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HIV-1 Elite Controllers Are Characterized by Elevated Levels of CD69-Expressing Natural Killer Cells

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Background: HIV type 1 ((human immunodeficiency virus) HIV-1) elite controllers (ECs) are a rare subset of people living with HIV-1 (PLWH) who control viral replication in the absence of antiretroviral treatment (ART) and may provide a model for a functional cure. We investigated the role of natural killer (NK) cells in HIV-1 ECs from South Africa.

Methods: Phenotypic (CD69, CD38, CD57, PD-1), functional (CD107a, IFN-γ (inferferon gamma)), and nutrient transporter profiles (glucose transporter 1, CD98) of NK cells from ECs (n = 20), viremic progressors (VPs; n = 19), PLWH on ART (n = 20), and people without HIV-1 (PWOH; n = 21) were analyzed using flow cytometry. The Kruskal–Wallis test and followed by the Mann–Whitney *U* test were used to determine differences among the study groups. The Spearman rank correlation coefficient was used to determine significant associations.

Results: Compared with the other study groups, the percentage of CD69-expressing NK cells was higher in ECs, whereas the percentage of CD38-expressing NK cells was higher in VPs. Percentages of $CD69^{+}CD38^{-}$ NK cells were elevated in ECs compared with VPs ($P = 0.003$), but were not different to PLWH on ART and PWOH. Differentiation, exhaustion, and metabolic profiles were not different in ECs compared with PLWH on ART and PWOH; however, NK cell function was lower than in PWOH.

Conclusions: These findings demonstrate that NK cells from ECs have an activated, mature profile with low levels of immune exhaustion and a reduced metabolic phenotype suggesting functional competence. This insight could inform the development of novel immunotherapeutic strategies for treating HIV-1.

Key Words: HIV-1, elite controllers, natural killer cells, spontaneous control, immunometabolism

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INTRODUCTION

In the absence of antiretroviral treatment (ART), people living with human immunodeficiency virus (HIV-1) (PLWH) typically progress to AIDS. In rare cases, PLWH can control their viral load to undetectable levels without ART. These individuals termed elite controllers (ECs) maintain undetectable viral loads and normal $CD4^{+}$ T-cell counts (>450 cells/μL) and serve as models of a functional HIV-1 cure.[1] Although the mechanisms that underlie HIV-1 spontaneous control remain unclear, data suggest that natural killer (NK) cells mediate responses that contribute to controlling viral replication.[2–4]

NK cells are innate immune cells that secrete cytotoxic proteins to control viral infection and tumor growth.[5] NK cells are phenotypically identified by the lack of CD3 together with the expression of CD56 and/or CD16.[6] On the basis of the relative expression of CD56 and CD16, NK cells are further subgrouped into $CD56^{bright}CD16^{-}$, $CD56^{dim}CD16^{+}$, and $CD56^{neg}CD16^{+}$ subsets.[6] $CD56^{dim}CD16^{+}$ NK cells are the major subset in peripheral blood and possess the highest cytotoxic activity. $CD56^{bright}CD16^{-}$ NK cells are the less mature precursor to $CD56^{dim}CD16^{+}$ NK cells and, upon stimulation, increase their cytokine production.[7]

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$CD56^{neg}CD16^{+}$ NK cells are associated with chronic viral infections and are considered dysfunctional.[8]

NK cells rely on oxidative phosphorylation at rest but switch to glycolysis upon stimulation.[9] Although glycolysis is less efficient in producing adenosine triphosphate (ATP), it is favored because it produces the precursor molecules important for NK cell proliferation and effector functions.[10] HIV-1 may disrupt NK cell nutrient availability; however, few studies have shown this.[11–13] NK cells from peripheral blood and tissues express different nutrient transporters, suggesting that NK cells adapt to different nutrient microenvironments.[14] HIV-1 is known to selectively infect metabolically active cells by upregulating glycolysis to complete its life cycle.[15] Because immunometabolism is a valuable area of research for drug targeting, there is a need for further investigation into the role of NK cells in HIV-1 control and pathogenesis.

Few studies have highlighted NK cell responses to HIV-1 in high-incidence settings, such as South Africa.[16–19] This study aimed to profile NK cells by assessing phenotypic, functional, and metabolic profiles during progressive disease versus spontaneous control of HIV-1 infection. We found that NK cells from ECs have an activated, mature profile with low levels of immune exhaustion and a reduced metabolic phenotype.

METHODS

Study Participants

The study included 21 people without HIV-1 (PWOH) and 59 PLWH from a previously described cohort (see Table 1, Supplementary Digital Content, http://links.lww.com/QAI/C348).[20] PLWH included 20 individuals on ART (viral suppression for 7+ years), 20 ECs (viral load $<$ 20 RNA copies/mL, $CD4^{+}$ T-cell count $>$ 400 cell/μL), and 19 ART-naive individuals with progressive disease (viral load $>$ 10,000 RNA copies/mL, $CD4^{+}$ T-cell count $<$ 450 cells/μL). PWOH were recruited from the National Institute for Communicable Diseases, Sandringham, South Africa. PLWH were recruited from Brenthurst Clinic, Parktown, and the Soweto Matlosana Centre for HIV/AIDS and Tuberculosis Research, Soweto, South Africa. All participants were of African descent. Participants were excluded from the study if they presented with other comorbidities or respiratory infections and female participants were excluded if pregnant.

Phenotypic Assessment of NK Cells Using Multicolor Flow Cytometry

Approximately 1 million thawed peripheral blood mononuclear cells (PBMCs) were immunophenotyped using CD3 PE-CF594 (UCHT1), CD19 BV711 (HIB19), CD14 PerCP-Cy5.5 (M5E2), CD56 Alexa Fluor 700 (B159), CD16 APC-Cy7 (3G8), CD69 FITC (FN50), PD-1 BV421 (EH12.1), CD57 BV605 (QA17A04) (all (Becton, Dickinson and Company) BD Biosciences, San Jose, USA), and CD38 PE-Cy7 (HIT2) (BioLegend, San Diego, USA) in the presence of BD Horizon Brilliant Stain Buffer (BD Biosciences). Cells were fixed using fixation & (permeabilization) PERM Cell Permeabilization Kit (Invitrogen, Waltham) and were resuspended in dPBS and stored at 4°C for a maximum of 1 hour before acquisition.

Functional Assessment of NK Cells Using Multicolor Flow Cytometry

Approximately 1 million cells/mL were stained with CD107a PE-Cy5 (H4A3) (BD Biosciences) in the presence of BD GolgiStop protein transport inhibitor (0.67 μL/mL R10 media) (BD Biosciences) and Brefeldin A (5 μg/mL) (Sigma-Aldrich, St. Louis). PBMCs were stimulated using the K562 MHC-devoid cell line (effector-to-target ratio of 10:1) and 10 μg/mL recombinant human IL-2, IL-15, and IL-18 (R&D Systems, Minneapolis), for 18 hours at 37°C in a humidified incubator with 5% CO_2. PBMCs were stained with CD3 PE-CF594 (UCHT1), CD14 APC (M5E2), CD56 BV650 (NCAM16.2), CD16 APC-Cy7 (2G8), CD98 BV421 (UM7F8) (all BD Biosciences), CD19 Alexa Fluor 700 (HIB19) (BioLegend), and glucose transporter 1 (Glut1) RBD-eGFP (Metafora Biosystems, Paris, France) in BD Horizon Brilliant Stain Buffer. The cells were fixed and intracellularly stained with IFN-γ (inferferon gamma) PE-Cy7 (B27) (BD Biosciences) in the presence of a permeabilizing medium (Invitrogen, Waltham). The cells were resuspended in dPBS and stored in the dark at 4°C until acquisition.

Flow Cytometry Analyses

All samples were acquired on a BD LSRFortessa X-20 Cell Analyser (BD Biosciences). The flow cytometer was standardized using Sphero Rainbow Calibration Particles (BD Biosciences). Approximately 1 million events were acquired per PBMC sample. Samples were analyzed using BD FlowJo version 10.9 (BD Biosciences). The gating strategy for NK cells is shown in Figure 1 (Supplemental Digital Content, http://links.lww.com/QAI/C345) and the gating strategy (lymphocytes, single cells, alive, $CD3^{+}$, $CD14^{-}$, $CD19^{-}$, $CD56^{-}$) was applied to all phenotypic T-cell analyses. Fluorescent minus one controls were prepared for all markers of interest in the phenotypic assessment of NK cells to discriminate positive from negative signals (see Figure 2A, Supplemental Digital Content, http://links.lww.com/QAI/C346), and similarly for that of T cells. Fluorescent minus one positive signals were subtracted from fully stained samples. In the phenotypic panel, the markers of interest were quantified on the total NK and T-cell population, because NK cell subsets could not be distinguished because of low CD56 Alexa Fluor 700 resolution and the panel did not include T-cell population differentiators, respectively. Unstimulated negative control tubes were included for each patient sample to differentiate positive from negative signals and results are reported after background subtraction (see Figure 2B, C, Supplemental Digital Content, http://links.lww.com/QAI/C346). The expression of the various markers was analyzed as a percentage or as median fluorescence intensity.

Statistical Analyses

Fisher exact test was used to compare sex representation between the groups. The differences in age and the expression of markers of interest between the study groups were analyzed using the nonparametric Kruskal–Wallis test followed by the Mann–Whitney *U* test. Spearman rank correlation coefficients were used to analyze relationships between the percentage of NK cells and markers of disease severity, and the association between the percentage of NK cells and corresponding T cells coexpressing CD69 and/or CD38. Statistical analysis was performed using GraphPad Prism software version 8.0.1 (GraphPad Prism Software Inc., San Diego). Differences between groups were considered statistically significant at $P < 0.05$.

RESULTS

Clinical Characteristics of Study Participants

We examined 80 participants across 4 study groups (see Table 1, Supplemental Digital Content, http://links.lww.com/QAI/C348). Most participants were women (65%) and the frequencies were similar across the study groups ($P = 0.598$). PLWH on ART and ECs were older than PWOH and VPs (PLWH on ART vs PWOH ($P = 0.0004$) and VPs ($P = 0.0003$); ECs vs PWOH ($P = 0.023$) and VPs ($P = 0.043$)). The median viral load in PLWH on ART and ECs was lower than in the VP group ($P < 0.0001$). The median $CD4^+$ T-cell count was lower in VPs than in PLWH on ART and ECs ($P < 0.0001$).

ECs Had a Higher Percentage of $CD56^{neg}CD16^+$ NK Cells

The percentage of the total NK cell population ($CD56^{bright}CD16^-$, $CD56^{dim}CD16^+$, and $CD56^{neg}CD16^+$), calculated as a percentage of the total lymphocytes present in the sample, was similar among the study groups. In general, the percentage of the $CD56^{bright}CD16^-$ and $CD56^{dim}CD16^+$ NK cells was lower in VPs than in other study groups (Fig. 1A). The percentage of the $CD56^{neg}CD16^+$ NK cell subset was higher in VPs than in other study groups (Fig. 1A). The percentage of $CD56^{neg}CD16^+$ NK cells was increased in ECs compared with that in PWOH ($P = 0.007$; Fig. 1A). The shift in the distribution of NK cell subsets is shown in Figure 1B. Overall, the percentage of NK cells was similar in ECs compared with that in PWOH and PLWH on ART, except for $CD56^{neg}CD16^+$ NK cells. Notable differences were observed in the percentages of NK cell subsets in VPs compared with those in all other study groups.

ECs Had a Higher Percentage of $CD69^+$ NK Cells

Next, we assessed the expression of CD69 and CD38 on total NK cells. CD69 is a marker of early activation and tissue retention, whereas CD38 is a marker of chronic activation.[21,22] The percentage of CD69-expressing total NK cells was higher in ECs than in all other study groups and lower in PLWH on ART than in the other study groups (Fig. 2A). The percentage of CD38-expressing total NK cells was higher in VPs than in all study groups, and in ECs than in PLWH on ART ($P = 0.018$; Fig. 2B). The percentage of CD38-expressing total NK cells correlated positively with viral load and negatively with $CD4^+$ T-cell counts when all PLWH were combined (Fig. 2B). Overall, the percentage of NK cells expressing the early activation marker, CD69, was higher in ECs than in all groups.

We next evaluated the total NK cells expressing combinations of CD69 and/or CD38 to interrogate the differences noted above in ECs (Fig. 2C - 2F). The percentage of $CD69^+CD38^-$ was increased in ECs ($P = 0.003$), PLWH on ART ($P = 0.018$), and PWOH ($P = 0.015$) compared with that in VPs, correlated negatively with viral load ($P = 0.003$, $r = -0.378$), and positively with $CD4^+$ T-cell counts ($P = 0.010$, $r = 0.337$; Fig. 2C). No differences were observed in the expression of $CD69^-CD38^+$ total NK cells between the study groups, however, the percentage of $CD69^-CD38^+$ total NK cells correlated positively with viral load ($P = 0.025$, $r = 0.291$) and negatively with $CD4^+$ T-cell counts ($P = 0.027$, $r = -0.291$; Fig. 2D). A positive correlation between the percentage of NK cells and T cells expressing CD69 and/or CD38 was observed (see Figure 3A, B, Supplemental Digital Content, http://links.lww.com/QAI/C347). Overall, an expansion of $CD69^+CD38^-$-expressing total NK cells was noted in ECs and immune cell functionality was similar across NK and T-cell types.

ECs Had Similar Percentages of $CD57^+$ and $PD\text{-}1^+$ NK Cells Compared With PLWH on ART and PWOH

We next assessed NK cell maturation and exhaustion by quantifying CD57 and PD-1 expression, respectively.[23,24] The ECs, PLWH on ART, and PWOH had comparable percentages of CD57 and PD-1–expressing total NK cells (Fig. 2G and 2H). VPs had a lower percentage of CD57-expressing NK cells than the other study groups and a higher percentage of PD-1–expressing NK cells than PWOH ($P = 0.001$; Fig. 2G and 2H). The percentage of CD57-expressing NK cells correlated negatively with viral load ($P = 0.002$, $r = -0.389$) and positively with $CD4^+$ T-cell counts ($P = 0.014$, $r = 0.323$) in the total group of PLWH combined (Fig. 2G). The percentage of PD-1–expressing total NK cells correlated negatively with $CD4^+$ T-cell count in the EC group ($P = 0.016$, $r = -0.533$; data not shown) and in the combined group of PLWH ($P = 0.007$, $r = -0.351$; Fig. 2H). Overall, the expression of CD57 and PD-1 on NK cells was not different in ECs compared with that in PLWH on ART and PWOH, suggesting that NK cell function is preserved in individuals with nonprogressive HIV-1 disease.

Reduced NK Cell Function in all PLWH

NK cell effector function was analyzed by measuring the degranulation capacity (CD107a) and cytokine production (IFN-γ) of the total NK cell population and its subsets. All PLWH had a lower percentage of CD107a-expressing total

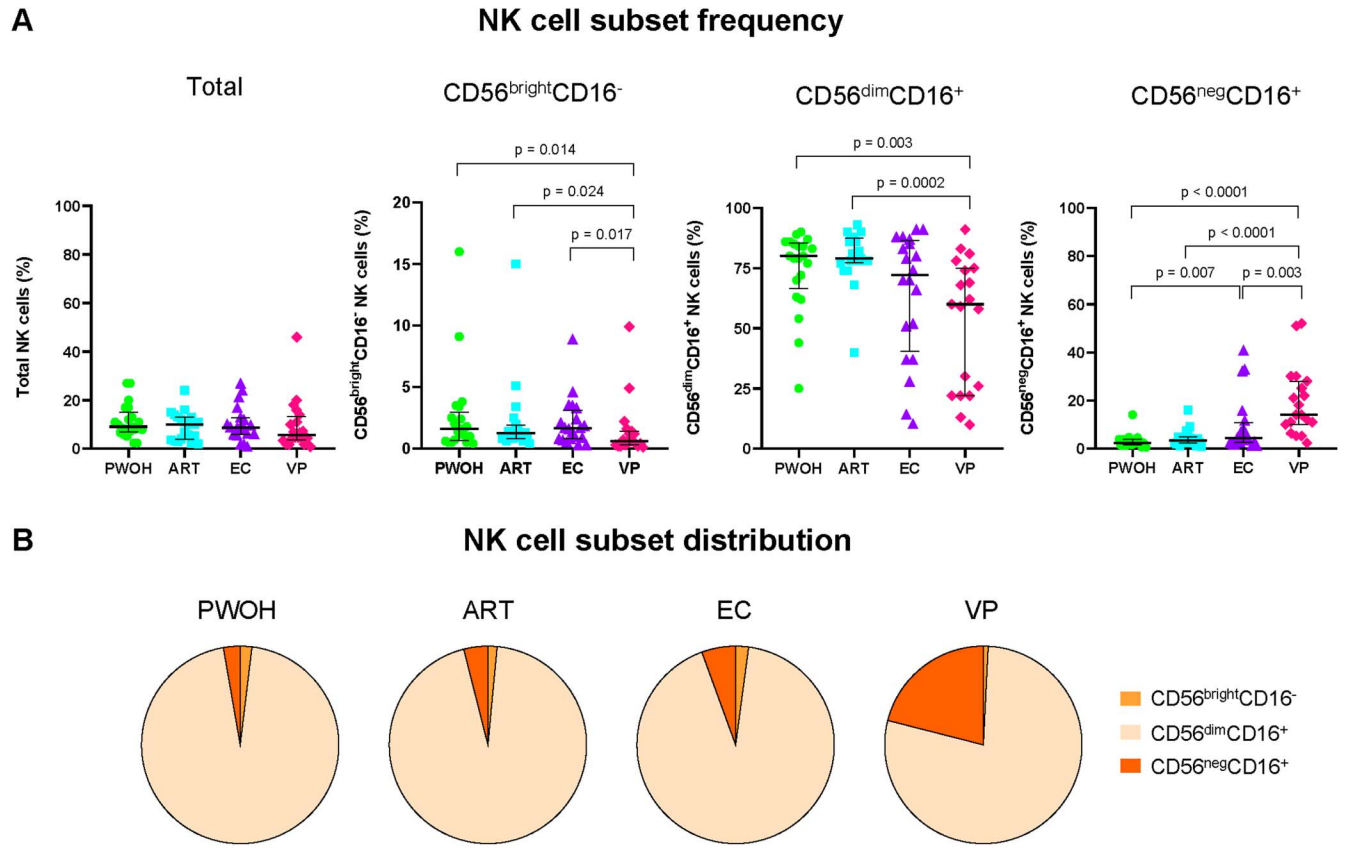

FIGURE 1. A, The percentage of total NK cells, CD56^{bright}CD16^{-}, CD56^{dim}CD16^{+}, and CD56^{neg}CD16+ NK cell subsets in people without HIV-1 (PWOH), PLWH on ART (ART), ECs, and VPs. Each symbol represents a study participant. The horizontal lines represent the medians, and the error bars represent the 25th and 75th percentiles. Significant *P* values are shown ($P < 0.05$). B, The distribution of CD56^{bright}CD16^{-}, CD56^{dim}CD16^{+}, and CD56^{neg}CD16+ NK cell subsets in PWOH, ART, EC, and VPs. Areas represent the median percentages.

NK cells than PWOH (Fig. 3A). A decreased percentage of CD107a-expressing CD56^{dim}CD16^{+} NK cells was observed in ECs compared with that in PWOH ($P = 0.004$). No differences in the percentage of CD107a-expressing CD56^{bright}CD16^{-} and CD56^{neg}CD16^{+} NK cell subsets were observed between the study groups (Fig. 3A). No correlations were observed between the percentage of CD107a-expressing total NK cells and viral load or CD4^{+} T-cell counts in the combined group of PLWH (Fig. 3A). Interestingly, the percentage of CD107a-expressing total NK cells correlated negatively with CD4^{+} T-cell counts in PLWH on ART ($P = 0.049$; $r = -0.445$), but correlated positively in ECs ($P = 0.018$; $r = 0.524$) (Fig. 3B).

The percentage of total NK cells producing IFN-γ was lower in VPs than in PWOH ($P = 0.0003$) and PLWH on ART ($P = 0.012$) (Fig. 3C). Similarly, the percentage of IFN-γ–producing CD56^{dim}CD16^{+} NK cells was lower in VPs than in PWOH ($P = 0.014$). There were no differences in the percentage of IFN-γ–producing CD56^{bright}CD16^{-} and CD56^{neg}CD16^{+} NK cells between the study groups. The percentage of IFN-γ–producing total NK cells correlated negatively with viral load ($P = 0.008$, $r = -0.343$) and positively with CD4^{+} T-cell counts ($P = 0.007$, $r = 0.354$) in PLWH combined (Fig. 3C). Taken together, we observed a reduction in NK cell degranulation and cytokine expression in all PLWH compared with those in PWOH.

Similar Glut1^{+} and CD98^{+} NK Cell Expression in ECs Compared With That in PLWH on ART and PWOH

NK cell metabolic function was investigated by quantifying the expression of nutrient transporters, Glut1 and CD98, on total NK cells and their subsets. Glut1 and CD98 are responsible for glucose and amino acid transportation, respectively. There was no difference in Glut1 or CD98 expression between the study groups in total NK cells. In all NK cell subsets analyzed, the expression of Glut1 and CD98 was increased in VPs compared with that in the other study groups—except for the Glut1 expression on CD56^{neg}CD16^{+} cells, which was similar across study groups (Figs. 4A, B). Glut1 and CD98 expression on total NK cells correlated positively with viral load but not with CD4^{+} T-cell counts (Figs. 4A, B). Overall, Glut1^{+} and CD98^{+} NK cell and subset expression was not different in ECs compared with that in PLWH on ART and that in PWOH but was increased in VPs.

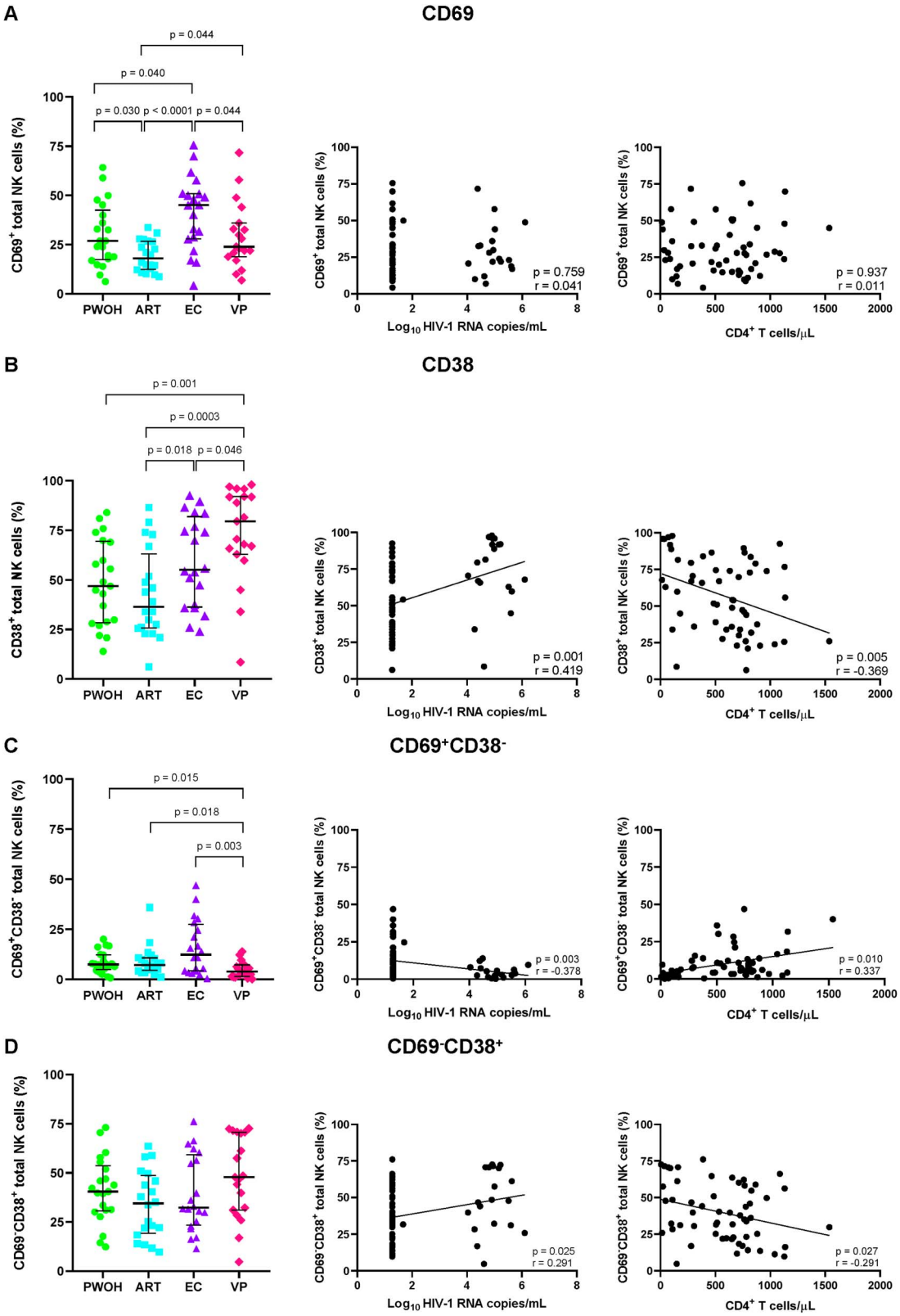

FIGURE 2. Continued

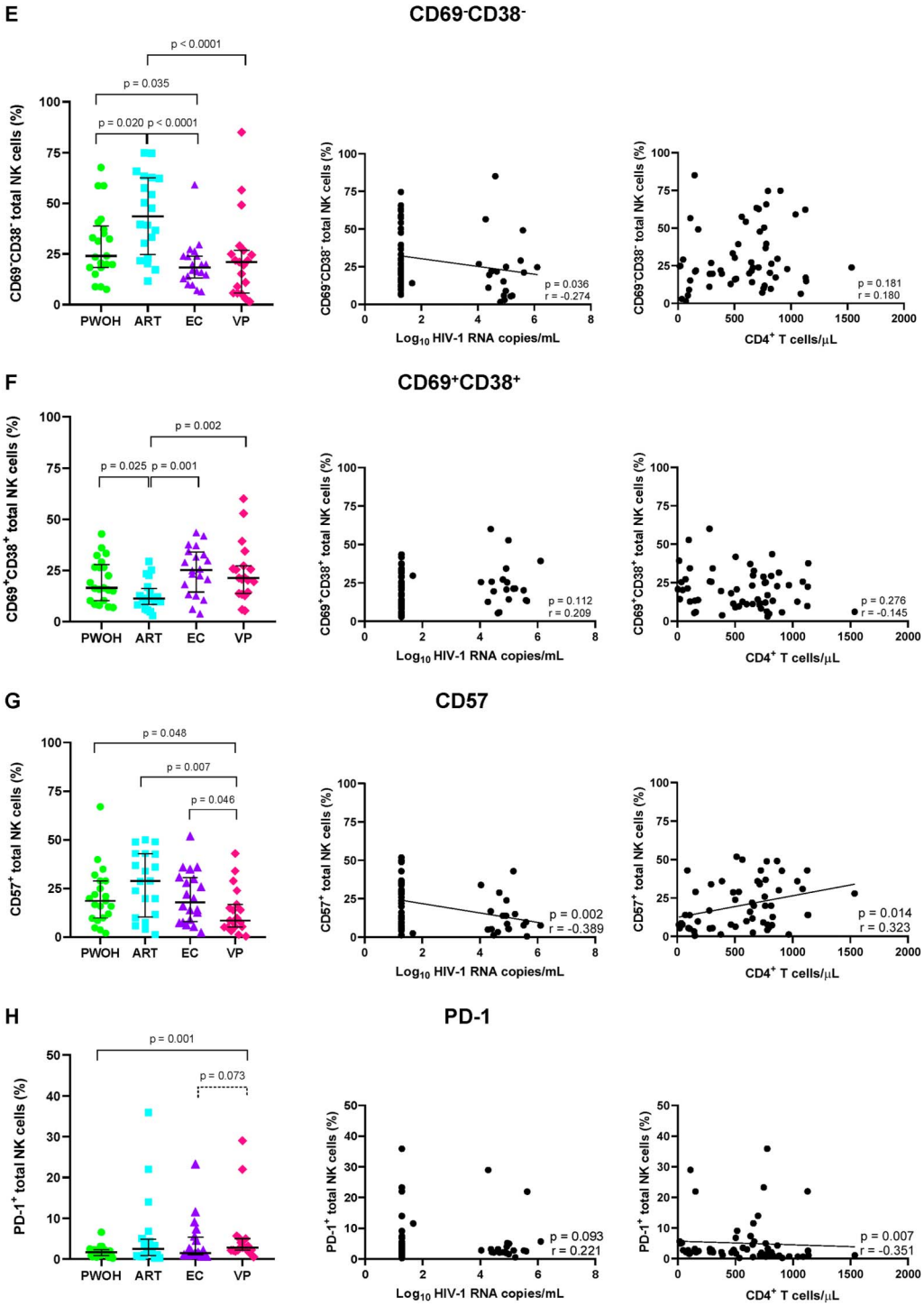

FIGURE 2. The percentage of (A) CD69⁺, (B) CD38⁺, (C) CD69⁺CD38⁻, (D) CD69⁻CD38⁺, (E) CD69⁻CD38⁻, (F) CD69⁺CD38⁺,(E) CD57⁺, and (F) PD-1⁺ total NK cells in people without HIV-1 (PWOH), PLWH on ART (ART), ECs, and VPs. The horizontal lines represent the medians, and the error bars represent the 25th and 75th percentiles. Correlations between the percentage of (A) CD69⁺, (B) CD38⁺, (C) CD69⁺CD38⁻, (D) CD69⁻CD38⁺, (E) CD69⁻CD38⁻, (F) CD69⁺CD38⁺, (E) CD57⁺, and (F) PD-1⁺ total NK cells, and viral load and CD4⁺ T-cell counts in PLWH combined. Lines are calculated using linear regression. Each symbol represents a study participant. Significant *P* values ($P < 0.05$; solid line), trends ($0.05 < P < 0.08$; dotted line), nonsignificant ($P > 0.05$; no line), and Spearman rho (r) values are shown.

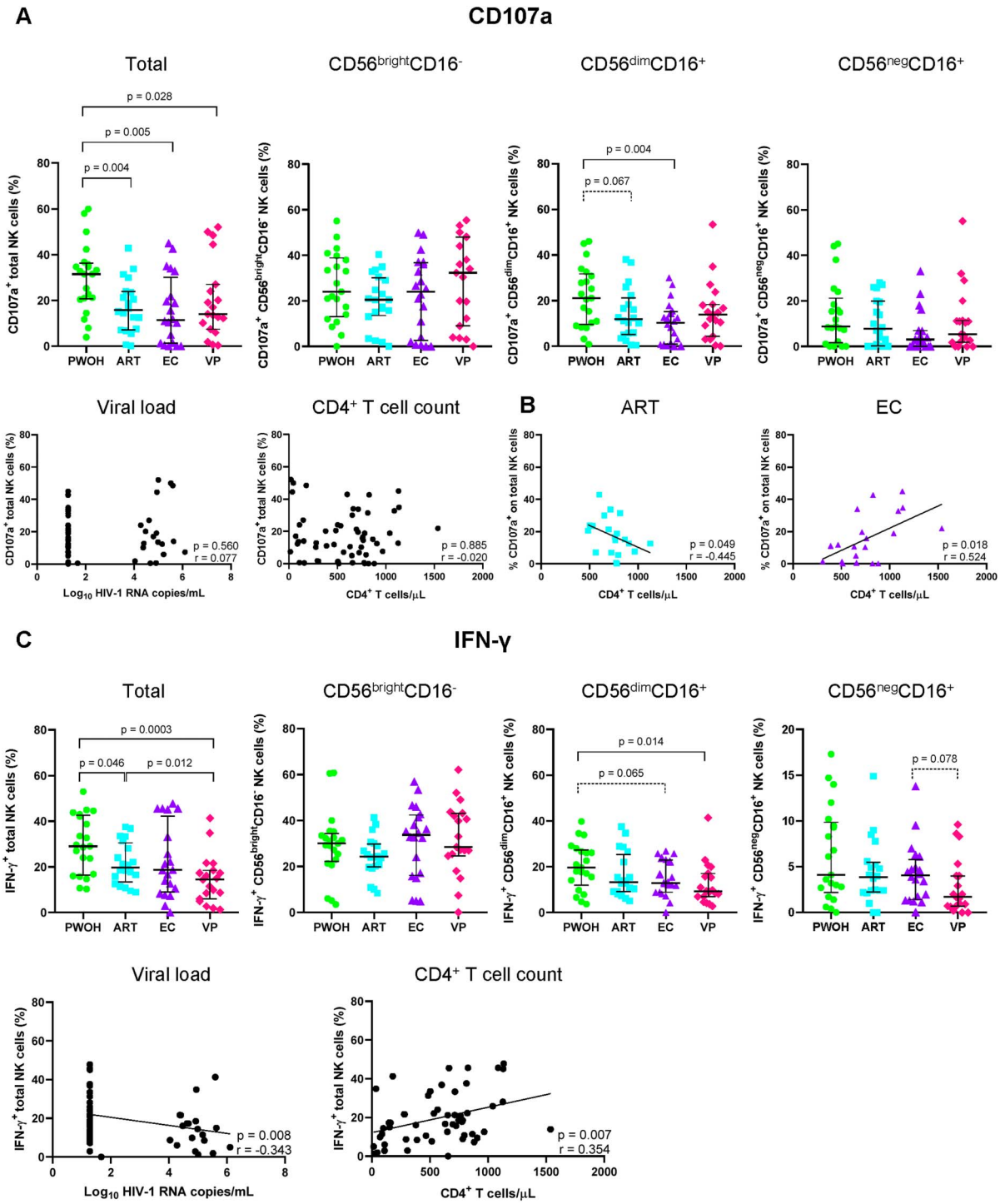

FIGURE 3. A, The percentage of CD107a$^+$ total NK cells, CD56^{bright}CD16$^-$, CD56^{dim}CD16$^+$, and CD56^{neg}CD16$^+$ NK cell subsets in people without HIV-1 (PWOH), PLWH on ART (ART), ECs, and VPs. Correlations between the percentage of CD107a$^+$ total NK cells and viral load and CD4$^+$ T-cell counts in PLWH combined. B, Correlations between the percentage of CD107a$^+$ total NK cells and CD4$^+$ T-cell counts in PLWH on ART and EC. C, The percentage of IFN-γ$^+$ total NK cells, CD56^{bright}CD16$^-$, CD56^{dim}CD16$^+$, and CD56^{neg}CD16$^+$ NK cell subsets in PWOH, ART, EC, and VPs. Correlations between the percentage of IFN-γ$^+$ total NK cells and viral load and CD4$^+$ T-cell counts in PLWH combined. The horizontal lines represent the medians, and the error bars represent the 25th and 75th percentiles. Lines are calculated using linear regression. Each symbol represents a study participant. Significant P values ($P < 0.05$; solid line), trends ($0.05 < P < 0.08$; dotted line), nonsignificant ($P > 0.05$; no line), and Spearman rho (r) values are shown.

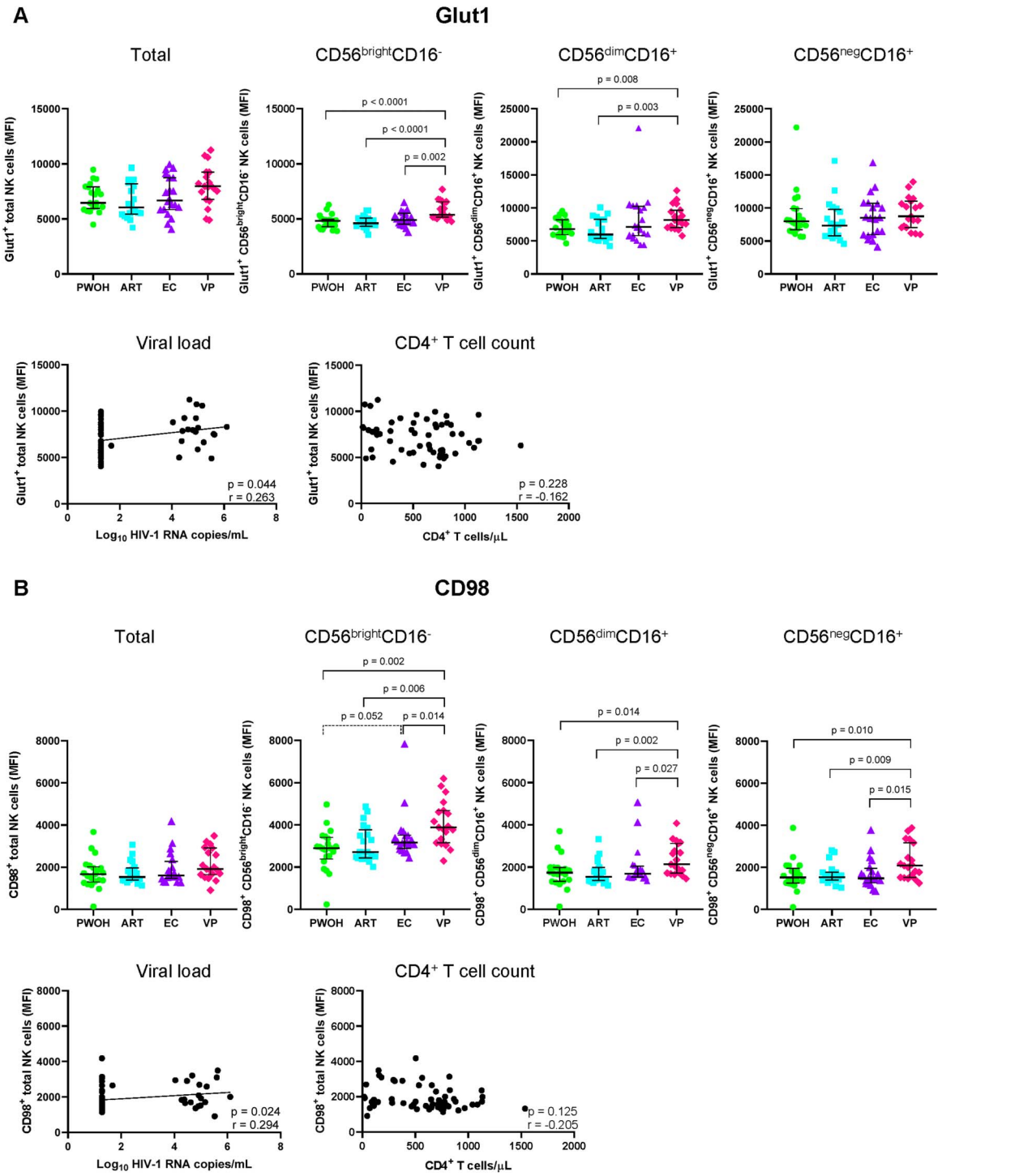

FIGURE 4. A, Glut1$^+$ and (B) CD98$^+$ expression, measured as median fluorescence intensity (MFI), on total NK cells, CD56^{bright}CD16$^-$, CD56^{dim}CD16$^+$, and CD56^{neg}CD16$^+$ NK cell subsets in people without HIV-1 (PWOH), PLWH on ART (ART), ECs, and VPs. The horizontal lines represent the medians, and the error bars represent the 25th and 75th percentiles. Correlations between the expression of (A) Glut1$^+$ and (B) CD98$^+$ NK cells and viral load, and CD4$^+$ T cells in PLWH combined. Lines are calculated using linear regression. Each symbol represents a study participant. Significant P values ($P < 0.05$; solid line), trends ($0.05 < P < 0.08$; dotted line), nonsignificant ($P > 0.05$; no line), and Spearman rho (r) values are shown.

DISCUSSION

HIV-1 and AIDS-related deaths remain one of the leading causes of mortality in sub-Saharan Africa. HIV-1 ECs are understudied in African populations and may hold clues for a functional cure. This study compared NK cell phenotypic and functional characteristics of ECs, VPs, PLWH on ART, and PWOH. Although the percentage of total NK cells was comparable among all the study groups, the distribution of the various NK cell subsets was altered as a consequence of HIV-1 infection. Second, total NK cells from ECs showed elevated expression of CD69 when compared with those from all study groups, and elevated expression of CD38 when compared with that of PLWH on ART. In addition, the percentage of $CD69^{+}CD38^{-}$ NK cells was higher in ECs than in VPs and was similar in PLWH on ART compared with that in PWOH. Third, CD57 and PD-1 expression on NK cells did not differ between ECs, PLWH on ART, and PWOH but differed in VPs. Fourth, the cytotoxic capacity of the total NK cells was reduced in all PLWH, and cytokine production was similar in ECs and PWOH. Lastly, we found increased expression of $Glut1^{+}$ and $CD98^{+}$ $CD56^{bright}CD16^{-}$ and $CD56^{dim}CD16^{+}$ NK cell subsets in VPs when compared with those in the other study groups.

The redistribution of NK cell subsets in response to a chronic viremic infection has previously been described.[8,25–27] Similarly, we showed a reduction in $CD56^{bright}CD16^{-}$ and $CD56^{dim}CD16^{+}$, and increased $CD56^{neg}CD16^{+}$ NK cell subsets in VPs compared with those in the other study groups. Interestingly, we observed an expansion of the $CD56^{neg}CD16^{+}$ NK cell subset in ECs when compared with that in PWOH. The presence of the $CD56^{neg}CD16^{+}$ NK cell subset in ECs is paradoxical because this subset is associated with progressive viral infections and has not previously been reported in ECs. A recent meta-analysis of $CD56^{neg}CD16^{+}$ NK cells showed that this subset has a defined killer cell immunoglobulin-like receptor (KIR) pattern implying a unique capacity for KIR-mediated education and hence could be advantageous in HIV-1 immune control.[28] Altogether, we demonstrated the preservation of the $CD56^{bright}CD16^{-}$ and $CD56^{dim}CD16^{+}$ NK cell subsets in ECs. Despite undetectable viral loads, low-level viremia in ECs drives the accumulation of $CD56^{neg}CD16^{+}$ NK cells that may have increased functional capabilities contributing to viral control.

Intriguingly, our study showed increased CD69 expression on NK cells in ECs compared with that in all other study groups and increased $CD69^{+}CD38^{-}$ NK cells percentage in ECs compared with that in VPs, which correlated negatively with viral load and positively with $CD4^{+}$ T-cell counts. Studies from HIV-1–exposed, seronegative cohorts showed that the seronegative status was attributed to an increase in $CD69^{+}$ NK cells.[29,30] In addition, an in vitro study in women showed that the upregulation of $CD69^{+}$ $CD4^{+}$ T cells was associated with a novel combination of cytokines that supported HIV-1 spontaneous control.[31] CD69 regulates T-cell differentiation and is a tissue-resident memory marker.[21] A recent study showed that effective HIV-1 immune control was attributed to the upregulation of tissue-resident memory $CD69^{+}CD8^{+}$ T cells that was not exclusively driven by activation.[32] Our findings in ECs further support that antigenic load itself cannot explain expansions of CD69-expressing NK or T cells and that CD69 may play a more protective role. Given the correlation of CD69 and CD38 subsets on T cells and NK cells, it is tempting to speculate that high CD69 expression in ECs measured here may reflect events in lymphoid tissues and thus warrants further investigation. The identification of a novel NK cell population coexpressing CD69 and chemokine receptor CXCR6 in human lymphoid tissues, which is distinct from the conventional $CD56^{bright}CD16^{-}$ and $CD56^{dim}CD16^{+}$ NK cell subsets, may provide further insights into the role of CD69 expression on NK cells in future studies.[33]

The upregulation of CD38 on T cells is indicative of chronic immune activation and disease progression, thus the increase in CD38 expression on total NK cells observed in VPs compared with that in the other study groups was expected.[34,35] The upregulation of $CD38^{+}$ NK cells in ECs compared with that in PLWH on ART supports data that showed higher levels of $CD8^{+}$ T-cell activation in ECs than that in PLWH on ART.[36] We also showed a similar expression of $CD69^{+}CD38^{-}$ total NK cells in ECs compared with that in PLWH on ART and PWOH. Higher frequencies of (human leukocyte antigen-DR isotype) $HLA\text{-}DR^{+}CD38^{-}$ T cells in ECs have been shown to have increased proliferative and functional capabilities compared with their CD38-expressing counterparts,[37] suggesting that chronically activated CD38-expressing cells become less functional. Taken together, a low viral antigen concentration may favor the generation of $CD69^{+}$ NK cells, which could serve as the initial activation signal required for the priming of effector functions and may constitute a unique feature of ECs.

The upregulation of CD57 is associated with NK cell terminal differentiation and superior functional characteristics.[38,39] The immune checkpoint inhibitor, PD-1, is often upregulated because of chronic viral infections and is indicative of immune exhaustion. We show comparable expressions of $CD57^{+}$ and $PD\text{-}1^{+}$ NK cells in ECs, PLWH on ART, and PWOH. We also show decreased $CD57^{+}$ NK cells in VPs compared with that in the other groups and increased PD-1–expressing NK cells in VPs compared with those in PWOH. The coexpression of CD57 and NKG2C is associated with an adaptive memory-like NK cell subset that is usually expanded after human cytomegalovirus (HCMV) exposure.[27,40] Given the prevalence of HCMV in South Africa and the fact that almost all PLWH are coinfected with the virus,[41] it is reasonable to surmise that most PLWH have an adaptive NK cell subset driven by HCMV rather than by HIV-1, although it has been suggested that HCMV has less of an impact on PLWH from Africa.[42] Together, these findings demonstrate that ECs preserve a mature and unexhausted NK cell profile that is not different to PLWH on ART and PWOH.

Degranulation in total NK cells was reduced in ECs, PLWH on ART, and VPs compared with that in PWOH. We

also found that IFN-γ production in NK cells was reduced in VPs compared with that in PWOH and increased in PLWH on ART compared to that in VPs. These findings are similar to a single-cell transcriptomic analysis of NK cells from PLWH, which showed a reduction in cytotoxic and cytokine-producing genes compared with that in healthy individuals.[43] Interestingly, we found the percentage of CD107a-expressing total NK cells correlated negatively with $CD4^+$ T-cell counts in PLWH on ART but positively in ECs. This suggests that although ART may restore peripheral blood $CD4^+$ T-cell counts, it does not fully restore NK cell cytotoxic function.

Lastly, we showed that the expression of Glut1 and CD98 was upregulated on NK cells in VPs compared with that in the other study groups and was positively associated with viral load. Similar nutrient transporter expression was observed in ECs, PLWH on ART, and PWOH. The upregulation of the nutrient transporters during progressive disease may result from an HIV-1–induced metabolic switch from oxidative phosphorylation to glycolysis on NK cells as previously described.[44] This is supported by data that showed the upregulation of CD98 on NK cells after retroviral exposure in a mouse model.[45–47] Elite control may be achieved by maintaining low viral stimulation sufficient to induce a partially activated state that supports oxidative phosphorylation for NK cell function maintenance. Our data suggest that chronic viral activation may lead to increased nutrient uptake, metabolic dysregulation, and diminished viral control.

The study limitations include the small sample size and cross-sectional study design. In addition, we were unable to distinguish bright and dim $CD56^+$ NK cells in the phenotypic analyses and, therefore, data were analyzed from the total NK cell population. Furthermore, our analyses of peripheral circulating NK cells may not reflect the NK cell tissue compartments. Age-related immune cell variations could confound the data. Seahorse assays could provide a more thorough understanding of the role of metabolic pathways and mitochondrial dysfunction during controlled and progressive HIV-1 disease.

In this study, we demonstrated that elite control was associated with an increased percentage of CD69-expressing NK cells, a mature, unexhausted NK cell profile together with preserved low metabolic activity—all of which support superior functional competencies. These new insights could inform novel immunotherapeutic designs for PLWH.

ETHICS AND INTEGRITY POLICIES

The study received ethical approval from the University of the Witwatersrand Human Research Ethics Committee.

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