

# B7-H6-mediated downregulation of NKp30 in natural killer cells contributes to HIV-2 immune escape

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**Objective:** HIV-1 and HIV-2 differ notably in their *epidemiology*, with worldwide HIV-1 spread and HIV-2 mainly confined to West Africa. Natural killer (NK) cells are critical antiviral effectors of the immune system; however, limited information is available about these innate effector cells during HIV-2 infection.

**Method:** In this study, 24 untreated HIV-2-infected patients were analyzed and compared with 21 long-term nonprogressor and 10 controller HIV-1<sup>+</sup> patients, and healthy donors. Extensive phenotype and functional NK-cell characteristics, as well as ligands of activating NK receptors involved in NK lysis were determined by flow cytometry.

**Results:** We report in HIV-2<sup>+</sup> patients a very significant reduced expression of the activating NKp30 receptor ( $P < 0.0001$ ) on NK cells, much higher than observed in HIV-1<sup>+</sup> patients. The impaired expression of NKp30 is correlated negatively with HLA-DR ( $r = -0.5970$ ;  $P = 0.0002$ ), and positively with both NKG2A ( $r = 0.5324$ ;  $P < 0.0001$ ) and Siglec-7 ( $r = 0.5621$ ;  $P = 0.0004$ ). HIV-2<sup>+</sup> patients with NKp30<sup>low</sup> NK cells displayed overproduction of IFN- $\gamma$  ( $P < 0.0001$ ) associated with impaired cytolytic function when tested against target cells expressing surface B7-H6. This cellular ligand of NKp30 is strongly detectable as a surface molecule on CD4<sup>+</sup> T cells infected by HIV-2.

**Conclusion:** Altogether, our data suggested that the defective expression of NKp30 may be induced by the chronic engagement of this receptor by B7-H6 expressed on HIV-2-infected target cells. This represents a novel mechanism by which the chronic ligand exposure by the viral environment may subvert NK-cell-mediated function to establish persistent HIV-2 infection. Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

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**Keywords:** B7-H6, HIV-2, natural killer cells, NKp30, viral escape mechanism

## Introduction

In recent decades, intensive research has focused on HIV-1 infection. Much less attention has been paid to the related HIV-2, which is endemic in West Africa, and in countries with strong socioeconomic ties to this region [1]. Compared

with HIV-1 infection, the clinical course of HIV-2 infection is generally characterized by a longer asymptomatic stage and, even without combined antiretroviral therapy, by lower plasma HIV viral loads and a lower mortality rate. HIV-2-infection can however progress to AIDS over time [2]. Spontaneous control of infection is nonetheless more

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common among HIV-2-infected individuals, with HIV controllers (HIC) accounting for 9.1% of HIV-2<sup>+</sup> [3], compared with 0.2% of HIV-1<sup>+</sup> individuals [4].

Natural killer (NK) cells act as bridge between innate and adaptive immune processes. In infectious settings, NK cells critically contribute to effector antiviral innate immune responses, as shown by the high susceptibility to viral diseases of individuals with inherited NK deficiencies [5]. NK cells, while engaged in immune surveillance processes, distinguish their cellular targets from healthy cells as a consequence of the balance between the triggering of their various inhibitory versus activating receptors, the former recognizing ligands expressed on healthy cells to maintain self-tolerance and the latter recognizing ligands specifically induced on 'stressed' cells to allow their elimination [6]. When the activating signals begin to outweigh their inhibitory counterparts, NK cells produce an array of cytokines (e.g. IFN- $\gamma$  and TNF- $\alpha$ ) and perform cytotoxic functions [7,8]. In HIV-1 infection, various genetic, epidemiological, and functional studies have shown that NK cells may be directly involved in preventing HIV-1 replication [9–11]. In HIV-2<sup>+</sup> individuals, the data collected until now have been limited to a few quantifications of NK cells and/or functional studies demonstrating the cytotoxicity of these cells, more so than for HIV-1<sup>+</sup> patients [12,13].

The sparseness of these data prompted us to perform an extensive phenotypic and functional characterization of NK cells from untreated HIV-2<sup>+</sup> patients of the ANRS (France Recherche Nord&Sud Sida-HIV Hépatites) CO5 HIV-2 cohort and compare them with those from long-term nonprogressor (LTNP) and controller (HIC) HIV-1<sup>+</sup> patients. We report herein that an intricate interplay between Nkp30 and its ligands leads to phenotypic and functional specific impairment of NK cells from HIV-2<sup>+</sup> patients.

## Materials and methods

### Study population and samples

The 24 HIV-2<sup>+</sup> patients were those included in the ANRS Immunovir-2/Reservoir study of the ANRS

HIV-2 CO5 cohort, previously described [14]. They were compared with 15 healthy African control individuals from West Africa [15], and to 21 nontreated LTNP and 10 HIC HIV-1<sup>+</sup> patients from the ANRS CO15 ALT cohort [10]. Peripheral blood samples from 20 uninfected Caucasian healthy controls were obtained from the French Blood Bank (Etablissement Français du Sang) and analyzed as controls for HIV-1<sup>+</sup> patients (Table 1). This study was conducted in accordance with the principles of the Declaration of Helsinki and with French statutory and regulatory law. Patients received information about research performed on biological samples and provided written informed consent to participate. This study was approved by the institutional ethics committee (Comité de Protection des Personnes of Ile de France XI).

### Flow cytometric analysis

CD3<sup>-</sup>CD56<sup>+</sup> NK cells were analyzed from frozen peripheral blood mononuclear cells (PBMC) by flow cytometry with an appropriate cocktail of mAbs described in Table Supplement 3, <http://links.lww.com/QAD/B388>. At least 20 000 CD45<sup>+</sup> cells were acquired on a Gallios flow cytometer (Beckman Coulter, Pasadena, California, USA) and then analyzed with Flow Jo version 9 (TreeStar, Ashland, Oregon, USA).

### Functional assay

NK cells were incubated with or without human leukocyte antigen (HLA) class-1 negative K562 target cells (ATCC CCL243), at an effector : target cell ratio of 1 : 1, in the presence of anti-CD107a mAb (#H4A3; Becton Dickinson, Franklin Lakes, New Jersey, USA), to measure degranulation. Cells were thereafter incubated for 5 h in the presence of Golgi Stop and Golgi Plug solutions (BD Biosciences) and then stained with NK-cell-surface markers. Cells were fixed, permeabilized with a cytofix/cytoperm kit (Becton Dickinson), and then intracellularly stained for IFN- $\gamma$  and TNF- $\alpha$  production (Table Supplement 3, <http://links.lww.com/QAD/B388>) [10]. In some experiments of degranulation, K562 target cells were preincubated with 10  $\mu$ g/ml of 17B1.3 anti-B7-H6 blocking mAb. Data were analyzed with Flow Jo version 9 (TreeStar).

**Table 1. Summary of clinical characteristics of the individual studies.**

	AFR-Ctl <sup>a</sup>	HIV-2 <sup>+</sup> <sup>a</sup>	CAU-Ctl <sup>a</sup>	LTNP-HIV-1 <sup>+</sup> <sup>a</sup>	HIC-HIV-1 <sup>+</sup> <sup>a</sup>
Number	15	23	20	21	10
Male/female	7/9	10/13	9/11	11/10	5/5
Age (year)	39 $\pm$ 13	49 $\pm$ 10	43 $\pm$ 12	39 $\pm$ 9	44 $\pm$ 15
CD4 <sup>+</sup> cell count (cells/ $\mu$ l)	1104 $\pm$ 137	958 $\pm$ 324	902 $\pm$ 66	758 $\pm$ 335	699 $\pm$ 169
Time since HIV diagnosis (years)	–	14.1 $\pm$ 5.6	–	10.1 $\pm$ 1.8	10.6 $\pm$ 1.3
Plasma viral load (copies/ml)	–	14 $\pm$ 6	–	51 006 $\pm$ 87 573	74 $\pm$ 40
HIV DNA (copies/10 <sup>6</sup> PBMC)	–	156 $\pm$ 164	–	1131 $\pm$ 1626	42 $\pm$ 29

Results are presented as mean  $\pm$  SD. Plasma viral load was quantified by an ultrasensitive realtime polymerase chain reaction (qPCR) assay with a lower limit detection of 1 copy per milliliter of plasma; PBMC, peripheral blood mononuclear cells.

<sup>a</sup>AFR, samples from African individuals; CAU, samples from Caucasian individuals; Ctl, healthy controls; HIC, HIV controllers; LTNP, long-term nonprogressor.

## HLA genotyping

DNA samples were extracted from patients' whole blood with the QIAamp DNA blood mini kit (Qiagen, Hilden, Germany). HLA class-I alleles were hybridized with the LABType SSO kit (One Lambda, Canoga Park, USA). HLA sequences were read with a LABScan 200 (Luminex, Austin, TX) and computer-assisted HLA Fusion software.

## Staining and analysis of uninfected and HIV-infected target cells for ligands of natural killer-cell receptors

CEM or purified CD4<sup>+</sup> T cells were infected with 10<sup>3</sup> 50% tissue culture infective dose of the HIV-1<sub>JR-CSF</sub> or HIV-2<sub>ROD</sub> strains [16,17]. A period of 7–9 days after infection, cells were stained with 10 µg/ml of soluble fusion proteins (NKp30, NKp44, and NKp46-Ig from R&D Systems, Minneapolis, Minnesota, USA) or mAbs (ULBP-1 to ULBP-6 and B7-H6 from R&D Systems, BAT-3 from Abcam, anti-HLA-ABC from Beckton Dickinson) for 2 h at 4 °C. Cells were then incubated with rat antimouse IgG Ab (1/50) or Fcγ-fragment-specific conjugated affinity-purified F(ab')<sub>2</sub> fragments of goat antihuman IgG (1/50; Jackson ImmunoResearch, West Grove, Pennsylvania, USA), as described [18]. To measure viral protein levels from HIV-1 and HIV-2, cells were fixed, permeabilized with a cytofix/cytoperm kit (Becton Dickinson), and then intracellularly stained with KC-57 mAb (Beckman Coulter). Data were analyzed with Flow Jo version 9 (TreeStar).

## Statistical analysis

Statistical analyses were performed with Prism software (GraphPad, La Jolla, California, USA). The nonparametric Mann–Whitney *U*, Kruskal–Wallis, and Wilcoxon tests were used as appropriate for comparison of continuous variables between groups. Correlations between variables were calculated with the nonparametric Spearman rank-order test. Principal component analysis (PCA) was performed with XLSTAT (Addinsoft, Paris, France). The component loadings were calculated as correlations between measured expression values and principal component scores. *P* values more than 0.05 were not considered significant.

## Results

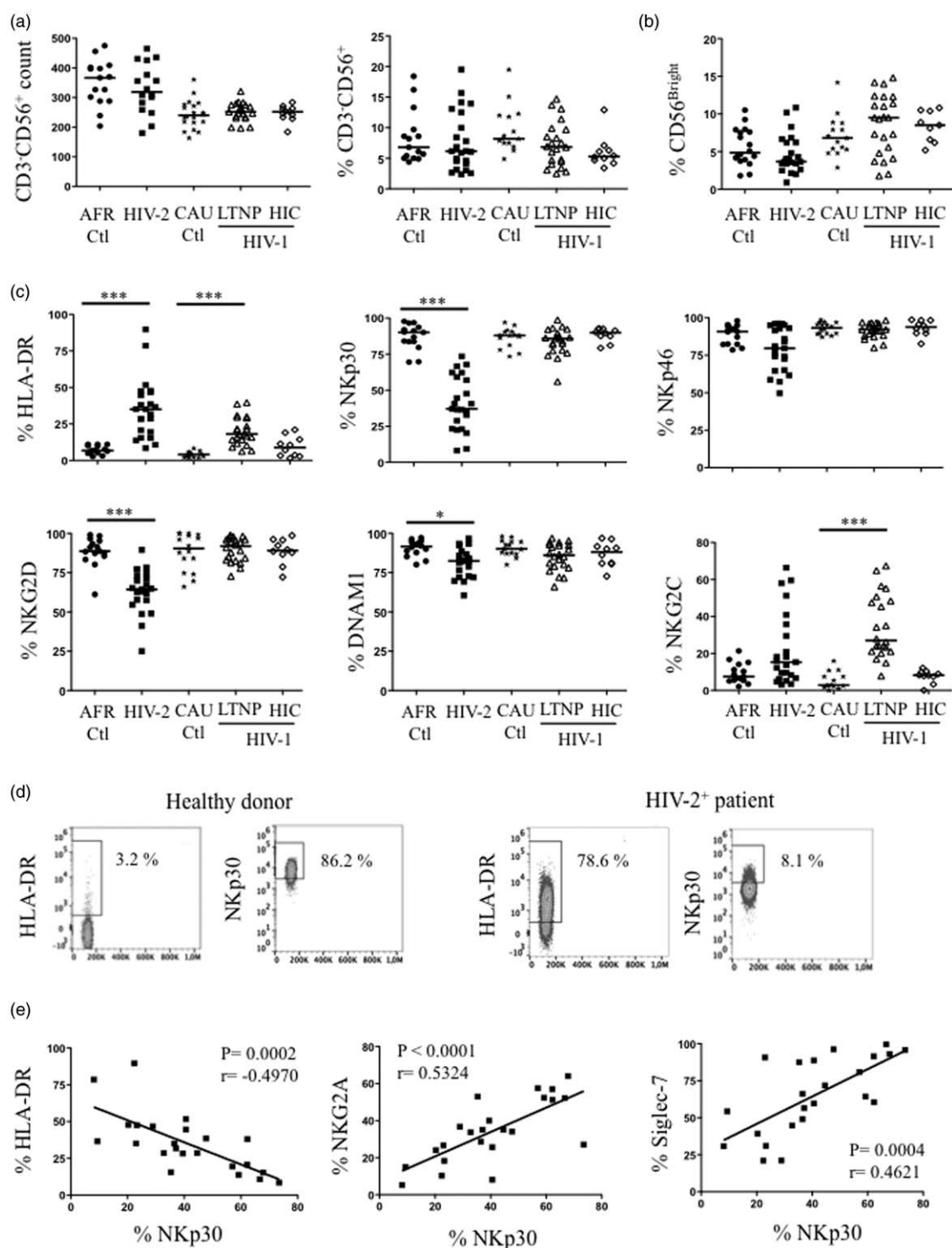
### NKp30 downmodulation in HIV-2<sup>+</sup> infection

An extensive phenotypic study was performed in NK cells from asymptomatic and treatment-naïve people infected with HIV-2<sup>+</sup>; all had been infected for at least 5 years, a CD4<sup>+</sup> T-cell count more than 400 cells/µl, and almost all an undetectable viral load (Table 1 and Supplementary Table 1, <http://links.lww.com/QAD/B388>). Both the frequency and absolute counts of circulating CD3<sup>+</sup>CD56<sup>+</sup> NK cells were similar in HIV-2<sup>+</sup> patients and healthy African controls; their absolute

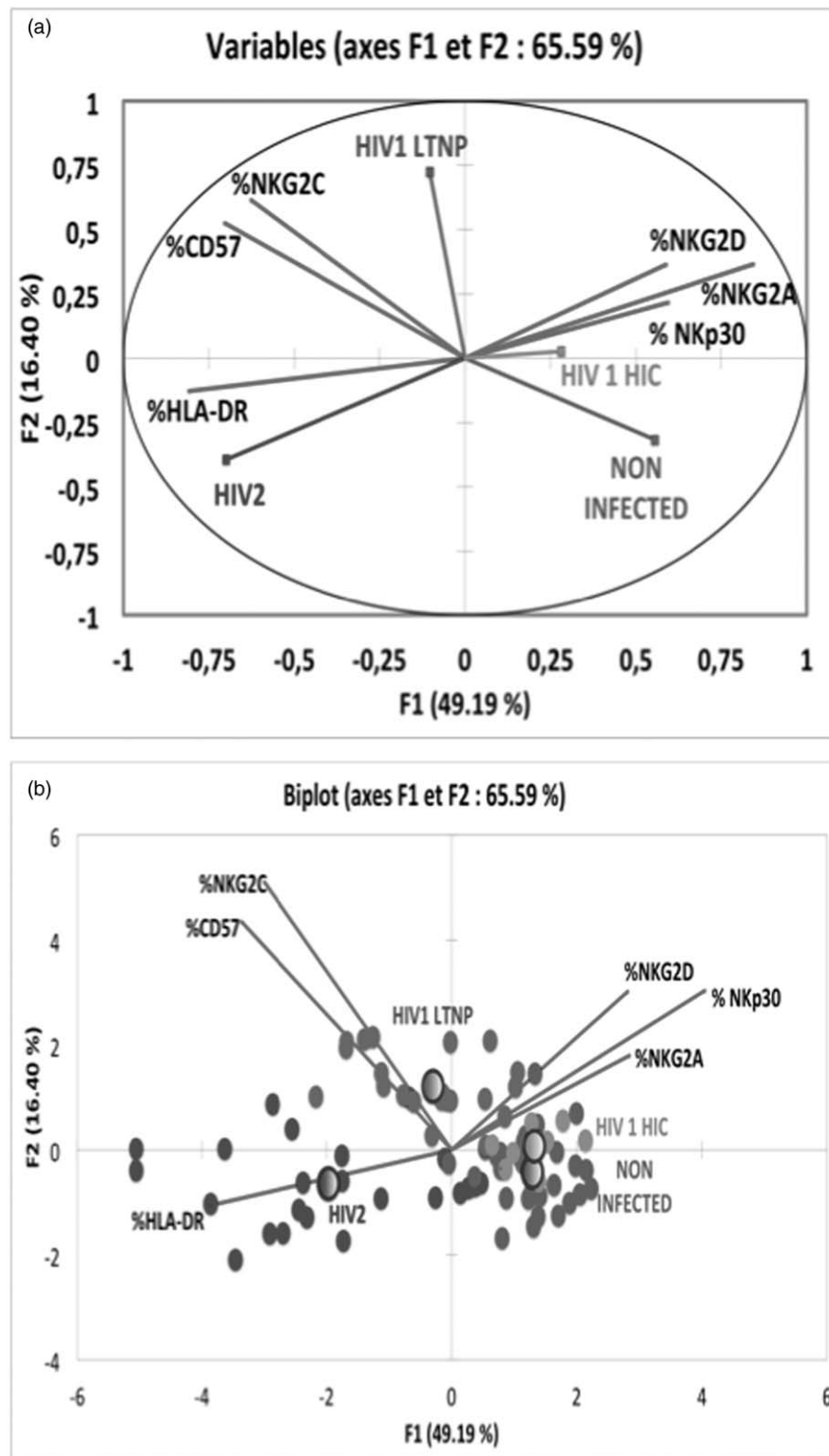
counts were slightly higher than those in uninfected and HIV-1<sup>+</sup> (LTNP and HIC) Caucasian (Fig. 1a). The CD56<sup>bright</sup> and CD56<sup>dim</sup> NK-cell subsets were equivalent in the different groups (Fig. 1b), but the proportion of CD56<sup>+</sup> NK cells was much higher in viremic HIV-1<sup>+</sup> patients (data not shown), as described [19].

A hierarchical clustering analysis of CD3<sup>+</sup>CD56<sup>dim</sup> NK cells revealed that HIV-2<sup>+</sup> patients were easily distinguishable from healthy African donors, whereas healthy African and Caucasian controls expressed similar levels of NK cell markers, excepted CD161 (Supplementary Fig. 1, <http://links.lww.com/QAD/B388>), as described [15]. However, NK cells from HIV-2<sup>+</sup> patients highly expressed the late cell-activating human leukocyte antigen (HLA - DR isotype) marker [median: 35.0% (8.4–89.7)], compared with healthy donors (less than 11.0%) and HIV-1<sup>+</sup> patients [median 18.1% (6.2–39.6) for LTNP and 8.8% (1.5–21.0) for HIC] (*P* < 0.0001) (Fig. 1c and d). Similar effects were observed in the median fluorescence intensity (Supplementary Fig. 2a, <http://links.lww.com/QAD/B388>). Of note, expression of HLA-DR was not increased in CD3<sup>+</sup> T (data not shown), indicating that lymphocyte activation was exclusively restricted to NK cells from HIV-2-infected patients. NK cells from HIV-2<sup>+</sup> patients were however indistinguishable from all other groups, in their cell-surface expression of inhibitory receptors, including NKG2A, KIR2DL1, and KIR2DL2/DL3 receptors (Supplementary Figs. 2 and 3, <http://links.lww.com/QAD/B388>), excepted KIR3DL1, which is significantly higher in HIV-2<sup>+</sup> patients than in any other group, regardless of HLA genotype (Supplementary Fig. 3, <http://links.lww.com/QAD/B388>; Supplementary Table 2, <http://links.lww.com/QAD/B388>). In contrast, expression of activating NK receptors [NKp30, NKp46, NKG2D, and DNAX accessory molecule-1 (DNAM1)] was lower in HIV-2<sup>+</sup> patients. The frequency of NKp30 was drastically decreased in HIV-2 [median: 37.1% (8.1–73.5)] compared with HIV-1<sup>+</sup> patients and healthy donors (medians between 85.8 and 90.1%, respectively) (*P* < 0.0001) (Fig. 1c; Supplementary Fig. 2, <http://links.lww.com/QAD/B388>). Of note, CD3ζ, a coreceptor of NKp30 [20], was similarly expressed in infected and noninfected samples (Supplementary Fig. 4, <http://links.lww.com/QAD/B388>). More importantly, in HIV-2<sup>+</sup> patients NKp30 is correlated negatively with HLA-DR (*r* = -0.4970; *P* = 0.0002) and positively with NKG2A (*r* = 0.5324; *P* < 0.0001) and Siglec-7 (*r* = 0.4621; *P* = 0.0004) (Fig. 1; Supplementary Fig. 2, <http://links.lww.com/QAD/B388>) [21]. Nonetheless, none of these markers was correlated with either biological parameters (CD4<sup>+</sup> T-cell count, CD4<sup>+</sup> T-cell nadir, plasma viral load, or HIV-2 DNA level).

These data were strengthened by a PCA. Figure 2a shows that HIV-2<sup>+</sup> patients segregated distinctly from all the other groups; HLA-DR expression was linked to the



**Fig. 1. Quantitative and phenotypic characteristics of natural killer cells in HIV-2<sup>+</sup> patients.** (a) Absolute count and percentage of CD3<sup>-</sup>CD56<sup>+</sup> natural killer cells. (b) Percentage of CD56<sup>bright</sup> natural killer cells among CD3<sup>-</sup>CD56<sup>+</sup> natural killer cells. (c) Percentage of CD3<sup>-</sup>CD56<sup>+</sup> natural killer cells expressing HLA-DR and major activating markers (NKp30, NKp46, NKG2D, DNAX accessory molecule-1, and NKG2C). Data are shown for African healthy donors (closed circles), HIV-2<sup>+</sup> patients (closed squares), long-term nonprogressor HIV-1<sup>+</sup> patients (open triangles), HIV controllers HIV-1<sup>+</sup> patients (open diamonds), and healthy Caucasian controls (stars). Horizontal bars represent the median. Intergroup comparisons were assessed with the Kruskal–Wallis test and Dunn’s posttest. \* $P < 0.05$ ; \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . (d) Flow cytometry expression of HLA-DR and NKp30 on CD3<sup>-</sup>CD56<sup>+</sup> natural killer cells from representative HIV-2<sup>+</sup> patient and healthy donor. (e) Correlation of NKp30 expression with HLA-DR, NKG2A, and Siglec-7 expression on CD3<sup>-</sup>CD56<sup>+</sup> natural killer cells from HIV-2<sup>+</sup> patients.



**Fig. 2. Principal component analysis of natural killer cell markers from HIV-2<sup>+</sup> patients.** (a) Statistical proximity between different natural killer-cell markers tested. (b) Statistical distribution of the individuals according to the differential expression of natural killer cell markers. Analyses are performed for HIV-2<sup>+</sup> patients (blue), HIV-1<sup>+</sup> long-term nonprogressor (violet), HIV-1<sup>+</sup> HIV controllers (orange), and healthy donors (green).

HIV-2<sup>+</sup> patients, NKG2C and CD57 markers were mostly associated with LTNP HIV-1<sup>+</sup> status, whereas HIC HIV-1<sup>+</sup> patients and healthy controls were close together but not directly associated with any specific NK-cell marker (Fig. 2b). All together these data strongly suggested that HIV-2 induces a different NK-cell signature than that found in LTNP and HIC HIV-1<sup>+</sup> patients.

### NKp30 downmodulation in natural killer cells is associated with upregulation of B7-H6 on HIV-2<sup>+</sup> target cells

The role of specific ligands of major NK receptors was assessed by infecting target cells *in vitro* by HIV-2<sub>ROD</sub> or HIV-1<sub>JR-CSF</sub> strains. The frequency (mean ± SD) of infected cells was 25.2 ± 8.1% and 27.5 ± 7.9% for HIV-2 and HIV-1, respectively (data not shown). A specific and significant downmodulation of major histocompatibility complex (MHC) class-I molecules was observed in HIV-2-infected cells (mean ± SD: 50.3 ± 7.5%, compared with uninfected cells, mean ± SD: 94.3 ± 3.8%;  $P < 0.0001$ ; Fig. 3a), as previously reported for HIV-1 [23,24]. To take into account the profound modulation of several activating NK receptors in HIV-2-infected patients (Fig. 1c), we tested the expression of their ligands on target cells. Figure 3b shows that both infected and uninfected bystander cells expressed ULBP-1 to ULBP-6 in both HIV-1 and HIV-2 infected cultures, whereas none of MHC class-I polypeptide-related sequence (MIC)-A, MIC-B, or ligands for NKp46 were induced after infection by HIV-1 or HIV-2 (Fig. 3b; data not shown). Ligands for NKp44 were not induced by HIV-2, but overexpressed on bystander HIV-1<sup>+</sup> cells, as previously reported [22,23]. Inversely, ligands for NKp30 were not induced in HIV-1, but increased in cells infected by HIV-2, compared with bystander cells; significant difference was only reached with uninfected cells ( $P = 0.0144$ ) (Fig. 3b). However, we cannot completely exclude that at least a fraction of bystander cells that upregulated B7-H6 were infected but did not express sufficient amounts of HIV antigens for detection. To more directly specify expression of NKp30 ligand on target cells infected by HIV-2, purified CD4<sup>+</sup> T cells were IL-2-activated, infected by HIV<sub>ROD</sub> and then tested for surface-expression of BAT-3 and B7-H6, two major cellular ligands of NKp30 [24,25]. Figure 3c shows that BAT-3 expression is not, or very slightly, modulated after HIV-2 infection, whereas B7-H6 seems to be mainly induced in CD4<sup>+</sup> T cells *in-vitro* infected by HIV-2<sub>ROD</sub>. Of note, soluble forms of B7-H6 and BAT-3 were not detectable in the serum of patients infected with either form of HIV or of healthy donors (data not shown).

### NKp30 downmodulation in natural killer cells is associated with aberrant functional capacity in HIV-2<sup>+</sup> patients

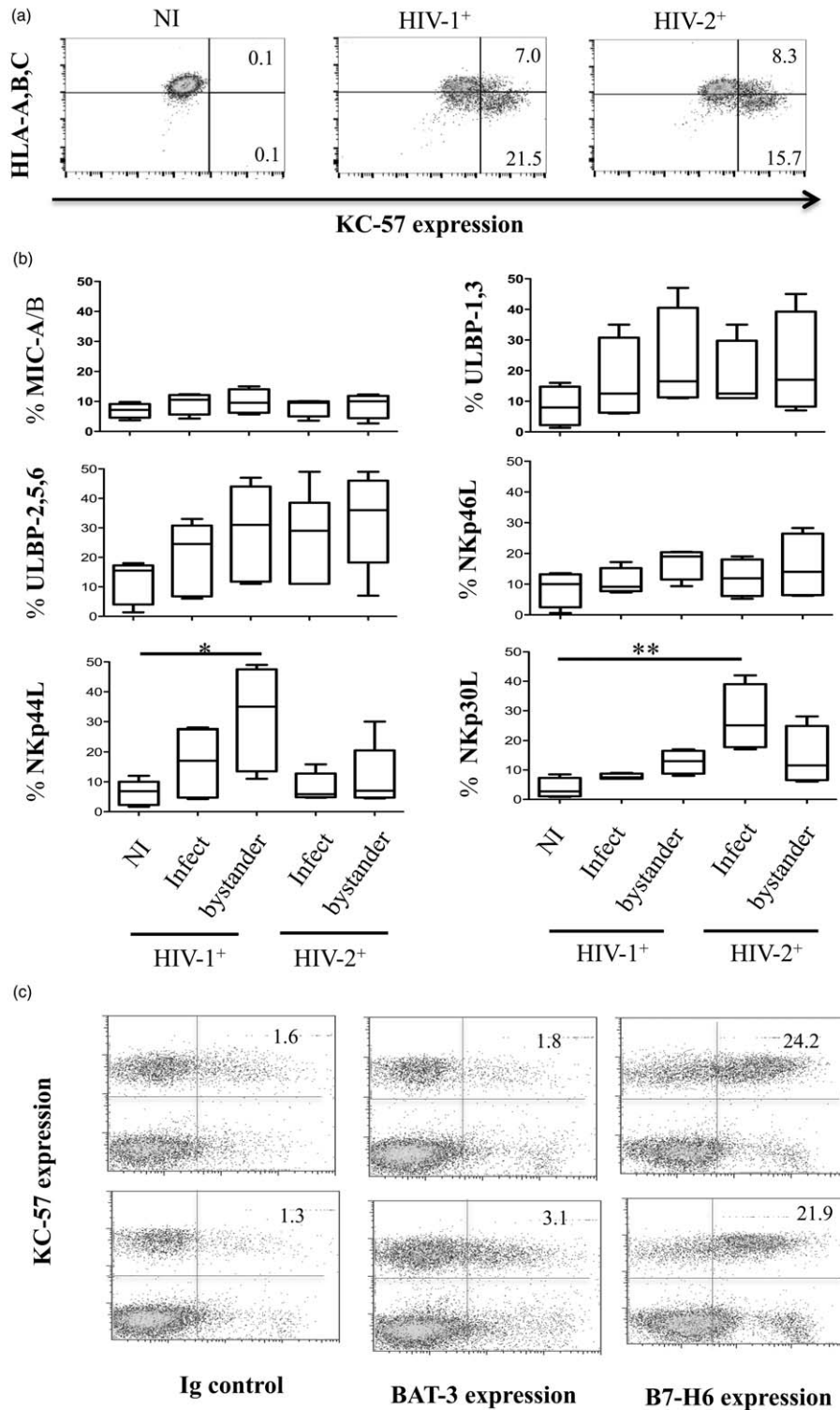
To determine the functional significance of these findings, we examined the overall functional capacity of NK-cell

subsets from HIV-2<sup>+</sup> patients. Figure 4a shows that in untreated condition, a median of 22.6% of NK cells from HIV-2 patients produced IFN- $\gamma$ , compared with less than 1.6% for healthy donors ( $P < 0.0001$ ). After stimulation by IL-12/IL-18 (Fig. 4a), the level of IFN- $\gamma$  was not significantly increased in NK cells from HIV-2 patients, compared with healthy donors (Fig. 4a). of note, level of TNF- $\alpha$  was similar in untreated NK cells from healthy and HIV-2<sup>+</sup> individuals and not strongly increased after stimulation with IL-12/IL-18 (data not shown).

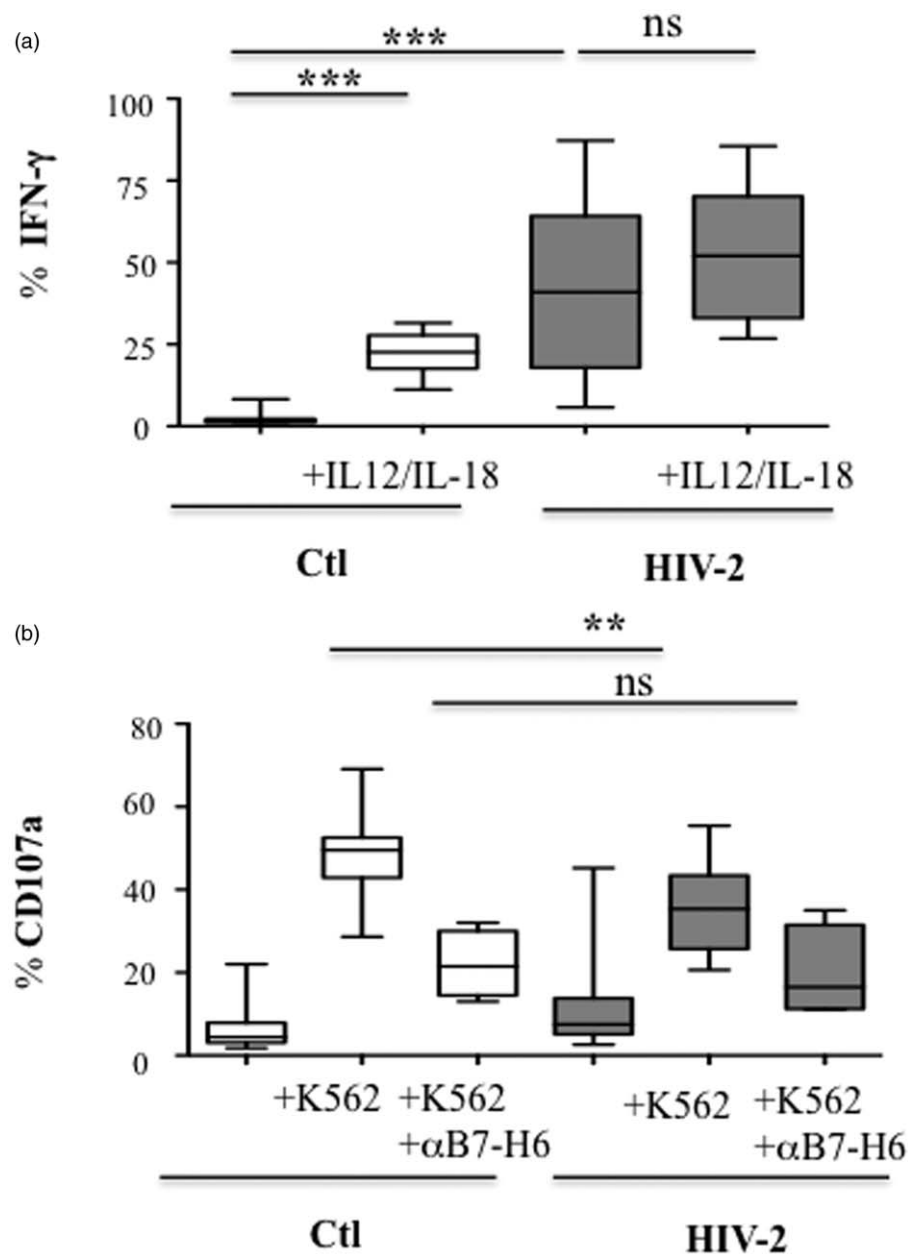
Next, NK cells were tested in degranulation assay against MHC-class I-negative K562 target cells. This cell line was previously shown to express high level of surface B7-H6. Figure 4b show that degranulation of NK cells was significantly less increased in HIV-2<sup>+</sup> patients, compared with healthy donors (Mann-Whitney test,  $P = 0.005$ ), whereas, similar expression of the different cytotoxic granule components (perforin, granzyme B, and granulysin) was observed in samples from HIV-2<sup>+</sup> and healthy individuals (Supplementary Fig. 5, <http://links.lww.com/QAD/B388>). To more directly assess the role of NKp30 in the degranulation of NK cells from HIV-2<sup>+</sup> patients, blocking experiments were performed with the 17B1.3 anti-B7-H6 blocking mAb, able of disrupting the recognition of NKp30. In the presence of anti-B7-H6 Ab, similar level of degranulation was observed in NK cells from HIV-2<sup>+</sup> and healthy individuals (Fig. 4b).

## Discussion

Our in-depth analysis revealed that HIV-2<sup>+</sup> patients and healthy donors can be separated in two distinct groups of patients by their specific NK cell profiles. On the other hand, the difference between HIV-2<sup>+</sup> and HIV-1<sup>+</sup> (LTNP and HIC) patients, suggests that NK cells play distinct roles in these two infections. Specifically, we observed that HIV-2<sup>+</sup> patients harbored an uncommon NK-cell signature, highlighted by a low expression of activating receptor associated with chronic cell-activation, suggesting that HIV-2 might sustain constitutive NK-cell activation, even in the absence of a detectable viral load. High level of HLA-DR expression is already considered as a hallmark of HIV-1<sup>+</sup> infection [10], however in HIV-2, HLA-DR expression inversely correlated with NKp30. Expression of this activating receptor is also markedly impaired in HIV-1, but only in viremic HIV-1<sup>+</sup> patients [26], and mainly among the CD56<sup>-</sup> NK-cell-subset, which largely accounts for the highly defective NK-cell-mediated lysis of autologous dendritic cells [27,28], a subset of NK cells not over-presented in HIV-2<sup>+</sup> patients. Decreases of NKp30 was previously reported in numerous other diseases, including hepatitis C virus (HCV) infection [29], papillomavirus-induced cervical cancer [30], acute myeloid leukemia [31], neuroblastoma [32], Sjögren syndrome [33], and anti-synthetase syndrome [34], suggesting a key role of this



**Fig. 3. Expression of ligands for natural killer cell receptors on target cells infected by HIV-2.** (a) Representative flow cytometry expression of MHC class I molecules (HLA-A, B, and C) on uninfected (NI) or CEM infected by HIV-1<sub>JR-CSF</sub> (CEM-HIV-1<sup>+</sup>) or HIV-2<sub>Rod</sub> (CEM-HIV-2<sup>+</sup>) according to HIV infection (KC-57 expression). (b) Expression of ligands for NKG2D (MIC-A/B, ULBP1/3, and ULBP 2/5/6), and NCRs (NKp30L, NKp44L, and NKp46) on uninfected (NI) or CEM infected by HIV-1<sub>JR-CSF</sub> (HIV-1<sup>+</sup>) or HIV-2<sub>Rod</sub> (HIV-2<sup>+</sup>). Data are shown for infected (infect; KC-57<sup>+</sup>) and uninfected bystander (bystander; KC-57<sup>-</sup>) CEM cells. Intergroup comparisons were assessed with the Kruskal–Wallis test and Dunn’s posttest. \**P* < 0.05; \*\**P* < 0.01. (c) Expression of NKp30 ligands (B7-H6 and BAT-3) on purified CD4<sup>+</sup> T cells from two healthy donors infected by HIV-2<sub>Rod</sub> strain. Percentage of HIV-2-infected cells (KC-57<sup>+</sup>) that expressed ligand of NKp30 are shown. NI, non infected.



**Fig. 4. Functional correlations with NKp30 in natural killer cells from HIV-2<sup>+</sup> patients.** (a) Intracellular expression of IFN- $\gamma$  in natural killer cells from healthy donors (Ctl) and HIV-2<sup>+</sup> patients. Cells were tested without treatment or after an overnight treatment with IL12 and IL18 (+IL12/IL18). (b) Degranulation assay by expression of CD107a on natural killer cells from healthy donors (controls) and HIV-2<sup>+</sup> patients. Cells were untreated or tested in the presence of K562 target cells (+K562) treated or not by 10 mg/ml anti-B7-H6 blocking mAb (+ $\alpha$ B7-H6). Effector and target were used at a 1:1 ratio. Intergroup comparisons were assessed with the Kruskal–Wallis test and Dunn’s posttest. \*\*\* $P < 0.0001$ ; \*\* $P < 0.01$ . ns: nonsignificant.

activating NK receptor in pathology. Furthermore, in chimpanzees, which control infection transmitted by exposure to human-adapted HIV-1 variants, functionally competent NK cells were associated with NKp30 expression, during the course of infection [35]. It is however important to note that in contrast to HIV-1, cell ligands for NKp44 are not induced in HIV-2 [22], consistently with the absence of a 3S motif in the HIV-2 envelope protein [23,36].

Low expression of NKp30 on NK cells was mainly associated with the production of soluble forms of NKp30 ligands, including B7-H6 and BAT-3 [37,38]. In contrast, in this study we found that B7-H6 was not detectable as soluble forms in serum samples from HIV-2 infected patients but was expressed in large quantities as a membrane-bound form at the surface of infected target cells, whereas it was found on few, if any, HIV-1-infected cells as firstly described by Ward *et al.* [22]. These findings



support the hypothesis that decreased expression of NKp30 on NK cells from HIV-2<sup>+</sup> patients is mediated by a chronic exposure to B7-H6 expressed on target cells, as previously reported in other settings [31,37,39].

Very little is known, however, about the possible mechanisms involved in the downmodulation of activating NK receptors, although it appears to depend on physical contact between NK effector cells and target cells expressing various specific ligands. Examples of chronic ligand exposure-induced downregulation of NK-cell-activating receptors, other than NKp30, include DNAM1, CD16, and NKG2D [40]. Thus, several lines of evidence indicate that NKG2D ligands induce receptor endocytosis and suggest that the rate of receptor internalization and degradation depends on the nature of the ligand. In addition, functional impairment is apparently associated with downmodulation of membrane-bound ligand-mediated NKG2D; for example, reduced receptor expression prevents NK cells from rejecting MICA-positive tumors in transgenic mice that overexpress MICA [41]. Similar data was reported for the downmodulation by NKp30 by cellular ligands in human cells infected by HCV [42] and in this study by HIV-2. Thus, a similar phenomenon may serve as a mechanism for evasion from NK-cell immune-surveillance in different situations, including HIV-2 infection. It seems however that in HIV-2, downmodulation of NK30 was independent of the expression of the CD3 $\zeta$  signaling NKp30 adaptor protein [20], whereas functional impairment of this coreceptor was shown after stimulation of CD3<sup>+</sup> T and NK cells in a context of persistent ligand exposure for NKG2D [43]. Together, these data suggest that disruption of natural cytotoxicity receptor (NCR)/NCR-ligand expression is a significant event that must be considered in evaluating the balance between NK-cell activation and inhibition.

Another remarkable point is that NK cells from HIV-2<sup>+</sup> patients produce very high amounts of IFN- $\gamma$ , regardless of the environmental pressure. This could be explained by the sustain activation status of NK cells, in accordance with the 'discontinuity theory' [44], in which the immune system responds to sudden changes in antigenic stimulation and is rendered tolerant by slow or continuous stimulation. The recent study of Huot *et al.* [45] concerning the control of simian immunodeficiency virus (SIV) replication by NK cells in lymph node follicles of African green monkeys would encourage us to test this hypothesis.

In conclusion, we have shown that the decreased expression of the activating NKp30 NK-cell receptors in HIV-2 results in impaired function caused by a by permanent exposure of B7-H6. This could represent a novel molecular mechanism of viral escape that may participates in immune subversion mediated by a chronic HIV-2 infection, or alternatively for a very efficient

control of HIV-2 replication through NKp30-mediated recognition and killing of infected cells by NK cells.

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## Conflicts of interest

There are no conflicts of interest.

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