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Influence of NKG2C Genotypes on HIV Susceptibility and Viral Load Set Point

Khlood Alsulami,[a,b,c] Naomi Bolastig,[a,c] Franck P. Dupuy,[a,c] Tsoarello Mabanga,[a,c] Louise Gilbert,[a,c] Zahra Kiani,[a,c] Jean-Pierre Routy,[a,b,c,d,e] Julie Bruneau,[f,g] Réjean Thomas,[h] Cécile Tremblay,[f,i] Christos M. Tsoukas,[a,b,c,j] Jason Szabo,[c,g,h] Pierre Côté,[k] Benoit Trottier,[k] Roger LeBlanc,[l] Danielle Rouleau,[i] Nicole F. Bernard,[a,b,c,e,j] for the investigators in the Montreal Primary HIV Infection cohort

[a]Research Institute of the McGill University Health Centre (RI-MUHC), Montreal, Quebec, Canada
[b]Division of Experimental Medicine, McGill University, Montreal, Quebec, Canada
[c]Infectious Diseases, Immunology and Global Health Program, Research Institute of the McGill University Health Centre, Montreal, Quebec, Canada
[d]Division of Hematology, McGill University Health Centre, Montreal, Quebec, Canada
[e]Chronic Viral Illness Service, McGill University Health Centre, Montreal, Quebec, Canada
[f]Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), Montreal, Quebec, Canada
[g]Department of Family Medicine, Université de Montréal, Montreal, Quebec, Canada
[h]Clinique Médicale l'Actuel, Montréal, Quebec, Canada
[i]Department of Microbiology, Infectiology, and Immunology, University of Montreal, Montreal, Quebec, Canada
[j]Division of Clinical Immunology, McGill University Health Centre, Montreal, Quebec, Canada
[k]Clinique de Médecine Urbaine du Quartier Latin, Montréal, Quebec, Canada
[l]Clinique Médicale Opus, Montréal, Quebec, Canada

ABSTRACT NKG2C is an activating NK cell receptor encoded by a gene having an unexpressed deletion variant. Cytomegalovirus (CMV) infection expands a population of NKG2C$^+$ NK cells with adaptive-like properties. Previous reports found that carriage of the deleted *NKG2C*$^-$ variant was more frequent in people living with HIV (PLWH) than in HIV$^-$ controls unexposed to HIV. The frequency of NKG2C$^+$ NK cells positively correlated with HIV viral load (VL) in some studies and negatively correlated with VL in others. Here, we investigated the link between *NKG2C* genotype and HIV susceptibility and VL set point in PLWH. NKG2C genotyping was performed on 434 PLWH and 157 HIV-exposed seronegative (HESN) subjects. Comparison of the distributions of the three possible *NKG2C* genotypes in these populations revealed that the frequencies of *NKG2C*$^{+/+}$ and *NKG2C*$^{+/-}$ carriers did not differ significantly between PLWH and HESN subjects, while that of *NKG2C*$^{-/-}$ carriers was higher in PLWH than in HESN subjects, in which none were found ($P = 0.03$, χ^2 test). We were unable to replicate that carriage of at least 1 *NKG2C*$^-$ allele was more frequent in PLWH. Information on the pretreatment VL set point was available for 160 *NKG2C*$^{+/+}$, 83 *NKG2C*$^{+/-}$, and 6 *NKG2C*$^{-/-}$ PLWH. HIV VL set points were similar between *NKG2C* genotypes. The frequency of NKG2C$^+$ CD3$^-$ CD14$^-$ CD19$^-$ CD56^{dim} NK cells and the mean fluorescence intensity (MFI) of NKG2C expression on NK cells were higher on cells from CMV$^+$ PLWH who carried 2, versus 1, *NKG2C*$^+$ alleles. We observed no correlations between VL set point and either the frequency or the MFI of NKG2C expression.

IMPORTANCE We compared *NKG2C* allele and genotype distributions in subjects who remained HIV uninfected despite multiple HIV exposures (HESN subjects) with those in the group PLWH. This allowed us to determine whether *NKG2C* genotype influenced susceptibility to HIV infection. The absence of the *NKG2C*$^{-/-}$ genotype among HESN subjects but not PLWH suggested that carriage of this genotype was associated with HIV susceptibility. We calculated the VL set point in a subset of 252 *NKG2C*-genotyped PLWH. We observed no between-group differences in the VL set point in carriers of the three possible *NKG2C* genotypes. No significant correlations were seen between

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Address correspondence to Nicole F. Bernard, nicole.bernard@mcgill.ca.

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the frequency or MFI of NKG2C expression on NK cells and VL set point in cytomegalovirus-coinfected PLWH. These findings suggested that adaptive NK cells played no role in establishing the in VL set point, a parameter that is a predictor of the rate of treatment-naive HIV disease progression.

KEYWORDS adaptive NK cells, HIV exposed seronegative, HIV load set point, injection drug users, men who have sex with men, people living with HIV, human immunodeficiency virus

Natural killer (NK) cells are cytotoxic lymphocytes that generate early immune responses to virus-infected and cancer cells (1). The activation state of NK cells is determined by the integration of signals received from activating and inhibitory receptors (2, 3). Among the types of receptors present on NK cells are the NKG2 receptors, which belong to the C-type lectin family. The genes encoding these receptors are located in the 12p13 region of chromosome 12, within the NK receptor complex (4, 5). The NKG2C activating receptor, like its inhibitory counterpart NKG2A, is expressed as a heterodimer with CD94 (6). The ligand for NKG2C and NKG2A is HLA-E, a nonclassical major histocompatibility complex class Ib (MHC-Ib) molecule, stabilized by peptides derived from classical MHC-I antigens and HLA-G (7, 8). HLA-E molecules complexed with epitopes from the human cytomegalovirus (CMV)-encoded viral protein UL40 leader sequences are ligands for NKG2C (9–12). Among $CD56^{dim}$ NK cells, $NKG2C^+$ NK cells are typically $NKG2A^-$ (13, 14). The interaction of NKG2C with its ligands transmits signals that activate cells bearing this receptor (7, 15).

Although NK cells are traditionally thought to be part of the innate immune system, $NKG2C^+$ NK cells, which often coexpress CD57, can undergo clonal expansion in response to CMV infection (9, 13, 16, 17). Because the expansion of $NKG2C^+$ cells resembles that seen in adaptive immune responses, these NK cells are called adaptive NK cells. Expanded adaptive NK cells frequently lack the signaling proteins Ewing's sarcoma's/FLI-1 activated transcript-2 (EAT-2), spleen tyrosine kinase (SYK), and FcεRγ, as well as the transcription factor promyelocytic leukemia zinc finger (PLZF) (18, 19). This is due to DNA methylation-dependent epigenetic modifications, which distinguish adaptive from conventional NK cells. Adaptive $NKG2C^+$ cells exhibit enhanced CD16-dependent cytokine secretion due to epigenetic remodeling of the gamma interferon (IFN-γ) and tumor necrosis factor alpha (TNF-α) promoter regions (20–22).

Some individuals do not express NKG2C at the NK cell surface due to homozygous deletion of an ~16-kb genomic region that includes the *nkg2c* gene (also called *klrc2*), which encodes NKG2C (23, 24). In several Caucasian populations and one Japanese and Tanzanian population each, the frequency (percentage) of the *NKG2C* deletion haplotype is close to 20%, with a homozygous deletion frequency of approximately 4% (22, 24–26). However, frequencies of the *NKG2C* deletion haplotype were found to be as low as 10.3% in Mexican mestizos and as high as 36.8% in West African populations from the Gambia and Guinea-Bissau (26–28). NK cells expressing NKG2C have been shown to play a role in the immune surveillance of CMV (17).

CMV infection also drives the expansion of $NKG2C^-$ NK cells in people who are $NKG2C^{-/-}$ (29). $NKG2C^-$ NK cells having an epigenetic footprint characteristic of $NKG2C^+$ adaptive NK cells are observed in $NKG2C^{-/-}$ carriers (22). Comparisons of the phenotypes and functions of adaptive NK cells from $NKG2C^{-/-}$ with those from $NKG2C^{+/+}$ and $NKG2C^{+/-}$ carriers found few differences, suggesting that the contribution of NKG2C to NK cell adaptation to CMV infection can be compensated for in NK cells from $NKG2C^{-/-}$ carriers in CMV-monoinfected as well as in HIV-CMV-coinfected subjects (22, 30). In adaptive NK cells from $NKG2C^{-/-}$ carriers, CD2 costimulation plays an important role in compensating for the absence of NKG2C in antibody-dependent responses (22).

HIV-CMV coinfection has been reported by many to drive the expansion of $NKG2C^+$ NK cells over that seen in CMV-monoinfected persons (31, 32). Several studies have

TABLE 1 Study population demographics[a]

Population	No. (%) in population		*P* value
	PLWH (*n* = 434)	HIV$^-$ (*n* = 157)	
Sex			
Males	408 (94.0)	141 (89.8)	
Females	25 (5.8)	23 (14.6)	
HIV exposure risk group			
Sexually exposed	371	78	
MSM	337	67[b]	
Heterosexually exposed			
Men	9	3	
Women	25	8	
IDUs	63	79	
Low-risk control		11	
Ethnicity			
Caucasian	384 (88.5)	146 (92.9)	0.11
American/African black	19 (4.5)	4 (2.5)	0.25
Latino	27 (6.2)	5 (3.2)	0.19
Asian	4 (0.9)	2 (1.3)	0.76

[a]Abbreviations: PLWH, people living with HIV; MSM, men who have sex with men; IDUs, injection drug users.
[b]At risk for sexual exposure.

questioned whether NKG2C$^+$ cells play a role in protection from HIV infection or in a slower disease course in those infected. Supporting a role for the NKG2C$^-$ variant in susceptibility to HIV infection was the observation that the percentage of carriers of an *nkg2c*$^-$ allele in either the homozygous or heterozygous form was higher in HIV-infected individuals than in HIV-uninfected individuals with no history of HIV exposure (33). Whether NKG2C$^+$ NK cells play a role in HIV control is unclear. Thomas et al. showed that among HIV-infected persons, the proportion of individuals with a pretreatment plasma viral load (VL) of <30,000 copies/ml was higher in carriers of the *NKG2C*$^{+/+}$ genotype than among those carrying an *nkg2c*$^-$ allele (33). Contrasting with the notion that the *NKG2C*$^{+/+}$ genotype was associated with lower VL control was the finding that the percentage of NKG2C$^+$ NK cells from seven *NKG2C*$^{+/+}$ carriers was positively correlated with a single pretreatment HIV VL (33). However, in two other studies, the percentage of NKG2C$^+$ NK cells was negatively correlated with VL in early infection (34, 35).

Here, we compared *NKG2C* genotypes in people living with HIV (PLWH) enrolled in the Montreal Primary HIV infection (PI) cohort with HIV-exposed seronegative (HESN) subjects who remained HIV uninfected despite multiple high-risk HIV exposures. We found that carriage of the *NKG2C*$^{-/-}$ genotype was associated with increased HIV susceptibility. However, neither the *NKG2C*$^{+/+}$ nor *NKG2C*$^{+/-}$ genotype alone nor the combination of both *NKG2C*$^{+/-}$ and *NKG2C*$^{-/-}$ genotypes was associated with changes in HIV susceptibility. We observed no differences in VL set points between HIV-infected carriers of the three possible *NKG2C* genotypes. We also observed no correlation between VL set point and the percentage of NKG2C$^+$ NK cells or the intensity of NKG2C expression. Thus, carriage of an *nkg2c*$^-$ allele does not appear to affect HIV VL set point, which is a determinant of the rate of HIV disease progression.

RESULTS

PLWH and HESN populations differ in *NKG2C*$^{-/-}$ genotype frequencies. Table 1 provides information on the racial/ethnic composition of the study population. Both populations were composed mainly of Caucasians (92.9 and 88.5% for PLWH and HESN participants, respectively) living in the same geographical region (Montreal, QC, Canada). There were no significant between-group differences in their ethnic/racial

TABLE 2 *NKG2C* allele and genotype frequencies in people living with HIV and HIV-exposed seronegative subjects[a]

NKG2C allotype/genotype	Frequency of allele or genotype in population		OR	95% CI	*P* value
	PLWH	HESN			
All, *n*	434	157			
Allele frequency, %					
nkg2c$^+$	80.5	80.9	1.0	0.5–2.1	1.00
nkg2c$^-$	19.5	19.1			
Genotype frequency, no. (%)					
NKG2C$^{+/+}$	276 (63.6)	97 (61.8)	1.1	0.7–1.6	0.70
NKG2C$^{+/-}$	147 (33.9)	60 (38.2)	0.8	0.6–1.2	0.38
NKG2C$^{-/-}$	11 (2.5)	0	8.6**	0.5–146.0**	0.04*
NKG2C$^{+/-}$ + *NKG2C*$^{-/-}$	158 (36.4)	60 (38.2)	0.9	0.6–1.5	0.70
Sexually exposed, *n*	371	78			
Allele frequency, %					
nkg2c$^+$	81.4	78.2	1.21	0.6–2.4	0.72
nkg2c$^-$	18.6	21.9			
Genotype frequency, no. (%)					
NKG2C$^{+/+}$	240 (64.7)	44 (56.4)	1.4	0.9–2.3	0.20
NKG2C$^{+/-}$	124 (33.4)	34 (43.6)	0.6	0.4–1.1	0.09
NKG2C$^{-/-}$	7 (1.9)	0	3.2**	0.2–57.2**	0.61*
IDUs, *n*	63	79			
Allele frequency, %					
nkg2c$^+$	75.4	83.5	0.6	0.3–1.2	0.21
nkg2c$^-$	24.6	16.5			
Genotype frequency, no. (%)					
NKG2C$^{+/+}$	36 (57.1)	53 (67.1)	0.6	0.3–1.3	0.29
NKG2C$^{+/-}$	23 (36.5)	26 (32.9)	1.2	0.6–2.3	0.72
NKG2C$^{-/-}$	4 (6.3)	0	12.0**	0.6–277.7**	0.04*

[a]Abbreviations: PLWH, people living with HIV; HESN, HIV-exposed seronegative; OR, odds ratio; 95% CI, 95% confidence interval; IDUs, injection drug users. Asterisks indicate statistical significance measured by Fisher's exact test (*) with Haldane's correction (**).

compositions ($P > 0.11$ for comparisons of Caucasians, Asians, Latinos, and American/African Blacks by two-tailed χ^2 tests).

The numbers and percentages of PLWH and HESN subjects carrying the wild-type (*nkg2c*$^+$) and deletion (*nkg2c*$^-$) alleles and the three *NKG2C* genotypes are shown in Table 2. The allele percentages were similar in both populations. The distribution of the three *NKG2C* genotypes *NKG2C*$^{+/+}$, *NKG2C*$^{+/-}$, and *NKG2C*$^{-/-}$ at this locus did not deviate statistically from the Hardy-Weinberg equilibrium (HWE) in PLWH ($P = 0.09$ by χ^2 test), while it did in the HESN subjects ($P = 0.003$ by χ^2 test). When the proportions of *NKG2C*$^{+/+}$, *NKG2C*$^{+/-}$, and *NKG2C*$^{-/-}$ genotypes were compared in PLWH and HESN subjects, there was a significantly higher frequency of *NKG2C*$^{-/-}$ individuals among PLWH than HESN subjects (odds ratio [OR], 8.60; 95% confidence interval [CI], 0.50 to 146; $P = 0.04$ by two-tailed Fisher's exact test), while the proportions of *NKG2C*$^{+/+}$ and *NKG2C*$^{+/-}$ genotypes in these two populations did not differ significantly (Table 2). Thomas et al. previously reported that HIV-uninfected persons at low risk for infection were significantly more likely than PLWH to carry the *NKG2C*$^{+/+}$ genotype, suggesting that carriage of at least 1 *NKG2C*$^-$ variant was associated with higher HIV susceptibility (33). Comparisons of the PLWH and HESN subjects revealed no between-population differences for either the *NKG2C*$^{+/+}$ or combined *NKG2C*$^{+/-}$ *NKG2C*$^{-/-}$ genotypes. In summary, carriage of the *NKG2C*$^{-/-}$ genotype was associated with higher susceptibility to HIV infection.

Risks for HIV transmission include sexual exposure to and needle sharing with PLWH. As the PLWH and HESN populations included individuals who were at risk for sexual exposure, as well as injection drug users (IDUs), we investigated whether there was evidence that carriage of *NKG2C*$^{-/-}$ genotype was linked to HIV susceptibility by mucosal or parenteral exposure. Of the sexually exposed (SE) subjects, 371 were PLWH

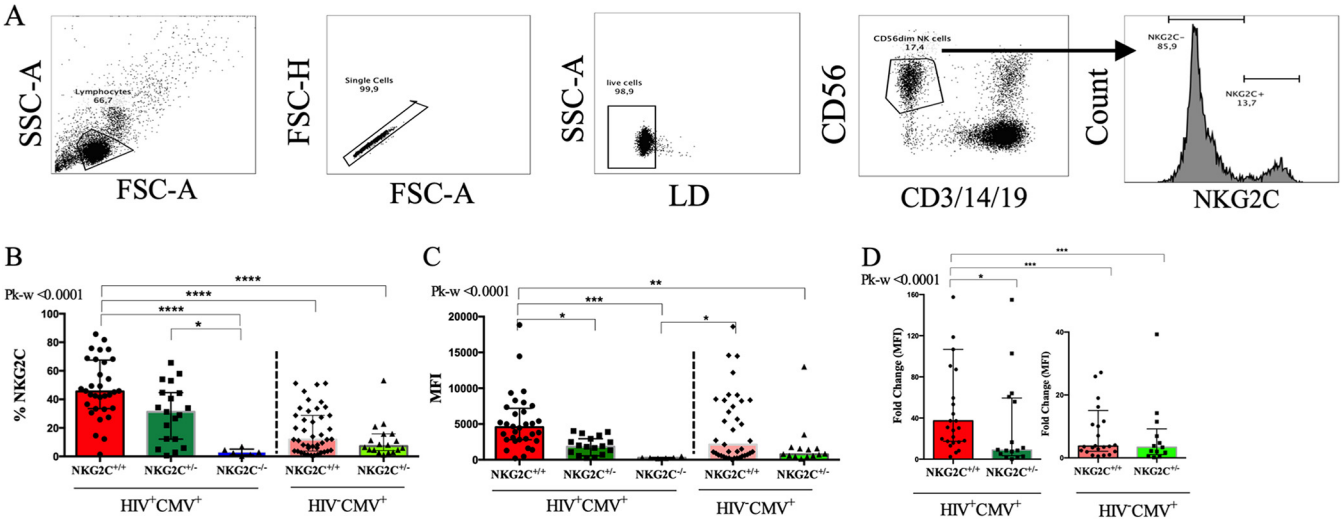

FIG 1 Evaluation of the frequency of NKG2C$^+$ NK cells and mean fluorescence intensity (MFI) of NKG2C expression. (A) Shown is the gating strategy used to detect the frequency and MFI of NKG2C expression. Peripheral blood mononuclear cells were stained for viability and cell surface CD3, CD56, CD14, CD19, and NKG2C. CD3$^-$ CD14$^-$ CD19$^-$ CD56^{dim} NK cells were gated on from the live singlet lymphocyte gate. From these, we determined the frequencies of NKG2C$^+$ CD56^{dim} NK cells and MFI of NKG2C expression on NK cells. The *y* axes show the frequency (B), MFI (C), and fold change over background in the MFI (D) of NKG2C expression on CD56^{dim} NK cells from CMV$^+$ people living with HIV (HIV$^+$ CMV$^+$) carrying the *NKG2C*$^{+/+}$ ($n = 32$), *NKG2C*$^{+/-}$ ($n = 19$), and *NKG2C*$^{-/-}$ ($n = 6$) genotypes and from CMV-monoinfected (HIV$^-$ CMV$^+$) individuals carrying the *NKG2C*$^{+/+}$ ($n = 43$) and *NKG2C*$^{+/-}$ ($n = 18$) genotypes. Each point represents a single individual. Bar graph heights and error bars represent medians and interquartile ranges for the group. FSC-A, forward scatter area; SSC-A, side scatter area; LD, live/dead; FSC-H, forward scatter height; Pk-w, *P* value for the Kruskal-Wallis test used to analyze the significance of differences between groups: *, $P < 0.05$; ***, $P < 0.001$; ****, $P < 0.0001$.

and 78 were HESN, with genotype distributions shown in Table 2. The *NKG2C* genotype distributions diverged from HWE in both the SE PLWH and HESN subjects ($P = 0.045$ and $P = 0.001$, respectively, by χ^2 tests). When the proportions of *NKG2C*$^{+/+}$, *NKG2C*$^{+/-}$, and *NKG2C*$^{-/-}$ genotypes were compared in SE PLWH and HESN subjects, no significant between-group differences were observed. Of the 63 PLWH and 79 HESN IDUs evaluated for *NKG2C* genotypes, the genotype distribution in both populations was in HWE ($P = 0.9$ and $P = 0.07$, respectively, by χ^2 tests). The proportion of *NKG2C*$^{-/-}$ genotypes was significantly higher among injection drug-using PLWH than HESN subjects (OR, 12; 95% CI, 0.6 to 277.7; $P = 0.04$ by Fisher's exact test); the proportions of *NKG2C*$^{+/+}$ and *NKG2C*$^{+/-}$ genotypes in the IDU PLWH and HESN subpopulations did not differ significantly from each other (Table 2). In summary, carriage of the *NKG2C*$^{-/-}$ genotype was significantly associated with higher HIV susceptibility in IDUs but not in SE subjects.

NKG2C cell surface expression is genotype dependent. We next compared the percentages of NKG2C$^+$ cells and the intensities of NKG2C expression on CD56^{dim} NK cells from carriers of the three *NKG2C* genotypes. As CMV infection drives the expansion of NKG2C$^+$ NK cells (9, 13, 31), for this analysis, we included only subjects who were CMV$^+$ from whom cells were available for staining. Cells from 32 *NKG2C*$^{+/+}$, 19 *NKG2C*$^{+/-}$, and 6 *NKG2C*$^{-/-}$ PLWH and 43 *NKG2C*$^{+/+}$and 18 *NKG2C*$^{+/-}$ HIV$^-$ subjects were tested. Figure 1A shows the strategy used to gate on live singlet CD3$^-$ CD14$^-$ CD19$^-$ CD56^{dim} NK cells, which is the predominant population expressing NKG2C (36). From these, NKG2C$^+$ cells were gated on. Figure 1B shows that CMV$^+$ PLWH who were *NKG2C*$^{+/+}$ and *NKG2C*$^{+/-}$ had a higher percentage of NKG2C$^+$ NK cells than did *NKG2C*$^{-/-}$ carriers, with medians of 45.5% (interquartile range [IQR], 33.5 to 67.5%) and 30.1% (IQR, 7.41 to 44.63%) for *NKG2C*$^{+/+}$ and *NKG2C*$^{+/-}$ carriers, respectively, and background levels of 2.7% (IQR, 1.04 to 5.14%) for *NKG2C*$^{-/-}$ carriers ($P < 0.001$ and $P < 0.05$ for comparisons of *NKG2C*$^{+/+}$ and *NKG2C*$^{+/-}$ carriers with *NKG2C*$^{-/-}$ carriers by Dunn's posttests). In a subanalysis comparing *NKG2C*$^{+/+}$ with *NKG2C*$^{+/-}$ carriers, we found that the percentage of NKG2C$^+$ CD56^{dim} NK cells was significantly higher in CMV$^+$ PLWH who were *NKG2C*$^{+/+}$ than in *NKG2C*$^{+/-}$ carriers ($P < 0.05$ by Mann-Whitney test). For intensity measurements, we examined the mean fluorescence intensity (MFI), the median fluorescence intensity, and the fold change

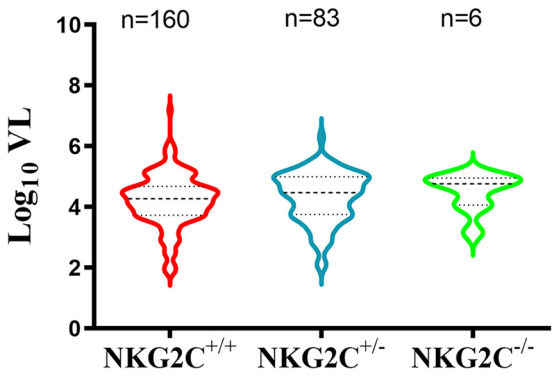

FIG 2 Log_{10} viral load (VL) set points in people living with HIV (PLWH) carriers of the *NKG2C*$^{+/+}$, *NKG2C*$^{+/-}$, and *NKG2C*$^{-/-}$ genotypes. Shown are violin plots of the median and interquartile range of the treatment-naive log_{10} VL set point in each *NKG2C* genotype group. The number of subjects included in each group is shown above each data set. A Kruskal-Wallis test was used to assess the significance of between-group differences in log_{10} VL set point.

over background in the MFI of NKG2C staining. The latter measure controls for between-experiment variations in MFI. Since values for mean and median fluorescence intensities did not differ substantially, we only report MFI values here. The MFI of NKG2C expression from *NKG2C*$^{+/+}$, *NKG2C*$^{+/-}$, and *NKG2C*$^{-/-}$ carriers were 4,562 (IQR, 2,813 to 7,175), 1,870 (IQR, 671 to 3,061), and 269.6 (IQR, 212.8 to 325.3), respectively (Fig. 1C). NKG2C expression was higher on CD56^{dim} NK cells from *NKG2C*$^{+/+}$ carriers than on those from *NKG2C*$^{-/-}$ carriers ($P < 0.001$ by Dunn's posttests). NK cells from *NKG2C*$^{+/+}$ carriers expressed higher levels of NKG2C than those from NKG2C$^{+/-}$ carriers ($P < 0.05$ by Dunn's posttest). The fold change in MFI over background for NKG2C expression intensity was also significantly higher on NK cells from *NKG2C*$^{+/+}$ than *NKG2C*$^{+/-}$ CMV^{+} PLWH carriers (Fig. 1D). We also investigated the percentage of NKG2C^{+} CD56^{dim} NK cells and the intensity of NKG2C expression on these cells from CMV-monoinfected subjects. Although the percentage of NKG2C^{+} NK cells was higher on cells from *NKG2C*$^{+/+}$ than *NKG2C*$^{+/-}$ carriers (11.8% [IQR, 3.77 to 28.7%] and 7.23% [IQR, 4.08 to 15.75%], respectively), as were the MFI and fold change in MFI intensity of NKG2C expression on CD56^{dim} NK cells, the difference did not achieve significance (Fig. 1B to D). Figure 1B D to D also show that the percentage of cells, MFI, and fold change in MFI over background of NKG2C expression were significantly higher among CD56^{dim} NK cells from *NKG2C*$^{+/+}$ carriers who were CMV^{+} PLWH than among *NKG2C*$^{+/+}$ and *NKG2C*$^{+/-}$ cells from CMV-monoinfected persons ($P < 0.006$ for all by Mann-Whitney tests).

***NKG2C* genotypes and HIV VL set point.** VL set points in pretreatment PLWH are measures of HIV progression associated with time to AIDS, CD4 counts of <200 copies/ml of plasma, and death (37, 38). When all *NKG2C*-genotyped PLWH for whom information on the VL set point was available were included, we found no significant differences between *NKG2C* genotypes in the VL set points ($P = 0.26$ by Kruskal-Wallis test) (Fig. 2).

We next investigated whether the percentage of NKG2C^{+} CD56^{dim} NK cells and/or the intensity of NKG2C expression correlated with the pretreatment VL set point. Forty-three *NKG2C*-genotyped CMV^{+} PLWH with a known HIV VL set point were included in this analysis: 21 *NKG2C*$^{+/+}$, 16 *NKG2C*$^{+/-}$, and 6 *NKG2C*$^{-/-}$ HIV^{+} CMV^{+} subjects. Neither the percentage nor the intensity of NKG2C expression (MFI or fold change over background in NKG2C MFI) was significantly correlated with VL set point when all observations were considered together or when results were stratified according to *NKG2C* genotype (Spearman's correlation tests) (Fig. 3A to L) As adaptive NK cells are typically also CD57^{+}, we also tested whether there was a correlation between the percentage of NKG2C^{+} CD57^{+} CD56^{dim} NK cells and HIV VL set point. Figure 4A shows the strategy used to gate on NKG2C^{+} CD57^{+} CD56^{dim} NK cells. No significant correlation was observed between these parameters

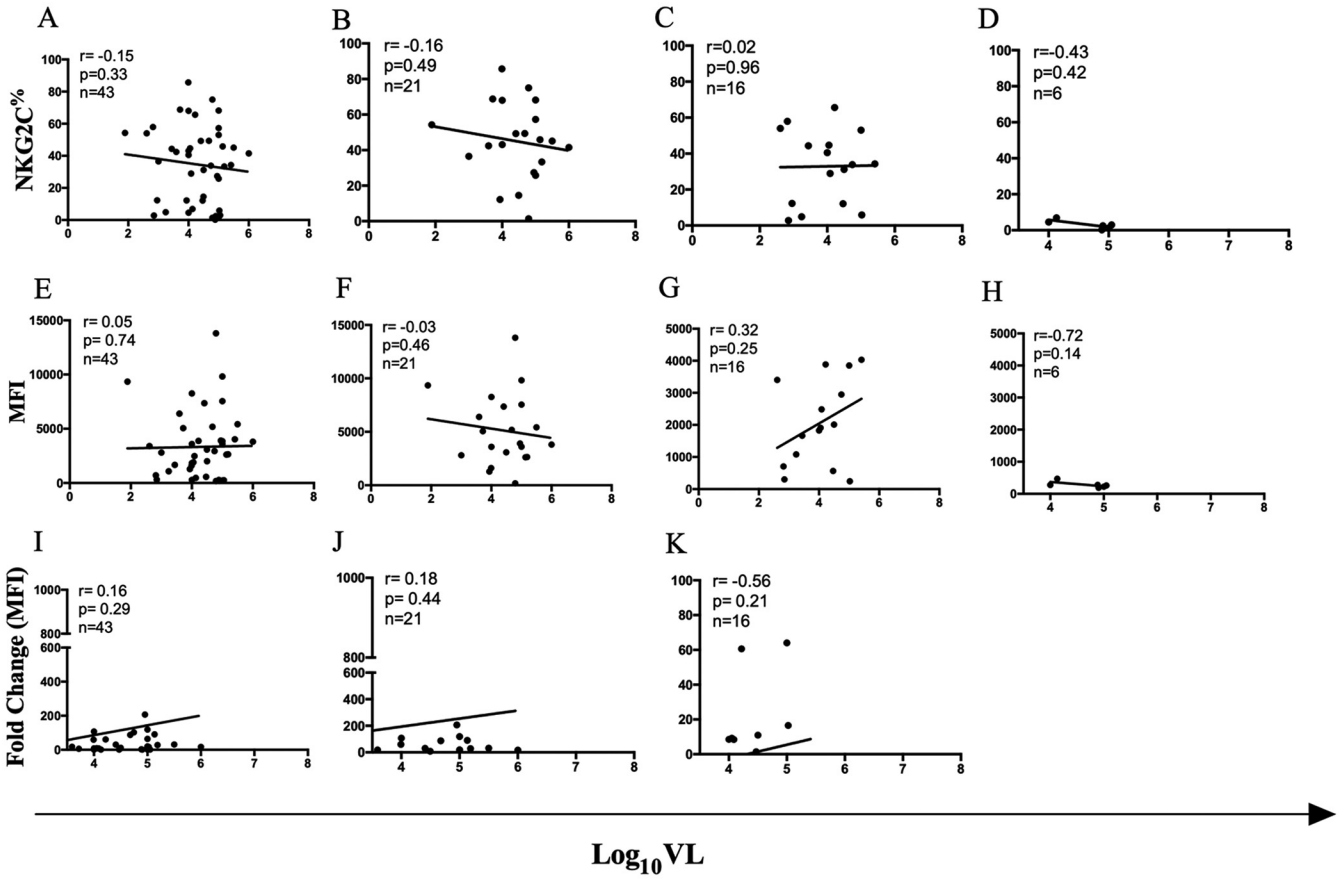

FIG 3 Correlation between $\log_{10}$ VL set point and frequency of NKG2C$^+$ NK cells, mean fluorescence intensity (MFI) of NKG2C expression and fold change in NKG2C MFI over background in cells from HIV$^+$ CMV$^+$ *NKG2C*$^{+/+}$, *NKG2C*$^{+/-}$, and *NKG2C*$^{-/-}$ carriers. Correlations between the frequency (A to D) MFI (E to H) of NKG2C expression and fold change in NKG2C MFI over background (I to K) on NK cells from CMV$^+$ PLWH with $\log_{10}$ VL for carriers of all *NKG2C* genotypes tested (A, E, and I) and stratified by *NKG2C*$^{+/+}$ (B, F, and J), *NKG2C*$^{+/-}$ (C, G, and K), and *NKG2C*$^{-/-}$ (D and H) genotypes. The number of subjects tested, the correlation coefficients (*r*), and the *P* values for each correlation are shown in the top left corner of the graphs.

for all NKG2C genotypes or for results stratified by NKG2C genotype (Spearman's test) (Fig. 4B to E).

As others have shown, the percentage of NKG2C$^+$ NK cells was significantly negatively correlated with the percentage of NKG2A$^+$ NK cells. This was the case for all genotypes together and for the *NKG2C*$^{+/+}$ and *NKG2C*$^{+/-}$ genotypes specifically (Fig. 5A). Figure 5B shows the strategy used to gate on NKG2A$^+$ NKG2C$^-$ CD56^{dim} NK cells. As for NKG2C$^+$ and NKG2C$^+$ CD57$^+$ CD56^{dim} NK cells, the percentage of NKG2A$^+$ NKG2C$^-$ CD56^{dim} NK cells did not correlate with the VL set point when results from all subjects were examined together or when results from *NKG2C*$^{+/+}$ and *NKG2C*$^{-/-}$ carriers were examined separately. For *NKG2C*$^{+/-}$ carriers, a negative correlation was observed ($r = -0.49$, $P = 0.04$) (Fig. 5D). However, application of a Bonferroni correction for multiple correlations reduced the significance of the correlation below the level of significance.

DISCUSSION

In this report, we assessed whether the *NKG2C* genotype distributions differed in a population of recently HIV-infected individuals compared to subjects who remained uninfected despite multiple documented exposures to HIV. We found that the *NKG2C*$^{-/-}$ genotype was more frequent among PLWH than HESN subjects. None of the 157 HESN subjects tested carried this genotype, which was present in 11 of 434 (2.53%) of PLWH. The distributions of *NKG2C* genotypes did not differ in the PLWH and HESN subpopulations who were exposed to HIV mucosally, while the *NKG2C*$^{-/-}$ genotype was more frequent in parenterally exposed PLWH than in HESN individuals. These

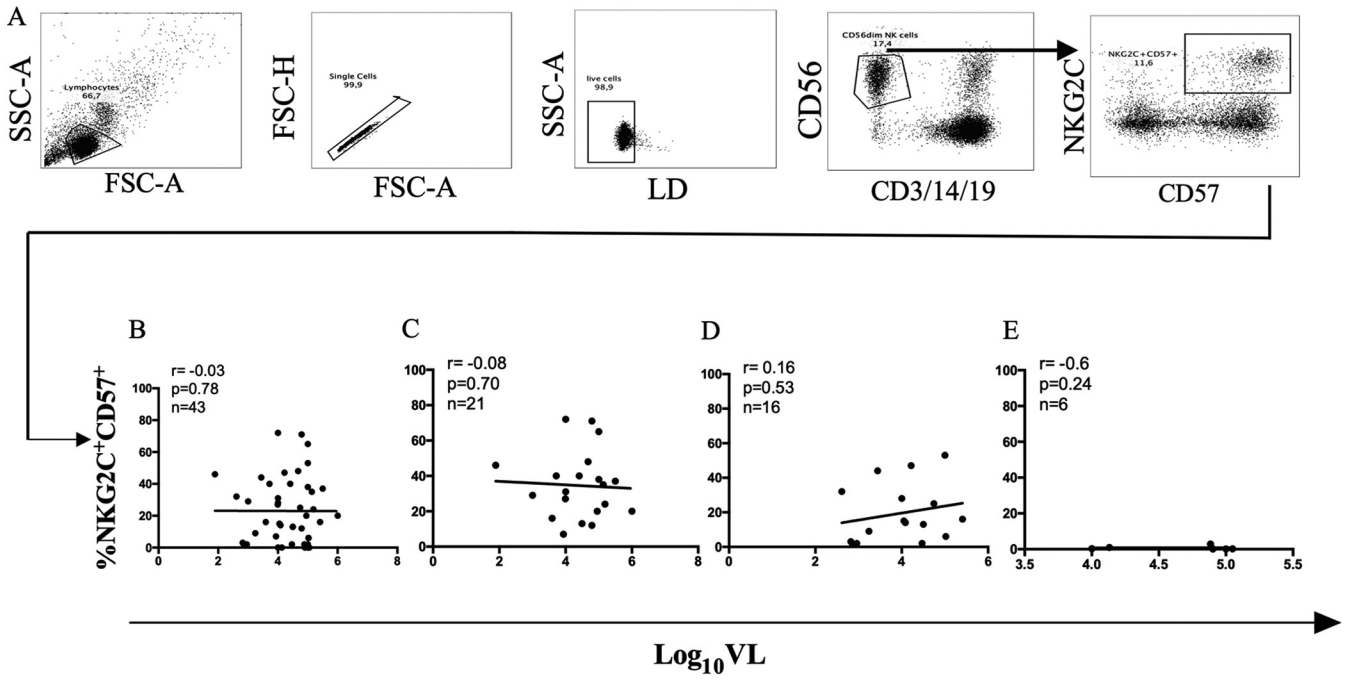

FIG 4 Correlation between $\log_{10}$ (VL) viral load set point and frequency of NKG2C$^+$ CD57$^+$ NK cells from CMV$^+$ PLWH carrying the three possible *NKG2C* genotypes. (A) From the live singlet lymphocyte gate, CD56^{dim} CD3$^-$ CD14$^-$ CD19$^-$ NK cells were gated on. From these NKG2C$^+$ CD57$^+$ NK cells were gated onto assess the frequency of these cells among CD56^{dim} NK cells. Correlations between the frequency of NKG2C$^+$ CD57$^+$ (B to E) CD56^{dim} NK cells with $\log_{10}$ viral load set point from for CMV$^+$ PLWH carrying all *NKG2C* (B), *NKG2C*$^{+/+}$ (C), *NKG2C*$^{+/-}$ (D), and *NKG2C*$^{-/-}$ (E) genotypes. The number of subjects tested, the correlation coefficients (*r*), and the *P* values for each correlation are shown in the top left corner of the graphs.

findings suggest that the *NKG2C*$^{-/-}$ genotype is associated with a higher risk of HIV infection. The PLWH population included individuals who remained treatment naive long enough to calculate a post-acute infection, pretreatment plasma VL set point. When this parameter was compared in carriers of the three *NKG2C* genotypes, we found no between-genotype differences in VL set point. Furthermore, neither the percentage of NKG2C$^+$ NK cells, MFI, nor fold change over background of the MFI of NKG2C expression on these cells correlated with VL set point in the CMV$^+$ PLWH.

There exists a variation in chromosome 12 where a 16-kb genomic region that includes the *nkg2c* gene is either present or entirely absent (23, 24). Genotyping of the mainly Caucasian study population described in this article found that the frequency of the *nkg2c*$^-$ variant was close to 20% in both the PLWH and HESN populations and the frequency of the homozygous *NKG2C*$^{-/-}$ genotype was 2.53% in PLWH. The *nkg2c*$^-$ allele frequency and the distribution of *NKG2C* genotypes in the PLWH are in line with those reported for several populations of European extraction, as well as in a Japanese population and an East African Tanzanian population (22, 24–26, 33). The allele frequency of *nkg2c*$^-$ was lower (10.3%) in a population of Mexican mestizos and higher (29.3 to 36.7%) in West African populations from the Gambia and Guinea-Bissau (26–28) In contrast with what we found in PLWH, we observed no *NKG2C*$^{-/-}$ carriers among 157 HIV-uninfected persons at risk for HIV exposure, a difference that was statistically significant. The non-Caucasian ethnic composition of the study populations was balanced between PLWH and HESN subjects. However, if only Caucasians were included in the analysis, proportional between-group differences in the percentage of the *NKG2C*$^{-/-}$ genotype fell below the level of significance ($P = 0.1$). This may be due to the smaller sample sizes. It was not possible to compare the proportional between-group differences in the percentages of the *NKG2C*$^{-/-}$ genotype for the other ethnicities included in the study populations due to the small numbers of subjects in these subgroups.

The *NKG2C* genotype distributions in the PLWH and the uninfected population described here differed from those reported by Thomas et al. (33). They compared the

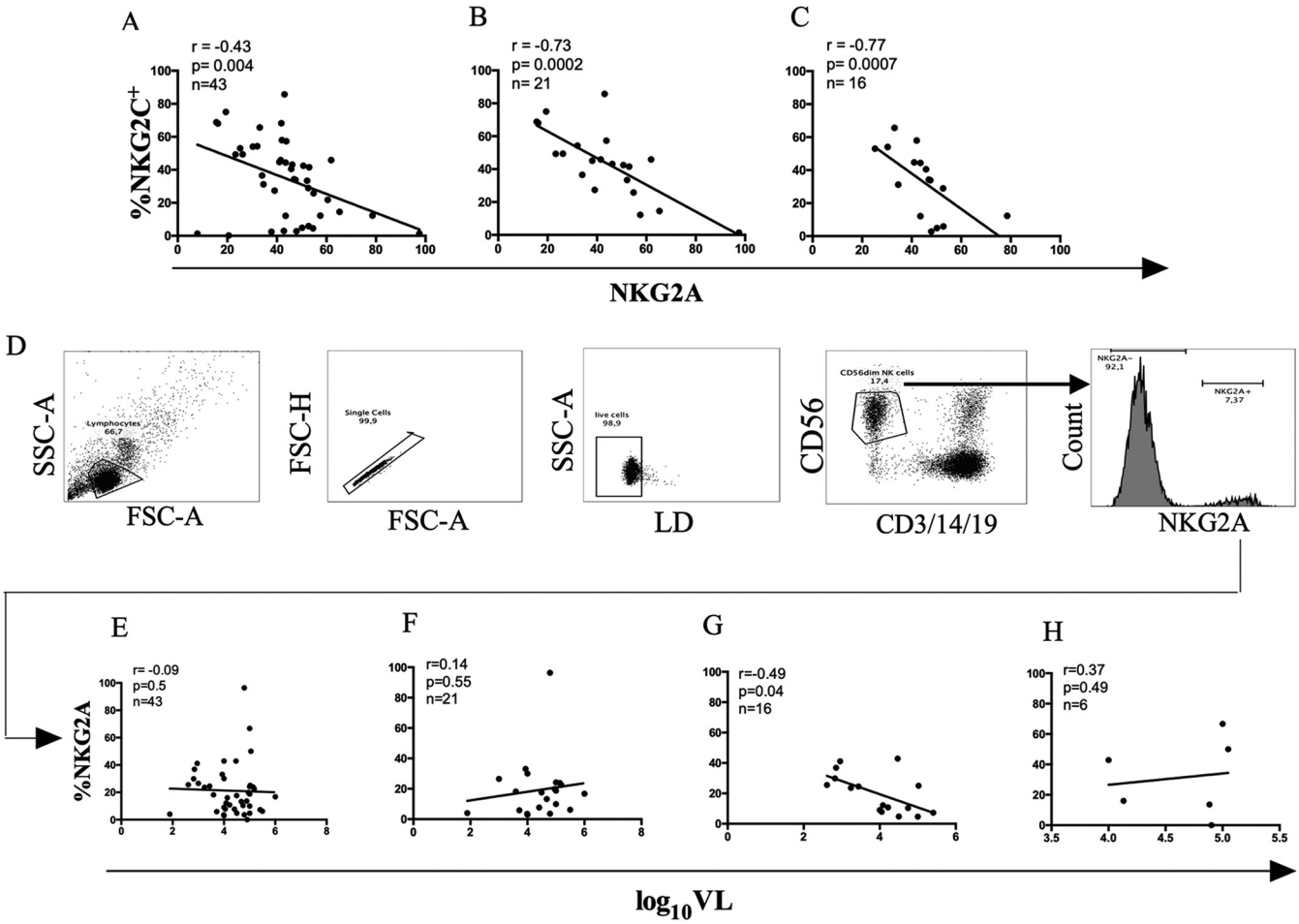

FIG 5 Correlation between $\log_{10}$ VL set point and frequency of NKG2A$^+$ NKG2C$^-$ CD56^{dim} NK cells from CMV$^+$ PLWH carrying the three possible *NKG2C* genotypes. Correlations between the frequency of NKG2C$^+$ CD56^{dim} and NKG2A$^+$ CD56^{dim} NK cells from CMV$^+$ PLWH for carriers of all *NKG2C* (A), *NKG2C*$^{+/+}$ (B), and *NKG2C*$^{+/-}$ (C) genotypes. The number of subjects tested, the correlation coefficients (*r*) and the *P* values for each correlation are shown in the inset at the top left corner of each graph. (D) Shown is the strategy used to gate on CD56^{dim} CD3$^-$ CD14$^-$ CD19$^-$ NK cells, from which NKG2A$^+$ NKG2C$^-$ cells were gated onto assess their frequency among CD56^{dim} NK cells. Correlations between the frequency of CD56^{dim} NKG2A$^+$ NKG2C$^-$ NK cells with $\log_{10}$ VL set point from for CMV$^+$ PLWH carrying all *NKG2C* (E), *NKG2C*$^{+/+}$ (F), *NKG2C*$^{+/-}$ (G), and *NKG2C*$^{-/-}$ (H) genotypes. The numbers of subjects tested, the correlation coefficients (*r*), and the *P* values for each correlation are shown in the top left corner of the graphs.

NKG2C genotype distribution in 433 PLWH with that in 280 controls who had no history of HIV exposure (33). They found *NKG2C*$^{-/-}$ subjects among their HIV-uninfected population, while we did not. They reported a significant association between carriage of an *nkg2c*$^-$ allele (i.e., combined *NKG2C*$^{+/-}$ and *NKG2C*$^{-/-}$ carriers) with HIV infection and that there was a higher proportion of *NKG2C*$^{+/+}$ carriers among uninfected controls than among PLWH. The main difference between the population reported by Thomas et al. and the one described here was the composition of the HIV-uninfected population. In the study by Thomas at al., the control population was not HIV exposed and thus was at a low risk for HIV infection. While it is possible that some of the people at high risk for HIV exposure we included remained HIV uninfected by chance, they represent a group that is likely to have a higher level of resistance to HIV infection than the HIV-uninfected population described by Thomas et al. The inclusion of HESN participants allowed us to explore more directly whether *NKG2C* genotypes were associated with HIV susceptibility. This may account for the discrepancy between our results and those reported by Thomas et al. regarding which *NKG2C* genotypes were associated with HIV susceptibility.

We stratified both PLWH and HESN subjects into those whose route of HIV infection/exposure was mucosal (SE) versus parenteral (IDU). When SE and IDU PLWH and HESN subjects were compared separately, we observed that the frequency of the

NKG2C$^{-/-}$ genotype was significantly higher in the IDU PLWH than HESN subjects, while this frequency did not differ significantly between SE PLWH and those at risk for sexual exposure to HIV. Many factors influence the per-act risk of HIV transmission, including the VL of the transmitting partner, the route of exposure, the presence of genital ulcers, circumcision, and the frequency of exposure, among others (39, 40). The SE PLWH and high risk for HIV exposure subpopulations were mainly men who have sex with men (MSM). Of these, all reported unprotected receptive (where the receptive partner was HIV seronegative) anal intercourse. This route of exposure averages at least a 10-fold higher risk of transmission per act than unprotected insertive anal or vaginal intercourse and a per-act risk that is close to that of injection drug use (41–44).

What accounts for the frequencies of the *NKG2C*$^{-/-}$ genotype not differing significantly between SE PLWH and those at risk for mucosal HIV exposure is unknown. The level of exposure to HIV may be a factor if a higher proportion of HIV-transmitting partners of SE than IDU HESN populations are on antiretroviral treatment (ART). In the context of NKG2C^{+} cells, the biology of HIV transmission by injection versus sexual exposure may be a factor. Parenteral exposure involves the introduction of needles contaminated with HIV-infected cells and/or virions into the circulation. Transmitted HIV-infected cells will express HLA-E, the ligand for NKG2C, and downmodulate HLA-A, -B, and -C, the ligands for inhibitory killer immunoglobulin-like receptors also present on NKG2C^{+} cells (2, 3, 7, 8, 36). The integration of these signals promotes NKG2C^{+} NK cell activation that may contribute to HIV clearance prior to the establishment of a productive infection. In this setting, the absence of NKG2C^{+} cells in *NKG2C*$^{-/-}$ carriers may be linked to heightened HIV susceptibility in those who became infected. In the case of sexual exposure, HIV-infected cells or virions must cross mucosal barriers to access the NKG2C^{+} NK cells in the circulation. Our knowledge of NKG2C^{+} NK cells at mucosal genital/anal sites is limited. NK, tissue-resident NK (TrNK), and NK-like innate lymphoid cells are present in tissues, including in the female genital tract (45, 46). The NK receptor profile of these cells differs from that of circulating NK cells, making it challenging to evaluate their stage of maturity, their ability to interact with HIV-infected cells, and the consequences of such an interaction in the context of what is known about circulating NK cells. Whether NK-like cells at portals of HIV entry express NKG2C is unknown. A study of the transcriptomic and protein expression patterns of TrNK cells in lung mucosal tissue did not report expression of NKG2C, while this receptor was shown to be expressed on adaptive NK cells in the liver, although these NK cells had distinct NK cell receptor profiles from those in the circulation (46, 47). If NKG2C^{+} NK cells were absent at the portals of HIV entry, it would reduce the relevance of *NKG2C* genotypes in modulating infection risk through a mucosal route. In sum, more information on the NK cell landscape at mucosal portals of HIV entry would aid in understanding the discrepancy between the percentage of *NKG2C*$^{-/-}$ carriers in SE versus IDU PLWH and HESN subjects.

The reason underlying why none of the 157 HESN subjects carried the *NKG2C*$^{-/-}$ genotype and how this may contribute to the maintenance of seronegative status despite multiple HIV exposures are unknown. CMV infection drives the expansion of adaptive NK cells (17, 29, 48). It is notable that NKG2C^{-} adaptive NK cells also expand in CMV-infected *NKG2C*$^{-/-}$ carriers (22, 36, 49, 50). Adaptive NKG2C^{-} and NKG2C^{+} NK cells are found at similar frequencies in those who do not and those who do carry an *nkg2c*$^{+}$ allele, and these cells share phenotypic, epigenetic, and functional properties that distinguish them from conventional NK cells (22, 30, 36, 49, 50). One of the differences between adaptive and conventional NK cells is that the former are more likely to express CD2. CD2 is a major coactivating receptor found on NK cells and T cell subsets, whose ligand is CD58 (LFA-3), which is expressed on many tissues (22, 51). CD2 is present on a higher percentage of adaptive NK cells from *NKG2C*$^{-/-}$ than *NKG2C*$^{+}$ carriers (51, 52). It compensates for the absence of NKG2C on adaptive NK cells from *NKG2C*$^{-/-}$ carriers in a manner that contributes to the activation of these cells. Although signaling through CD2 alone has little effect on adaptive NK cell activation, it synergizes with

CD16 signaling, to potently activate NK cells to secrete IFN-γ and TNF-α (22). It is tempting to speculate that CMV infection provides the costimulatory signals (i.e., CD16 cross-linking by anti-CMV antibody Fc regions and CD2-CD58 interactions) to activate adaptive NK cells. CMV has tropism for epithelial cells, fibroblasts, myeloid cells, and endothelial cells, all of which express CD58 and thus have the potential to be adaptive NK cell-interacting partners (53). CMV infection is a common infection, with a prevalence close to 40% in HIV-uninfected Canadians that increases with age (54–56). In ART-naive PLWH enrolled in the Montreal PI cohort, the prevalence of CMV coinfection is 84% (57). It would be interesting to investigate whether the higher frequency of the *NKG2C*$^{-/-}$ genotype in PLWH than in HESN subjects is linked to differential activation of these NKG2C$^-$ adaptive NK cells in PLWH than in HESN subjects due to factors such as differential levels of CMV infection or other factors that affect NK cell activity in a manner that influences HIV susceptibility.

We observed that the percentages of NKG2C$^+$ NK cells in CMV$^+$ PLWH and in CMV-monoinfected persons differed according to *NKG2C* genotype. CMV infection drives the expansion of NKG2C$^+$ NK cells (9, 13, 31). This was the rationale for confining this analysis to PLWH and HIV-uninfected subjects who were CMV seropositive. Cell surface NKG2C percentage, MFI, and intensity of fold change over background in the MFI of NKG2C expression results reported by others did not test for CMV serostatus, which if negative, would preclude the expansion of NKG2C$^+$ NK cells (33). In CMV-monoinfected subjects, differences in the percentages and intensities of NKG2C expression between *NKG2C*$^{+/+}$ and *NKG2C*$^{+/-}$ carriers were not significant. However, these values in CMV$^+$ PLWH compared to CMV-monoinfected persons were higher for cells from carriers of both *NKG2C*$^{+/+}$ and *NKG2C*$^{+/-}$ genotypes, as has been seen by others (31, 32, 58).

Treatment-naive VL set point is associated with the rate of HIV disease progression, as measured by time to CD4 counts of $<$200/mm^3, AIDS, and death (37, 38). We found no significant correlations between either the percentage of NKG2C$^+$ CD56^{dim} NK cells or the intensity of NKG2C expression on NK cells and the VL set point. This was also the case for correlations between the percentage of NKG2C$^+$ CD57$^+$ and NKG2A$^+$ CD56^{dim} NK cells and the VL set point. These results differ from those of others who correlated the percentage of NKG2C$^+$ cells with single VL measures in ART-naive individuals. Thomas et al. found a positive correlation between these parameters, although their analysis only included 7 untreated subjects in the chronic phase of infection (33). In contrast, Ma et al. found a negative correlation between the percentage of NKG2C$^+$ NK cells and concurrent VL in 22 treatment-naive PLWH infected at least 120 days, which corresponded to the VL set point (34). Gondois-Rey et al. also found a negative correlation between the percentage of NKG2C$^+$ NK cells and concurrent VL in 18 treatment-naive subjects tested at time points in acute/early infection (35). The analysis performed here was done on a larger group of 43 HIV$^+$ and CMV$^+$ individuals together and stratified by *NKG2C* genotype. To our knowledge this is the first report investigating correlations between the intensity of NKG2C expression on NK cells and VL set point. Overall, we found no evidence that NKG2C$^+$ NK cell parameters influenced VL set point, which is a determinant of the rate of HIV disease progression.

$\gamma\delta$ T cells also express NKG2C and have been shown to respond to HIV-infected cells (59, 60). Future studies should explore the link between NKG2C genotype, CMV infection, and frequency of NKG2C-expressing $\gamma\delta$ T cells at the level of susceptibility/resistance to HIV infection and at the level of HIV control.

In summary, our results support that carriage of the *NKG2C*$^{-/-}$ genotype is associated with higher susceptibility to HIV infection, particularly by the parenteral infection route. Although, *NKG2C* copy number was associated with percentage and intensity of NKG2C expression on NK cells, these parameters did not correlate with HIV VL set point.

MATERIALS AND METHODS

Ethics statement. This research study was approved by the Institutional Review Board of the Research Ethics Committee of the McGill University Health Centre (study identification code 2018-4501).

It was conducted according to the principles expressed in the Declaration of Helsinki. Written informed consent for the collection of each individual's specimens and subsequent analyses using these samples was obtained from all study subjects.

Study population. The study population included 591 individuals: 434 were PLWH enrolled in the Montreal PI study, and 157 were HESN subjects (61). Persons at high risk of being sexually exposed to HIV, which we will designate here are sexually exposed (SE) HESN ($n = 78$), included HIV-uninfected men who have sex with men (MSM) recruited from the Clinique Médicale l'Actuel ($n = 40$) and subjects enrolled in the Ipergay Pre-Exposure Prophylaxis (PrEP) on-demand study followed in Montreal ($n = 21$) (62). These MSM SE HESN subjects answered "yes" to the question "Have you had unprotected receptive anal intercourse with a partner of unknown HIV serostatus or known to be HIV-infected, at least 5 times in the last 6 months or at least 50 times in your lifetime before starting PrEP?" An additional 17 SE HESN subjects were HIV-negative partners in HIV-discordant couples who remained HIV uninfected despite multiple exposures that occurred before the availability of antiretroviral treatment (ART). These included 9 men and 8 women; 6 of the men were MSM (63). We also recruited HIV-negative injection drug user (IDU) HESN subjects from the St. Luc cohort ($n = 79$) (64). All IDU HESN subjects answered "yes" to the question "Have you shared needles and/or injection equipment with partners known to be HIV-infected at least 5 times?" Clinic visits for St. Luc cohort participants occurred approximately every 6 months, at which time information was collected regarding the frequency of their at-risk behavior for HIV exposure. All HESN subjects provided a blood sample from which peripheral blood mononuclear cells (PBMCs) and plasma were isolated and stored frozen until use. HIV serostatus was assessed using HIV enzyme immunoassays (EIAs) (65). Subjects enrolled in the Montreal PI cohort included individuals recruited within the first 6 months of HIV infection, who were then followed an average of every 3 months for up to 4 years (65). At each clinic visit, CD4 and CD8 counts and plasma VL were measured, ART status was recorded, and blood was drawn for isolation of PBMCs and plasma, which was stored frozen until use. For one experiment comparing the expression of NKG2C on cells from HIV^- CMV^+ persons, 11 additional subjects who had minimal HIV exposure, were included.

***NKG2C* genotyping.** Genomic DNA was extracted from the PBMCs of all study subjects with the QIAamp DNA blood minikit (Qiagen, Inc., Toronto, ON, Canada) according to the manufacturer's instructions. Full-length *nkg2c* (*nkg2c*$^+$) and the deletion variant (*nkg2c*$^-$) are alleles at the same locus (24). NKG2A is encoded at a separate locus. The presence of *nkg2c*$^+$ or *nkg2c*$^-$ alleles and the *nkg2a* locus, as a positive control present in all subjects, was determined by sequence-specific PCR. Three sets of forward and reverse sequence-specific primers for *nkg2c*$^+$, *nkg2c*$^-$, and *nkg2a* were used to amplify the allele groups at the *nkg2c* and *nkg2a* loci. The forward and reverse primers for amplification of the *nkg2c*$^+$ allele were NKG2CT/F (5′-ATCAATTATTGAAATAGGATGC-3′) and NKG2CT/R (5′-CGCAAAGTTACAACCATCACCAT-3′) (24). Those amplifying the *nkg2c*$^-$ allele were BREAK-F (5′-ACTCGGATTTCTATTTGATGC-3′) and BREAK-R (5′-ACAAGTGATGTATAAGAAAAAG-3′) (24). Those amplifying the *nkg2a* internal control were NKG2A3F (5′-TGTATCCACCTCTCCTTTCG-3′) and NKG2A4R (5′-TTTGTACAGCCTAAGATCAAG-3′) (24). Twenty-five nanograms per microliter of genomic DNA from each participant was amplified with Platinum *Taq* (Thermo Fisher Scientific, Burlington, ON, Canada) in a T100 Thermal Cycler (Bio-Rad Laboratories, Inc., Hercules, CA) using the following conditions: denaturation at 95°C for 2 min, then 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s, followed by a 5-min extension at 72°C. Amplicons were visualized by gel electrophoresis on a 2% agarose gel in 0.5× TBE buffer (45 mM Tris base, 45 mM boric acid, 1 mM EDTA) run at 125 V for 30 min in Fluo-DNA loading buffer (6×; Zmtech Scientifique, Montreal, QC, Canada) and imaged with an Omega Lum C imaging system (Gel Company, Inc., San Francisco, CA). Band sizes of 300 bp corresponded to *nkg2c*$^+$ alleles, 400 bp to *nkg2c*$^-$ alleles, and 800 bp to *nkg2a* (29). Samples were classified as homozygous for the presence of the *nkg2c*$^+$ allele when only the 300-bp band was present (*NKG2C*$^{+/+}$), homozygous for *nkg2c*$^-$ (*NKG2C*$^{-/-}$) when only the 400-bp band was present, and heterozygous for *nkg2c*$^+$ and *nkg2c*$^-$ when bands of both sizes ($NKG2C^{+/-}$) were present (29).

Flow cytometry analysis of the frequency of $NKG2C^+$ cells and the intensity of NKG2C expression. PBMCs from 32 *NKG2C*$^{+/+}$, 19 *NKG2C*$^{+/-}$, and 6 *NKG2C*$^{-/-}$ HIV^+ CMV^+ subjects were stained with an antibody cocktail that allowed for gating on live NK cells as $CD3^-$ $CD14^-$ $CD19^-$ $CD56^{dim}$ lymphocytes. We also stained PBMCs from 43 *NKG2C*$^{+/+}$ and 18 *NKG2C*$^{+/-}$ HIV^- CMV^+ subjects with this antibody cocktail; all belonged to the HESN group, except for 11 HIV^- CMV^+ low-risk controls. These were examined for differences in the percentage of $NKG2C^+$ $CD56^{dim}$, $NKG2C^+$ $CD57^+$ $CD56^{dim}$, and $NKG2A^+$ $CD56^{dim}$ NK cells and the intensity of NKG2C expression on $CD56^{dim}$ NK cells from subjects carrying each *NKG2C* genotype. The intensity of NKG2C staining was assessed by measuring the mean fluorescence intensity (MFI), the median fluorescence intensity, and the fold change over background in the MFI of NKG2C staining. Cryopreserved PBMCs were thawed and resuspended in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 50 IU/ml penicillin, and 50 mg/ml streptomycin (R10) (all from Wisent, St Jean Baptiste, QC, Canada). PBMCs (10^6 in 100 μl of R10) were cell surface stained for 25 min at 4°C with previously optimized concentrations of fluorochrome-conjugated antibodies to the following cell surface markers: CD3-BV785 (clone OKT3), CD19-BV785 (HIB19), CD14-BV785 (M5E2), and CD56-BV605 (HCD56) from Biolegend, San Diego, CA; CD16-allophycocyanin (APC)-Cy7 (3G8) from BD Biosciences, Baltimore, MD; NKG2C-phycoerythrin (PE)-Cy7 (REA250) and NKG2A-APC (REA110) from Miltenyi Biotec, Auburn, CA; CD57-PE (TB01) from Life Technologies, Burlington, ON, Canada); and Indo-Violet LIVE/DEAD (L/D) stain from Fisher Scientific, Waltham, MA. Cells were then washed twice with fluorescence-activated cell sorter (FACS) buffer (phosphate-buffered saline [PBS], 4% fetal bovine serum [FBS], 0.05% NaN_3) and fixed in 2% paraformaldehyde (Santa Cruz Biotechnology, Santa Cruz, CA). Between 5×10^5 and 7×10^5 cells were acquired using an LSRFortessa X-20 flow

cytometer (BD Biosciences, San Jose, CA). Results were analyzed using FlowJo v10.6.2 software (Tree Star, Ashland, OR).

VL set point determination. VL set points were calculated for 160 *NKG2C*$^{+/+}$, 83 *NKG2C*$^{+/-}$, and 6 *NKG2C*$^{-/-}$ HIV^{+} carriers. The average of the VLs from all treatment-naive time points 6 months after the estimated date of infection to the end of their follow-up in the Montreal PI cohort were used to calculate the VL set point.

Statistical analysis. Statistical analysis and graphical presentation of results were performed using GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, CA) and Statistical Analysis System (SAS) software version 9.4 (SAS Institute, Cary, NC). The statistical significance of differences in the racial/ethnic composition of the HIV^{+} and HESN populations and deviations in the distributions of *NKG2C* genotype from Hardy-Weinberg equilibrium (HWE) was assessed using χ^2 tests. Between-group differences in the frequency of *NKG2C* genotypes in PLWH and HESN populations were determined using two-tailed Fisher's exact tests with Haldane's correction. The statistical significance of between-genotype differences in the percentage of NKG2C^{+} NK cells, the intensity of NKG2C expression on CD56^{dim} NK cells, and VL set point in ART-naive PLWH was assessed using Kruskal-Wallis tests with Dunn's posttests. The significance of correlations between the percentages of NKG2C^{+}, NKG2C^{+} CD57^{+}, and NKG2A^{+} CD56^{dim} NK cells and intensity of NKG2C expression and VL set point in ART-naive CMV^{+} PLWH was assessed using Spearman's correlation tests. *P* values of <0.05 were considered significant.

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