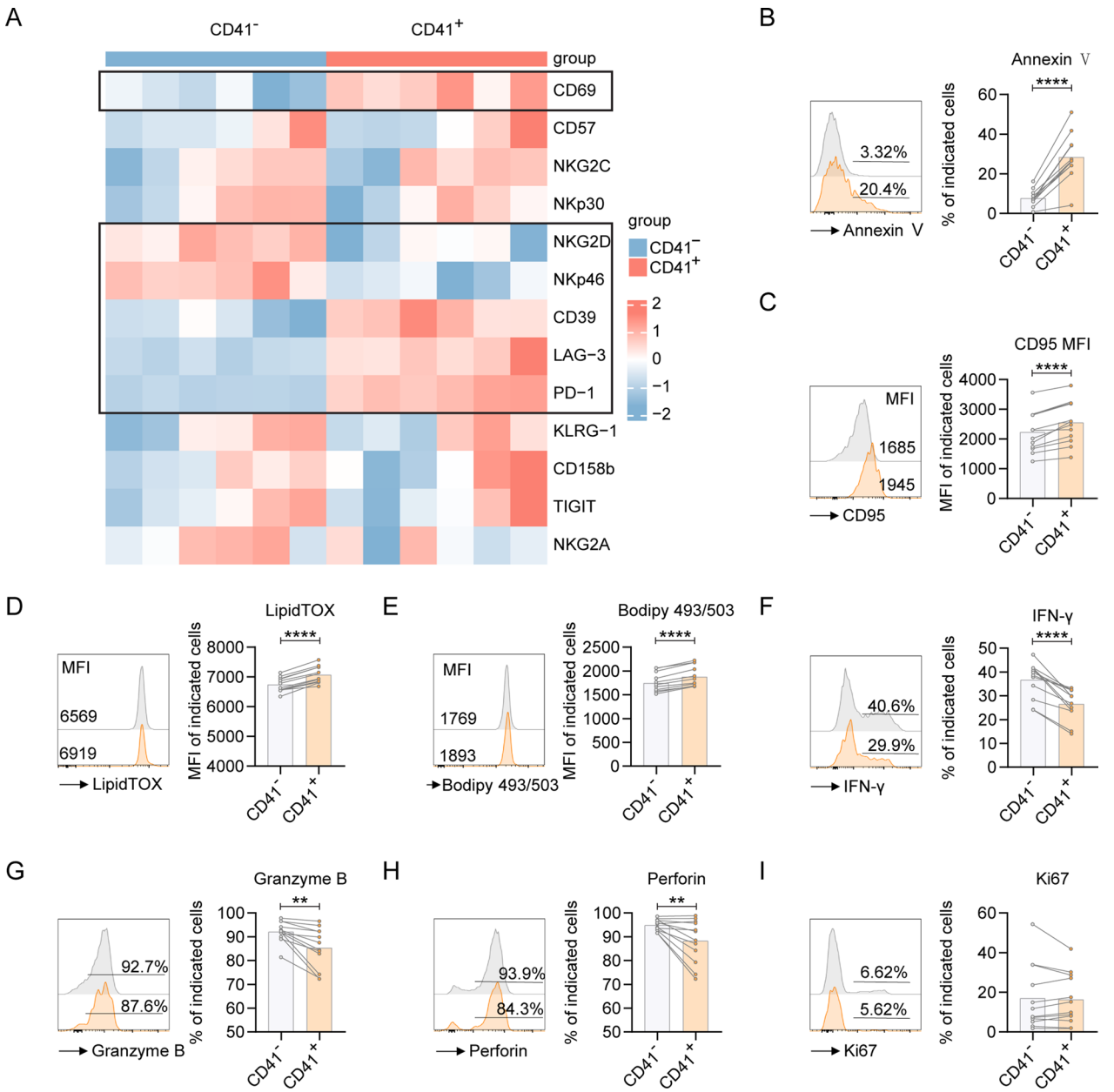

Fig. 4 Platelet-NK cell aggregates were characterized by exhaustion and functional impairment. **A** Heatmap showed the expression of activating and inhibitory receptors in platelet-free NK cells vs. platelet-NK cell aggregates (n = 5). **B**–**I** Representative histograms (left) and plots (right) displayed the expression of Annexin V (**B**), CD95 (**C**), LipidTOX (**D**), Bodipy 493/503 (**E**), IFN-γ (**F**), Granzyme B (**G**), Perforin (**H**), and Ki67 (**I**) in platelet-free NK cells vs. platelet-NK cell aggregates (n = 5). P values were obtained by paired t-test or Wilcoxon matched-pairs signed rank test. **P < 0.01, ****P < 0.0001

Platelets might induce signaling disturbances in NK cells via SLAMF5 among HIV-infected individuals

The signaling lymphocyte activating molecule (SLAM) family receptors serve as self-ligands, except SLAMF2 interacts with SLAMF4. We examined the expression of SLAM family receptors in isolated platelets and NK cells respectively. It was found that platelets partially expressed SLAMF4 and highly expressed SLAMF5. NK cells expressed varying degrees of SLAMF2-7, except SLAMF1 (Fig. 5A). Without SLAMF2, platelets couldn't transmit signals to NK cells via SLAMF2-SLAMF4. Then, we screened for SLAMF5 and verified through imaging flow cytometry that NK cells and platelets could express SLAMF5 (Fig. 5B). Additionally, compared to HDs, an

increased expression of SLAMF5 in platelets, not in NK cells, was observed among TNs (Fig. 5C, D). These results suggested that platelets may transmit signals to NK cells through SLAMF5.

The human SLAM-associated protein (SAP) family adapters include SAP and EAT-2 (Ewing's sarcoma-associated transcript 2), which exhibit a dual mechanism of action. Upon ligand stimulation of SLAM family receptors, SAP and EAT-2 facilitate the downstream transmission of activation signals. Conversely, in the absence of SAP and EAT-2, these connectors link to inhibitory phosphatases such as SHP-1, SHP-2, and SHIP-1, leading to the transmission of inhibitory signals. Subsequently, we assessed the downstream phosphorylation signals of the SLAM family by flow cytometry. Compared to platelet-free NK cells, platelet-NK cell aggregates exhibited elevated levels of activation-related signals (p-Fyn and p-PLC-γ2) and inhibition-related signals (p-SHP-1 and p-SHP-2), while p-PLC-γ1 showed no significant changes (Fig. 5E–I). Totally, the downstream phosphorylation signals of the SLAM family in platelet-NK cell aggregates indicated signaling disturbances in HIV-infected individuals.

Discussion

Rather than concentrating on the traditional functions of platelets in coagulation and hemostasis, we emphasized platelets' specific roles in activation and immunomodulation. Our findings demonstrated that increased platelet-NK cell aggregates were associated with HIV-1 disease progression. The aberrant platelet activation in HIV-infected individuals was correlated with both HIV-1 disease progression and the formation of platelet-NK cell aggregates. Generally, dysfunction in NK cells would impair the anti-infection and anti-tumor abilities of HIV-infected individuals [17]. In our study, the platelet-NK cell aggregates exhibited excessive activation, exhaustion, and functional impairment. Furthermore, platelets may transmit signals to NK cells via SLAMF5, leading to signaling disturbances in platelet-NK cell aggregates. Hence, platelet-NK cell aggregates may serve as valuable biomarkers for monitoring immune activation and as robust indicators of HIV-1 disease progression.

In a previous study of COVID-19 [18], platelet-NK cell aggregates were identified through scRNA-seq, and the rates of these aggregates were related to disease severity. According to the maturation score, platelet-NK cell aggregates exhibited a mature phenotype. However, in our study, there was no significant difference in the distribution of platelet-NK cell aggregates among the three NK cell subsets. This discrepancy may be attributed to the different disease models. There was an increase in platelet-NK cell aggregates in HIV-infected individuals, which was negatively correlated with disease progression. However, our findings were derived solely from a cross-sectional analysis. We speculated that inflammation or coagulation activation associated with the HIV-1 virus may induce the aggregation of platelet-NK cells. Activated platelets can interact with NK cells, potentially inhibiting the antiviral activity of these immune cells [19–22]. For instance, platelets may impair NK cell cytotoxicity and interfere with the production of IFN-γ by secreting transforming growth factor-beta (TGF-β) and down-regulating NKG2D [23–26]. This interaction could contribute to disease progression, creating a detrimental cycle. HIV-1 infection profoundly impacts NK cells, leading to heightened cellular activation, alterations in the NK cell receptor repertoire, and changes in NK cell metabolism and function [27–29]. Our study demonstrated that platelet-NK cell aggregates displayed over-activated and exhausted phenotypes, along with increased apoptotic markers and intracellular lipid droplet levels, and diminished functionality. These findings indicated that NK cells within the aggregates were in a state of exhaustion, which probably reflects the consequence of hyperactivation of NK cells during HIV-1 infection. Furthermore, platelet activation in turn contributes to persistent immune activation and an inflammatory vascular environment. Although HIV-1 can directly activate platelets [30], other mechanisms for sustained platelet activation should be considered, as our data indicated that platelet activation was also observed despite ART-mediated viral suppression. Further studies are necessary to enhance our understanding of platelet activation in the context of HIV-1 infection.

(See figure on next page.)

Fig. 5 Platelets might induce signaling disturbances in NK cells via SLAMF5 among HIV-infected individuals. **A** Representative histograms displayed the expression of SLAM receptors in platelets (left) and NK cells (right). The labeling of SLAM receptors is represented in grass green, and the fluorescence minus one (FMO) control was in gray. **B** Representative image of SLAMF5 expression obtained from imaging flow cytometry. NK cells (purple), SLAMF5 (yellow), and platelets (green). **C**, **D** Plots displayed the expression of different SLAM receptors in platelets (**C**) and NK cells (**D**) from HDs and TNs (n = 4–8 in each group). **E**–**I** Representative histograms (left) and plots (right) displayed the MFI of p-Fyn (**E**), p-PLC-γ1 (**F**), p-PLC-γ2 (G), p-SHP-1 (**H**), and p-SHP-2 (**I**) in platelet-free NK cells vs. platelet-NK cell aggregates (n = 12). P values were obtained by the Kruskal–Wallis test followed by Dunn's multiple comparisons test and paired t-test or Wilcoxon matched-pairs signed rank test. **P < 0.01, ***P < 0.001

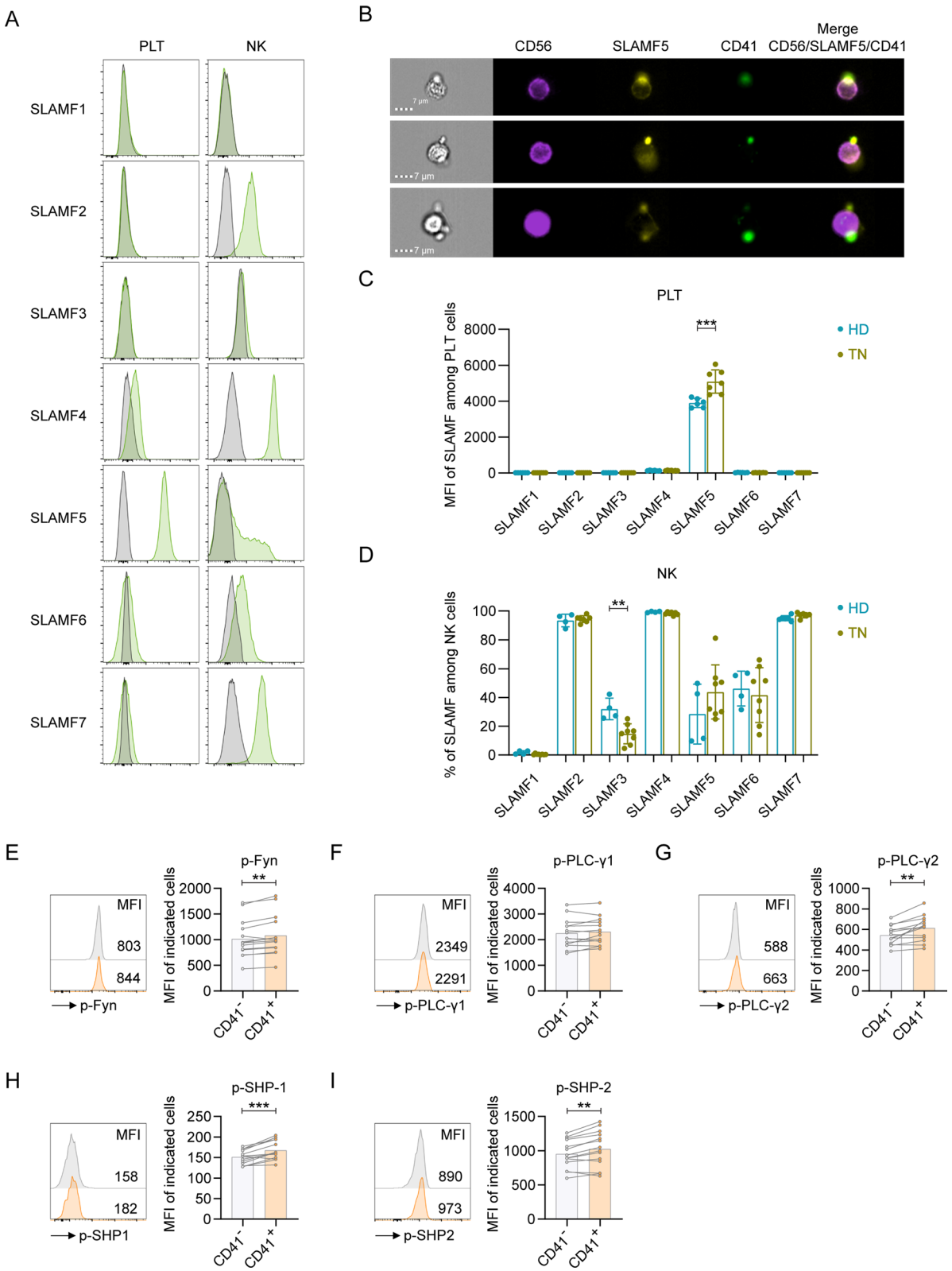

Fig. 5 (See legend on previous page.)

The linking between platelets and NK cells is primarily mediated by P-selectin on platelets and PSGL-1 on NK cells. PSGL-1 has previously been studied as an adhesion molecule [31, 32], and is now progressively recognized as an important regulator of various aspects of immune responses involving myeloid cells and T-cells in both homeostatic and inflammatory contexts [33, 34]. Although PSGL-1 is ubiquitously expressed across all hematopoietic lineages, its expression levels and functionality vary among these cell types. Currently, the effects of PSGL-1 on NK cells remain inadequately defined, which represents a direction for our future research. In the present study, we focused on the SLAM receptors. SLAMF5 is a self-ligand receptor expressed on both platelets and NK cells [35]. SLAMF5 receptor signals can be both activating or inhibitory, depending on balanced recruitment of the cytosolic signaling proteins p-Fyn, p-PLC-γ2, and inhibitory SHP-1, SHP-2 phosphatases to immunoreceptor tyrosine switch motifs in the SLAM family receptor cytoplasmic domain [36–39]. NK cell function is tightly regulated by the balance of activating and inhibitory receptors. We hypothesized that platelets within the conjugates interacted with NK cells through SLAMF5 molecules, transmitted excessive stimulatory and inhibitory signals, which led to intracellular signaling disturbances and consequently a significant reduction in functional response. In our results, an increased expression of SLAMF5 on platelets, not on NK cells, was observed among HIV-infected individuals compared to HDs. We speculated that platelets might mediate intercellular communication with NK cells through SLAMF5-mediated signaling pathways. Experimental results revealed significant alterations in downstream signaling within platelet-NK cell aggregates, characterized by concurrent elevation of both activation markers (p-Fyn, p-PLC-γ2) and inhibitory signals (p-SHP-1, p-SHP-2). This paradoxical signaling pattern suggests potential dysregulation of SLAM family receptor signaling networks, possibly resulting from excessive platelet-derived activation signals that may drive NK cells into a state of functional imbalance following overactivation. Consistent with this hypothesis, we observed that platelet-NK cell aggregates displayed: (1) Markers indicative of both hyperactivation (CD69 upregulation) and exhaustion (elevated CD39, LAG-3, PD-1); (2) Enhanced apoptotic signatures (Annexin V and CD95 positivity); (3) Accumulation of lipid droplets (Bodipy 493/503 and LipidTOX staining). Furthermore, functional assessments demonstrated significant impairment in aggregate-contained NK cells, manifesting as reduced cytokine production (IFN-γ secretion) and diminished cytotoxic capacity (decreased Granzyme B and Perforin expression). However, elucidation of the detailed mechanism requires further exploration.

Our study had several limitations. Firstly, we conducted a cross-sectional evaluation and did not perform follow-up assessments to monitor the changes. We need larger-scale prospective cohort studies for future validation of the longitudinal dynamics and long-term effects. Secondly, our research did not include a cohort of patients with other viral infections, which prevents us from determining whether our findings reflect HIV-specific outcomes or a general pattern associated with viral infections. Finally, the volumes of peripheral blood collected were occasionally insufficient for comprehensive biomarker analysis in certain participants. And not all individuals underwent viral load testing, resulting in a lack of viral load information.

Conclusion

The study confirmed an increase of platelet-NK cell aggregates in HIV-infected individuals, which was associated with HIV-1 disease progression. The findings also expanded the evidence of the formation of these aggregates associated with platelet activation. Importantly, ART didn't reduce the levels of platelet-NK cell aggregates or platelet activation. Moreover, the platelet-NK cell aggregates exhibited signs of over-activation and exhaustion, as well as heightened markers of apoptosis and intracellular lipid droplets. Additionally, the cytokine secretion and cytotoxic function of the NK cells in aggregates were impaired. It is noteworthy that platelets may transmit signals to NK cells via SLAMF5. Our results revealed disturbances in the downstream phosphorylation signals of the SLAM family within the aggregates. This study provided a new perspective on the regulation of NK cells under the formation of these aggregates.

Abbreviations

HIV-1	Human immunodeficiency virus type 1
NK	Natural killer
IFN-γ	Interferon-gamma
SLAM	Signaling lymphocyte activating molecule
ART	Antiretroviral therapy
PSGL-1	P-selectin glycoprotein ligand-1
PMA	Platelet-monocyte aggregates
scRNA-seq	Single-cell RNA sequencing
TNs	Treatment-naïve patients
ARTs	ART-experienced individuals
HDs	Healthy donors
IRs	Immunological responders
INRs	Non-immunological responders
HBV	Hepatitis B virus
HCV	Hepatitis C virus
PGE1	Prostaglandin E1
PRP	Platelet-rich plasma
SAP	SLAM-associated protein
ITSMs	Immunoreceptor tyrosine-based switch motifs
EAT-2	Ewing's sarcoma-associated transcript 2
FMO	Fluorescence minus one
TGF-β	Transforming growth factor-beta

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-025-06591-3.

Additional file1: Representative gating strategy for flow cytometry analysis of peripheral blood platelet-NK cell aggregates in HIV-infected individuals.

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Author contributions
JD and HXZ contributed to the conception and design. MJD performed the experiments, analyzed data, and wrote the manuscript. RJB, MQJ, and JTF selected participants and collected the blood samples. All authors read and approved the final manuscript.

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Availability of data and material
All data are provided in this study and raw data can be requested by the corresponding author.

Declarations

Ethics approval and consent to participate
This study was approved by the Committee of Ethics at Beijing Ditan Hospital, Capital Medical University in Beijing with informed consent acquired from all participants (No. 2021-22-01). Confidentiality and privacy were assured. The study complied with the Declaration of Helsinki.

Consent for publication
All listed authors consented to the submission, and all data were used with the consent of the person generating the data.

Conflict of interest
The authors declare no conflict of interest.

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