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Elevated levels of invariant natural killer T-cell and natural killer cell activation correlate with disease progression in HIV-1 and HIV-2 infections

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Objective: In this study, we aimed to investigate the frequency and activation of invariant natural killer T (iNKT) cells and natural killer (NK) cells among HIV-1, HIV-2, or dually HIV-1/HIV-2 (HIV-D)-infected individuals, in relation to markers of disease progression.

Design: Whole blood samples were collected from treatment-naive HIV-1 ($n = 23$), HIV-2 ($n = 34$), and HIV-D ($n = 11$) infected individuals, as well as HIV-seronegative controls ($n = 25$), belonging to an occupational cohort in Guinea-Bissau.

Methods: Frequencies and activation levels of iNKT and NK cell subsets were analysed using multicolour flow cytometry, and results were related to HIV-status, $CD4^+$ T-cell levels, viral load, and T-cell activation.

Results: HIV-1, HIV-D, and viremic HIV-2 individuals had lower numbers of $CD4^+$ iNKT cells in circulation compared with seronegative controls. Numbers of $CD56^{bright}$ NK cells were also reduced in HIV-infected individuals as compared with control study participants. Notably, iNKT cell and NK cell activation levels, assessed by CD38 expression, were increased in HIV-1 and HIV-2 single, as well as dual, infections. HIV-2 viremia was associated with elevated activation levels in $CD4^+$ iNKT cells, $CD56^{bright}$, and $CD56^{dim}$ NK cells, as compared with aviremic HIV-2 infection. Additionally, disease markers such as $CD4^+$ T-cell percentages, viral load, and $CD4^+$ T-cell activation were associated with CD38 expression levels of both iNKT and NK cells, which activation levels also correlated with each other.

Conclusion: Our data indicate that elevated levels of iNKT-cell and NK-cell activation are associated with viremia and disease progression markers in both HIV-1 and HIV-2 infections.

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Keywords: CD38, HIV-1, HIV-2, immune activation, invariant natural killer T cells, natural killer cells, viremia

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Introduction

HIV type 1 (HIV-1) and type 2 (HIV-2) are related viruses that can cause AIDS. Although HIV-1 has given rise to a pandemic, HIV-2 is mainly restricted to West Africa. In contrast to HIV-1, long-term nonprogressors are common in HIV-2 infection [1]. The reason for this is not fully understood, but HIV-2 infection is characterized by a large proportion of infected individuals with low or undetectable plasma viral load, and a viral load set point at least one log lower than in HIV-1 infection [2,3]. However, HIV-2-infected individuals with detectable viremia progress faster to AIDS and have higher mortality than HIV-2-infected aviremic study participants [4–6].

The innate and adaptive immune responses to HIV-1 have been studied extensively, whereas much less is known about immune responses in HIV-2 and HIV-1/2 dual (HIV-D) infections. Nevertheless, it has been reported that the breadth and potency of virus specific T-cell responses, as well as neutralizing antibodies, distinguish HIV-2 from HIV-1 [7–11]. Additionally, HIV-2 has been reported to delay subsequent HIV-1 disease progression in HIV-D-infected individuals [12,13]. Furthermore, HIV-2-infected individuals, compared with HIV-1, display lower levels of chronic T-cell immune activation [14]. Still, T-cell activation in HIV-2 infection appears to be related to $CD4^+$ T-cell levels as well as viral load, similar to HIV-1 infection [15–17].

HIV-1 disease progression is associated with chronic immune activation, characterized by elevated frequencies of T cells, expressing activation markers, such as CD38 and HLA-DR [18] (and reviewed in [19,20]). Still, the cause or consequence relationship between virus replication and chronic immune activation has not been fully elucidated. Chronic immune activation is also associated with augmented levels of bystander activating factors such as proinflammatory cytokines, markers of inflammation, and microbial translocation which are detected in progressive infection of both HIV-1 and HIV-2 [21–24]. Many of these bystander factors are produced by activated innate immune cells, which include monocytes, macrophages, and dendritic cells [19,20]. Increased activation of natural killer (NK) cells occurs in chronic HIV-1 infections [25–27], and such activation can be associated with dysfunctional NK cell responses [28].

Innate lymphocytes such as NK and invariant natural killer T (iNKT) cells play an important role in the host defence against viruses including HIV [29,30]. Dramatic shifts in the population dynamics of NK cells are observed during chronic HIV infection, for example the expansion of the $CD56^{neg}$ NK cell population [31]. The function of NK cells has been shown to be less impaired during HIV-2 compared with HIV-1 infection [32]. iNKT cells have characteristics of both NK and T cells and rapidly produce high levels of proinflammatory cytokines such as IFNγ and tumour necrosis factor [33] (and reviewed in [34]). About one-third of the iNKT population expresses CD4 and can be infected by HIV-1 [35–37]. Furthermore, during untreated HIV-1 infection, iNKT cells are known to decline in numbers and develop an exhausted, dysfunctional phenotype [36–39].

Comparative studies on the phenotypes of iNKT and NK cells during HIV-1, HIV-2, and HIV-D infections, and the relation to viremia and $CD4^+$ T-cell suppression, are lacking. In this cross-sectional study, we investigated the frequency and phenotype of NK and iNKT cells in individuals infected with, but not treated for, these viruses. Results revealed that iNKT and NK cell activation correlates with disease severity, as indicated by levels of $CD4^+$ T cells, viral load, and $CD4^+$ T-cell activation, in both HIV-1 and HIV-2 infections.

Materials and methods

Study population

The individuals included in this study were part of an occupational cohort of police officers in Guinea-Bissau [40,41] (Table 1 and Supplementary Methods in Supplemental Digital Content, http://links.lww.com/QAD/A929). Blood samples were from treatment-naïve HIV-1 ($n=23$), HIV-2 ($n=34$), or HIV-D ($n=11$) infected individuals, as well as from 25 HIV-seronegative individuals from the same cohort. The study was approved by the ethical committee in Guinea-Bissau and by the ethical committee at Lund University.

Blood sampling, HIV status, and $CD4^+$ T-cell determination

Venous blood samples were drawn and collected in vacutainer tubes (BD Biosciences, San Jose, California, USA) with EDTA as anticoagulant and in Cyto-Chex BCT tubes (Streck, Omaha, Nebraska, USA). Plasma obtained from EDTA tubes was kept frozen at −80°C until use. HIV infection status was determined by serology, and the percentage of $CD4^+$ T cells was determined on fresh whole blood by flow cytometry, as described [42]. The rational for choosing %$CD4^+$ T cells as a marker of disease progression in the HIV-infected cohort was based on findings suggesting that %$CD4^+$ T cells is a more stable disease marker than absolute $CD4^+$ T-cell count in settings with elevated pathogenic burden and comorbidities [43] and on the prior use of this marker in the studied cohort [44]. Determination of absolute number of $CD4^+$ T cells/μl ($CD4^+$ T-cell count) was performed at the Laboratory of Clinical Immunology and Transfusion Medicine, Skåne University Hospital, Lund, Sweden on Cyto-Chex stabilized whole blood.

Table 1. Cohort characteristics.

	HIV-1	HIV-2	HIV-D	Controls
Numbers (women/men)	23 (10/13)	34 (8/26)	11 (2/9)	25 (10/15)
Age (years)[a]	46 (41–53)	53 (48–58)	50 (44–52)	48 (40–55)
% $CD4^+$ T cells[a]	12.3 (6.4–25.6)	25.4 (17.9–35.9)	15.3 (11.6–21.6)	42.3 (38–50)
Viral load (RNA copies/ml)[a]	12 009 (2799–30067)	<75 (<75–134)	2230 (1147–6398)	NA

[a]Median (IQR).
NA, not applicable.

Plasma viral load

With minor modifications, plasma HIV-1 and HIV-2 viral loads were determined by in-house quantitiative PCR protocols, as described previously [45]. In brief, viral RNA was extracted and purified using the miRNeasy micro Kit (Qiagen, Hilden, North Rhine-Westphalia, Germany), and TaqMan qRT-PCR was performed using the Superscript III Platinum One Step qRT-PCR kit (Life Technologies, Carlsbad, California, USA). The detection limits for the HIV-1 and HIV-2 quantifications were 75 RNA copies/ml in plasma from HIV-1 or HIV-2 singly infected and 135 RNA copies/ml in plasma from HIV-D-infected.

Immunophenotyping

Immunophenotyping of lymphocytes was performed on whole blood collected and stabilized in Cyto-Chex BCT tubes (7–11 days) before multicolour flow cytometry [46]. Before antibody labelling, red blood cells were lysed using 1× lysis buffer (BD FACS) and washed with PBS containing 2 mmol/l EDTA. The panel for the antibody staining is shown in Table S1, Supplemental Digital Content, http://links.lww.com/QAD/A929. Multicolour flow cytometry was performed using a BD LSRFortessa, and data were analysed using FlowJo software Version 9.6.2 (LLC, Ashland, Oregon, USA) (for details see Supplementary Methods and Fig. S1, in Supplemental Digital Content, http://links.lww.com/QAD/A929). Prior to the analysis of whole blood samples from the study participants, the selected panel of antibodies was tested on whole blood collected in EDTA vacutainer tubes, stained and analysed the same day; and stabilized and stored in Cyto-Chex tubes from corresponding donors before staining and analysis. Results, as depicted in Supplemental Fig. S2, http://links.lww.com/QAD/A929, showed that the gated and analysed iNKT and NK cell subsets in the Cyto-Chex stabilized blood were distinct and similar to that of the freshly analysed blood. The absolute numbers of iNKT cells and NK cells/μl were calculated according to %iNKT cells (or NK cells)/100 × $CD4^+$ T-cell count/%$CD4^+$ T cells/100. Mean fluorescence intensities from cell subsets with 25 or more events were included in the analysis of CD38 and transcription factor (TF) expression.

Statistical analysis

Nonparametric statistical methods for comparison between groups included the Kruskal–Wallis test, with Dunns-post test, and the Mann–Whitney *U* test. Correlations were done with the nonparametric Spearman's rank-correlation test, and the analyses were performed using the GraphPad Prism 6.0 software (GraphPad Software, San Diego, California, USA). Multiparameter analysis using univariate general linear modelling was performed with SPSS version 22 (IBM Analytics, Armonk, New York, USA).

Results

Characteristics of study participants

Blood samples were obtained cross-sectionally from treatment-naive HIV-1 ($n = 23$), HIV-2 ($n = 34$), or HIV-D ($n = 11$) infected study participants and HIV-seronegative controls ($n = 25$) belonging to an occupational cohort residing in Guinea-Bissau (Table 1 and [40,41]). The median ages of the study groups ranged between 46 and 53 years, with HIV-1-infected study participants being 7 years younger than the HIV-2-infected participants ($P < 0.05$). With regard to the immunological status, the median level of % $CD4^+$ T cells was for all HIV-infected groups lower than for the uninfected group ($P < 0.001$). HIV-1, compared with HIV-2, infected individuals also had lower %$CD4^+$ T cells (median 12.3 vs 25.4; $P < 0.01$). Furthermore, all HIV-1-infected, singly or dually, had higher median viral loads than the HIV-2-infected participants (median 12 009, 2230 vs <75 RNA copies/ml, $P < 0.001$ and $P < 0.01$, respectively). Indeed, many (74%) of the HIV-2-infected individuals had undetectable plasma viral loads and were therefore classified as aviremic.

Invariant natural killer T and natural killer cell subsets are reduced in HIV-infected individuals

iNKT and NK cell numbers in peripheral blood are often reduced in HIV-1-infected individuals [36,37,39,47,48]. In contrast, studies investigating innate lymphocytes in HIV-2 are limited [1]. Here we compared the frequency of total and $CD4^+$ iNKT cells as well as $CD56^{dim}$ and $CD56^{bright}$ NK cells in whole blood of HIV-seronegative controls, HIV-1, HIV-2, and HIV-D-infected individuals (Fig. 1a and b). Absolute numbers of both total iNKT and $CD4^+$ iNKT cell subsets of HIV-1 and HIV-D-infected study participants were, or tended to be, decreased compared with controls ($P < 0.001$ to $P = 0.09$). Moreover, all HIV-infected groups had also reduced

numbers of CD56^{bright} NK cells compared with the controls ($P < 0.01$ to $P < 0.05$), whereas the impact on the CD56^{dim} NK cell numbers was less pronounced. The proportion of iNKT and NK cell subsets within the lymphocyte population was also less affected (Fig. S3 in Supplemental Digital Content, http://links.lww.com/QAD/A929).

Viremia is infrequently detected in HIV-2-infected individuals, but once occurring, it is linked to disease progression similar to that seen in HIV-1 infection [4,5,49]. By comparing HIV-2 singly infected study participants, being either viremic or aviremic (Fig. 1c), we found that the absolute numbers of CD4$^+$ iNKT cells were reduced in viremic HIV-2 infection ($P < 0.05$), and likewise the CD56^{dim} NK cells tended to be fewer ($P = 0.07$). The percentage of CD56^{dim} NK cells was lower in HIV-2 viremic study participants, and a similar trend was noted for the CD4$^+$ iNKT cells ($P < 0.05$ and $P = 0.05$, respectively, Fig. S3 in Supplemental Digital Content, http://links.lww.com/QAD/A929). However, there was no difference in the percentage or absolute numbers of total iNKT cells, nor CD56^{bright} NK cells (Fig. 1c and Fig. S3 in Supplemental Digital Content, http://links.lww.com/QAD/A929). Reduced numbers of CD56^{bright} NK cells were instead observed in the aviremic HIV-2 study participants compared with the HIV-seronegative controls ($P < 0.01$; data not shown).

Natural killer and invariant natural killer T-cell subsets display enhanced activation in HIV infections

In both HIV-1 and HIV-2 infections, excessive T-cell activation is correlated with the progression to AIDS [16,17]. However, less in known about the link between elevated activation of NK cells and iNKT cells and disease

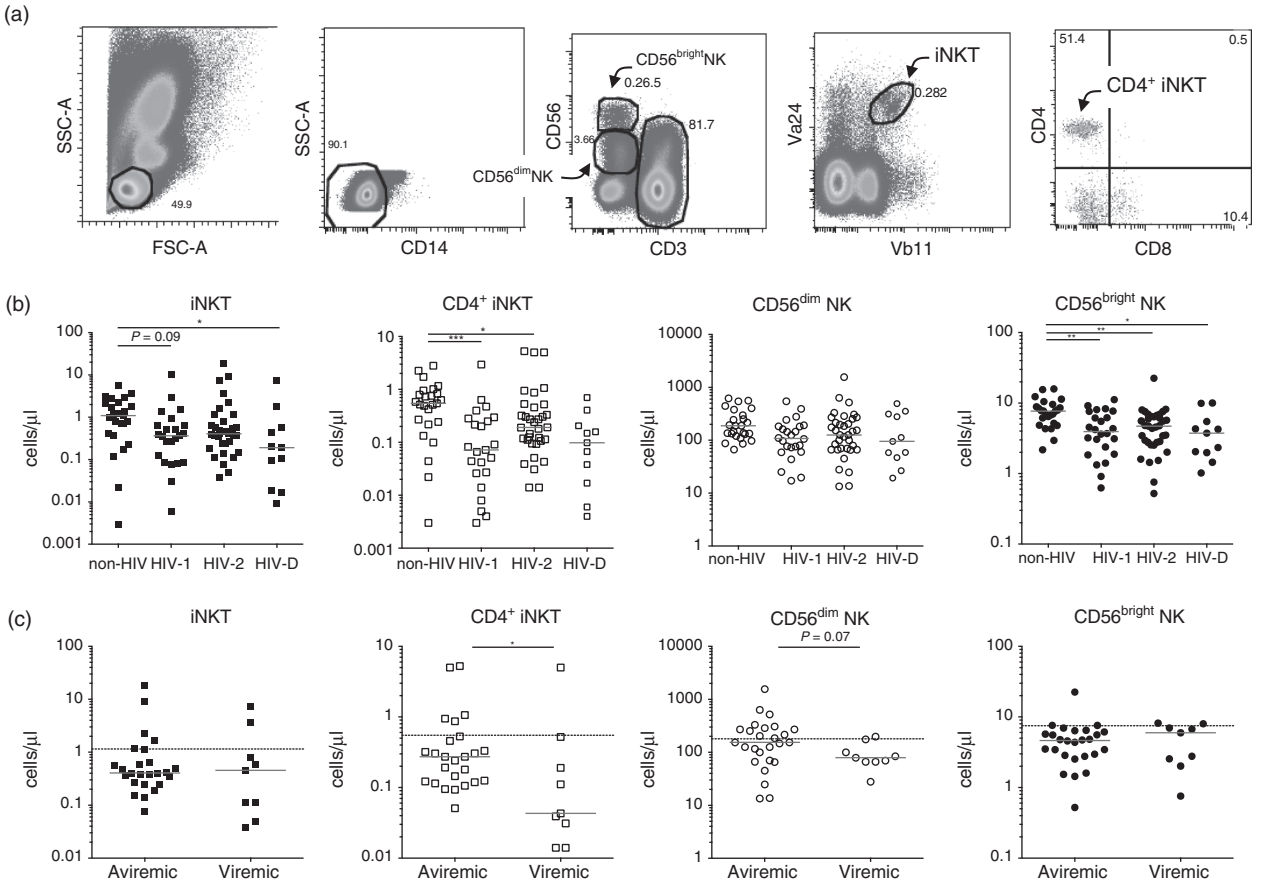

Fig. 1. Invariant natural killer T and natural killer cell subsets are reduced in HIV-infected individuals. Absolute numbers of invariant natural killer T (iNKT) and natural killer (NK) cell subsets assessed in blood samples of HIV-1, HIV-2, HIV-D, and seronegative control individuals. (a) The gating strategy for the different iNKT and NK cell subsets. (b) The total invariant natural killer T-cell (filled squares), CD4$^+$ iNKT cell (open squares) CD56^{dim} NK cell (open circles), and CD56^{bright} NK cell (filled circles) absolute numbers per µl of blood. Comparisons of absolute numbers of (c) total iNKT, CD4$^+$ iNKT, CD56^{dim} NK cell and CD56^{bright} NK cell subsets (labelled as described above) in HIV-2 aviremic (viral load ≤75 RNA copies/ml blood) and viremic individuals (viral load >75 RNA copies/ml). The dashed line represents the median absolute number of respective cell type in HIV-seronegative individuals. Median is depicted as solid bars. The Kruskal–Wallis test with Dunn's post test, and the Mann–Whitney test were used where appropriate. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

progression. Thus, we set out to analyse the level of CD38 expression on these cells in HIV-1, HIV-2, and HIV-D-infected individuals as well as HIV-seronegative controls (Fig. 2a). CD38 expression levels on total iNKT, $CD4^+$ iNKT, and $CD56^{bright}$ NK cells were significantly elevated in both HIV-1 and HIV-2 singly infected study participants ($P < 0.0001$ to $P < 0.05$) compared with the controls. HIV-D-infected individuals also had increased CD38 expression on iNKT cells and $CD56^{bright}$ NK cells ($P < 0.01$ and $P < 0.05$, respectively). Furthermore, the activation levels of both $CD56^{dim}$ and $CD56^{bright}$ NK cells were higher in HIV-1 compared with HIV-2-infected individuals ($P < 0.01$).

As T-cell activation has been shown to correlate with viral load in both HIV-1 and HIV-2 infections [15], we next compared CD38 expression levels on iNKT cells and NK cells in patients with viremic and aviremic HIV-2 infection (Fig. 2b). Elevated levels of CD38 expression on $CD4^+$ iNKT, $CD56^{dim}$ as well as $CD56^{bright}$ NK cells distinguished the viremic state of HIV-2 infection ($P < 0.05$). Furthermore, CD38 expression on total iNKT, $CD4^+$ iNKT, and $CD56^{bright}$ NK cells of aviremic HIV-2-infected study participants were elevated compared with controls ($P < 0.01$; Fig S4 in Supplemental Digital Content, http://links.lww.com/QAD/A929). Taken together, these findings suggest that elevated activation of iNKT and NK cell subsets is a shared feature between HIV-1 and HIV-2 infections.

Altered immune activation of T lymphocytes in HIV infections is potentially influenced by the expression of certain TFs [50]. Promyelocytic leukaemia zinc finger (PLZF) and T-bet have been implicated in the development and activation of iNKT cells and NK cells [51–55]. Here, no difference in relative PLZF or T-bet levels among iNKT cells was observed between the groups (Fig. S5 in Supplemental Digital Content, http://links.lww.com/QAD/A929). We noted instead that relative PLZF levels in $CD56^{dim}$ NK cells were reduced in HIV-D-infected individuals compared with the controls ($P < 0.001$), with a trend towards reduced levels in the single HIV-1 and HIV-2 infections. In the $CD56^{bright}$ NK cell subset, relative levels of T-bet were elevated in HIV-1 infection compared with controls ($P < 0.05$).

Invariant natural killer T and natural killer cell activation in HIV infection correlate with $CD4^+$ T cell level and viral load

We next analysed if iNKT cell and NK cell activation levels correlated with viral load or $\%CD4^+$ T cells among the HIV-infected individuals. CD38 levels on both total

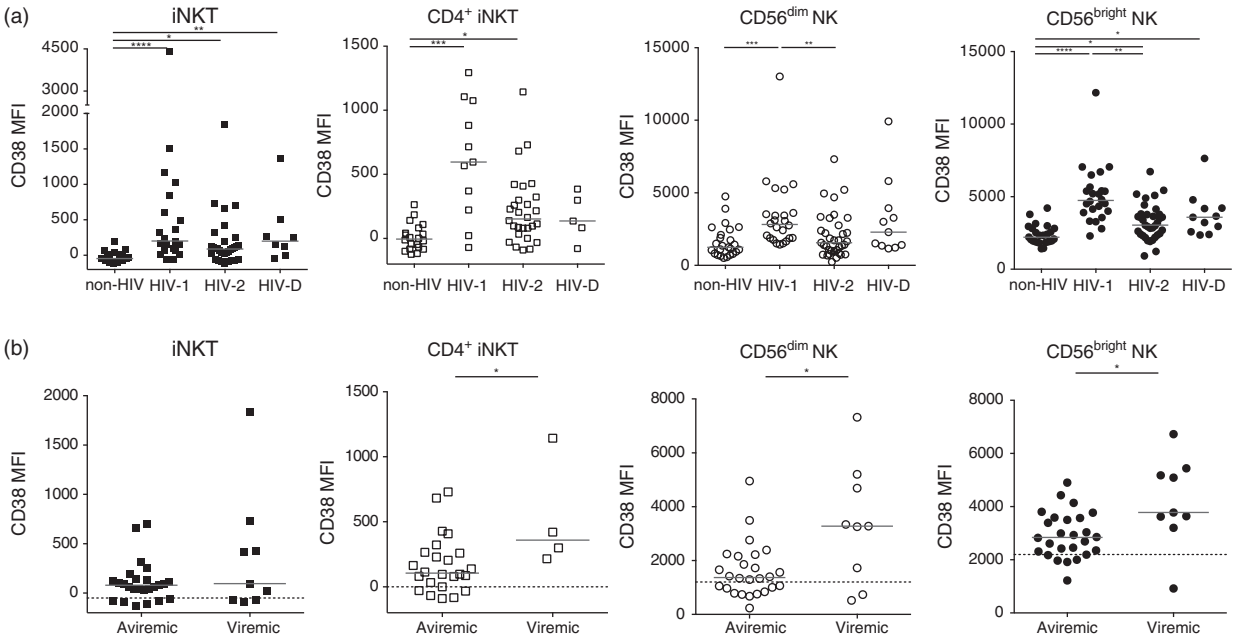

Fig. 2. Invariant natural killer T and natural killer cells display enhanced activation in HIV infections. Degree of activation represented by the median fluorescence intensity (MFI) level of CD38 surface expression was assessed in blood samples of HIV-1, HIV-2, HIV-D-infected individuals and controls. (a) Comparison of CD38 MFI expression levels on total invariant natural killer T (iNKT; filled squares), $CD4^+$ iNKT (open squares), $CD56^{dim}$ (open circles), and $CD56^{bright}$ natural killer cells (NK; filled circles) between HIV-infected groups and seronegative controls. (b) CD38 MFI levels on iNKT and NK cell subsets in aviremic and viremic HIV-2+ individuals (as defined in Fig. 1c). The dashed line represents the median CD38 MFI level of noninfected individuals. Median depicted as solid bars for the different groups. Kruskal–Wallis test with Dunn's post test was used for multiple comparisons, whereas the Mann–Whitney *U* test was used for comparison of two groups. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, 0.001, $^{****}P < 0.0001$.

iNKT cells and $CD4^+$ iNKT cells (Fig. 3a) correlated directly with viral load ($r = 0.33$, $P < 0.01$ and $r = 0.48$, $P < 0.001$, respectively), and inversely with $CD4^+$ T cells ($r = -0.36$, $P < 0.01$ and $r = -0.36$, $P < 0.05$, respectively). Furthermore, strong correlations were observed between levels of CD38 on $CD56^{dim}$ and $CD56^{bright}$ NK cells (Fig. 3b) and viral load ($r = 0.52$, $P < 0.0001$ and $r = 0.65$, $P < 0.0001$, respectively) and $\%CD4^+$ T cells ($r = -0.41$, $P < 0.001$ and $r = -0.57$, $P < 0.0001$, respectively). There were no significant correlations between the iNKT and NK cell expression levels of TFs and CD38 (data not shown).

To further delineate the modulators of CD38 expression, we performed analyses using univariate general linear modelling, including HIV status, age, sex, $log\%CD4^+$ T cells, and log viral load. These analyses supported the conclusion that $\%CD4^+$ T cells, independently of HIV infection type, associated with activation levels assessed by CD38 expression on $CD56^{dim}$ and $CD56^{bright}$ NK cells, as well as total iNKT cells (Table S2 in Supplemental Digital Content, http://links.lww.com/QAD/A929). Furthermore, the $CD4^+$ iNKT cell activation levels were associated with viral load.

Coordinated elevation of immune activation in innate and adaptive cell subsets

Little is known about the correlations between levels of activation of innate and adaptive lymphocyte subsets in HIV infections. We therefore analysed the relationships between CD38 expression levels on $CD4^+$ T cells and NK as well as iNKT cell subsets. Our data revealed relatively strong correlations between activation levels of $CD4^+$ T cells (Fig. 4a) and those of total iNKT cells ($r = 0.55$, $P < 0.0001$), $CD4^+$ iNKT cells ($r = 0.79$, $P < 0.0001$), $CD56^{dim}$ NK cells ($r = 0.67$, $P < 0.0001$), and $CD56^{bright}$ NK cells ($r = 0.67$, $P < 0.0001$). We also noticed that activation levels of the total iNKT and the $CD4^+$ iNKT cell subsets positively correlated with both NK cell subpopulations (Fig. 4b), and most significantly so between the $CD4^+$ iNKT cells and the $CD56^{bright}$ NK cells ($r = 0.76$, $P < 0.0001$). Thus, these findings suggest that elevated activation levels can be detected concomitantly in different lymphocyte populations, representing both innate and adaptive cells.

Discussion

Here we investigated frequencies and activation levels of iNKT cells and NK cells in treatment-naïve HIV-1 and HIV-2 singly-infected or dually-infected individuals, as well as HIV-seronegative controls. Our results indicate that chronic infection of both HIV-1 and HIV-2 type leads to elevated activation of both iNKT cells and NK cells.

The phenotype of iNKT cells in chronic HIV-1 infection is characterized by elevated expression of the PD-1 exhaustion marker, and an impaired ability to produce IFN-γ [38]. In this study, we found that individuals infected with different types of HIV had iNKT cells with elevated CD38 expression, reflecting an increased activation status. Our results indicated also that viremic HIV-2+ individuals have $CD4^+$ iNKT cells with increased levels of CD38, as compared with the study participants with aviremic HIV-2 infection. It should,

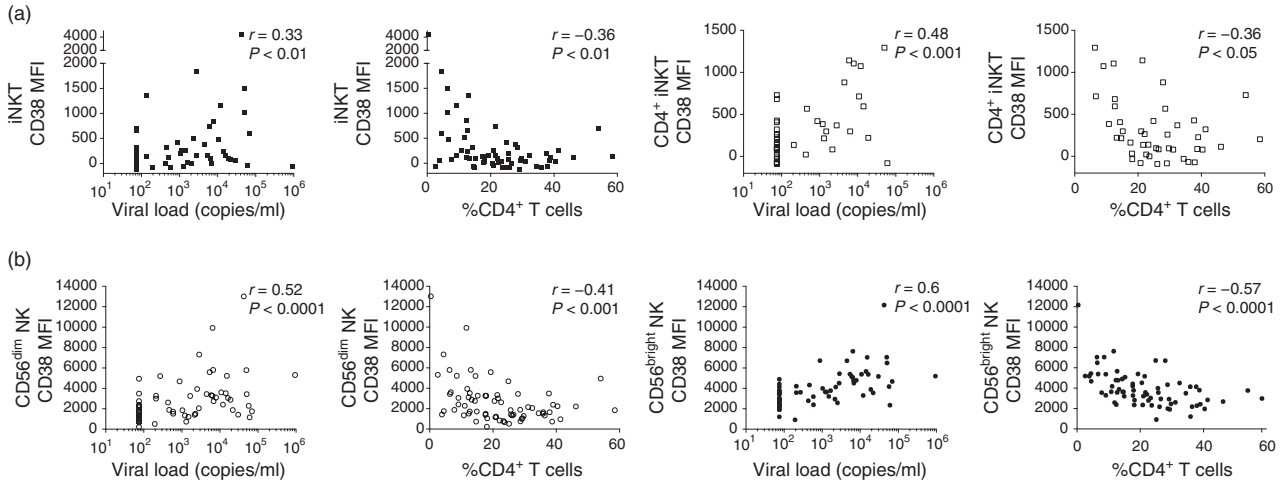

Fig. 3. Invariant natural killer T and natural killer activation correlates with $CD4^+$ T-cell levels and viral load. Level of activation represented by CD38 median fluorescence intensity on invariant natural killer T (iNKT) and natural killer (NK) subsets. (a) Correlations of CD38 levels on total iNKT (filled squares) and $CD4^+$ iNKT (open squares) to viral load (RNA copies/ml) and to $\%CD4^+$ T-cells among HIV-infected individuals. (b) Correlation of CD38 levels on $CD56^{dim}$ (open circles) and $CD56^{bright}$ (filled circles) NK cells with viral load (RNA copies/ml) and $\%CD4^+$ T cells among HIV-infected individuals. Correlations were calculated using the Spearman Rank correlation test.

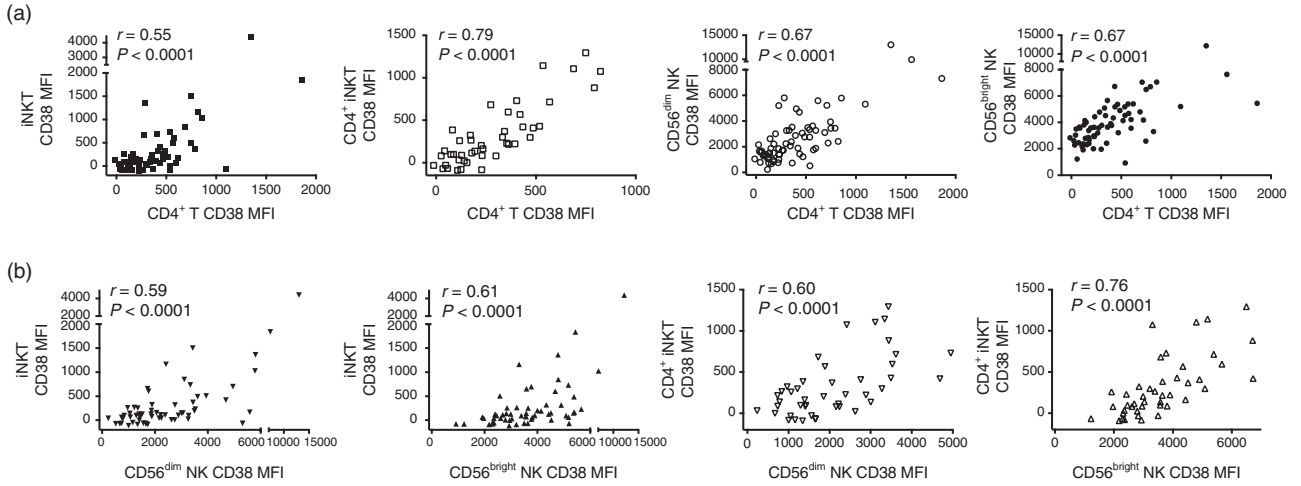

Fig. 4. Correlations between upregulation of CD38 expression on different invariant natural killer T and natural killer cell subsets, as well as CD4$^+$ T cells. Level of activation represented by CD38 median fluorescence intensity on invariant natural killer T (iNKT) and natural killer (NK) subsets, as well as CD4$^+$ T cells of HIV-infected individuals. (a) Correlation between CD38 expression on total iNKT (filled squares), CD4$^+$ iNKT (open squares), CD56^{dim} (open circles) and CD56^{bright} NK (filled circles) cells to CD38 expression on CD4$^+$ T cells. (b) Correlation between CD38 expression on total iNKT and CD4$^+$ iNKT to CD56^{dim} and CD56^{bright} NK cells. Correlations were calculated using the Spearman Rank correlation test.

however, be noted that the number of study participants with viremic HIV-2 infection was limited and firm conclusions are therefore difficult to draw. Still, a similar pattern has been described for conventional T cells in viremic HIV-2-infected individuals displaying T-cell activation comparable with that of HIV-1 infections [16]. Nevertheless, the aviremic HIV-2 study participants, with low or no viremia, had CD4$^+$ iNKT cells with CD38 expression levels that were higher than that of the HIV-seronegative controls, which concurs with earlier observations on increased T-cell activation in aviremic HIV-2 infected individuals [17] and HIV-1 elite controllers [56].

In agreement with previous studies describing an association between NK cell activation and HIV-1 disease progression [27,57], we observed that activation of both CD56^{dim} and CD56^{bright} NK cells was increased in HIV-1 singly infected individuals compared with the controls, but also to the HIV-2-infected individuals. NK cell activation levels in HIV-2 infected study participants with detectable viremia were comparable with those observed in HIV-1. Nevertheless, similar to CD4$^+$ iNKT cells, the activation level of CD56^{bright} NK cells of aviremic HIV-2 study participants were somewhat elevated compared with the HIV-seronegative controls. This finding stands in contrast to the lack of NK cell activation in HIV-1 elite and viremic controllers reported by others [27]. However, as the majority of the HIV-2 aviremic study participants had been infected for several decades, it is possible that prolonged HIV-2 infection may influence the activation state of the NK cells even in the absence of overt viremia. It is also clear that aviremic HIV-2-infected study participants can have elevated T-cell activation along with declining CD4$^+$ T-cell levels [17]. In line with this finding, our univariate general linear modelling analyses revealed that reduced CD4$^+$ T-cell level strongly influenced elevated activation of NK cells in an HIV type-independent manner. Analogous impact of CD4$^+$ T-cell levels on frequencies of CD38 expressing NK cells was recently reported for HIV-1 infection [27].

Here we observed that activation levels of iNKT and NK cell subsets are highly correlated and that associations exist between the activation levels of these innate lymphocytes and CD4$^+$ T-cell activation. This may suggest that iNKT and NK cells contribute to the systemic immune activation, or that this phenomenon is the consequence of such activation. The relationships and kinetics between different factors contributing to chronic immune activation in HIV infections are still not fully elucidated (reviewed in [58]), and may require longitudinal studies from the time of the primary infection, which, especially in the setting of HIV-2 infection, are challenging. Progressive disease in HIV-2 infection has been associated with reduced capacity of NK cells to degranulate, along with altered chemokine and cytokine secretion by CD56^{bright} NK cells [32], implying that although NK cell activation is elevated, these cells are not fully functional. Previously, we reported on increased levels of IL-12 in whole blood of HIV-1 and/or HIV-2-infected individuals with progressive disease, indicating that the overall cytokine milieu may activate NK cells [59].

The pattern with loss of CD4$^+$ iNKT cells in HIV-1 and dually-infected participants observed here is consistent with previous findings in HIV-1 single infections

[36,37,39]. Among the HIV-2-infected individuals, it was mainly those with viremia who had a decrease in $CD4^+$ iNKT cells, similar to that seen in the HIV-1-infected participants. Although the $CD4^+$ iNKT cell subset can be infected with HIV-1 [60], this has not yet been investigated for HIV-2. Although, not as stark as previously reported [61], we observed either trends or significant reductions in the numbers of $CD56^{dim}$ and $CD56^{bright}$ NK cell subpopulations during HIV-1 infection. This reduction appeared to be independent of the HIV type. Furthermore, HIV-2 viremia tended to lead to a reduction in $CD56^{dim}$ NK cells, whereas the $CD56^{bright}$ population did not change in numbers or proportion. This implies that viremia in HIV-2 infection may alter NK cell maturation, a phenomenon also observed in IFNα treatment of chronic hepatitis [62].

HIV-associated immune activation has been related to alterations of transcriptional regulation in $CD8^+$ T cells [63,64]. The TFs PLZF and T-bet are important for iNKT and NK cell development, maturation, and function [65–69]; however, their expression in human iNKT cells and NK cells from HIV-infected individuals remains unexplored. We noticed here decreased PLZF levels in the $CD56^{dim}$ NK cells of HIV-D-infected individuals, and a downward trend in the $CD56^{bright}$ NK cell subpopulation. Furthermore, increased T-bet levels were detected in $CD56^{bright}$ NK cells during HIV-1 infection. Additionally, we analysed relationships between the iNKT and NK cell expression levels of TFs and CD38, but found no significant correlations (data not shown). These results should spur further investigations on TFs and their role in functional and developmental modifications of iNKT and NK cells during different types and stages of HIV infections.

In summary, our findings reveal that activation levels of iNKT cells and NK cells are elevated and coordinated in untreated HIV-1, HIV-2, and HIV-D infections. Additionally, we show that elevated activation of both iNKT and NK cells is associated with reduced $CD4^+$ T-cell percentages and higher viral loads, in a largely HIV type-independent manner.

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Conflicts of interest

There are no conflicts of interest.

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