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NK cells in self-limited HCV infection exhibit a more extensively differentiated, but not memory-like, repertoire

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Summary

Natural killer (NK) cells have long been thought of as a purely innate immune cell population, but increasing reports have described developmental and functional qualities of NK cells that are commonly associated with cells of the adaptive immune system. Of these features, the ability of NK cells to acquire functional qualities associated with immunological memory and continuous differentiation resulting in the formation of specific NK cell repertoires has recently been highlighted in viral infection settings. By making use of a unique cohort of monitored, at-risk intravenous drug users in this study, we were able to dissect the phenotypic and functional parameters associated with NK cell differentiation and NK cell memory in patients 3 years after acute HCV infection and either the subsequent self-clearance or progression to chronicity. We observed increased expression of cytolytic mediators and markers $CD56^{bright}$ and $NKp46^{+}$ of NK cells in patients with chronic, but not self-limited HCV infection. Patients with a self-limited infection expressed higher levels of differentiation-associated markers CD57 and KIRs, and lower levels of NKG2A. A more extensively differentiated NK cell phenotype is associated with self-clearance in HCV patients, while the NK cells of chronic patients exhibited more naïve and effector NK cell phenotypic and functional characteristics. The identification of these distinct NK cell repertoires may shed light on the role NK cells play in determining the outcome of acute HCV infections, and the underlying immunological defects that lead to chronicity.

KEYWORDS
acute hepatitis, cytotoxicity, differentiation, hepatitis C, innate immunity, memory, Natural killer cells

1 | INTRODUCTION

Spontaneous clearance of an acute hepatitis C virus (HCV) infection occurs in approximately 25% of patients who contract a primary infection.[1] Clearance has been associated with strong T-cell responses and a rapid induction of neutralizing antibodies against the autologous virus,[2-5] but in recent years, the importance of natural killer (NK) cells in this process has received renewed interest.[6-9] NK cells act by eliminating HCV infection via two type of mechanisms: direct mechanisms, including the release of cytotoxic enzymes, such as granzymes and perforins, that result in lysis of the infected hepatocytes, and indirect

Abbreviations: ACS, Amsterdam Cohort Studies; HCV, hepatitis C virus; IDUs, injecting drug users; IFN, interferon; KIRs, killer cell immunoglobulin-like receptor; NK, natural killer; PBMC, peripheral blood mononuclear cells.

mechanisms that involve the release of cytokines, such as interferon (IFN)-γ, by activated NK cells that result in triggering of antiviral responses that inhibit viral replication in infected cells.

Natural killer cell activity is strictly governed by a balance of activating and inhibitory receptors on the cell surface. Some of the major classifications of these regulatory receptors include C-type lectin-like receptors (NKG2A, NKG2C, NKG2D), natural cytotoxicity receptors (NKp30, NKp44 and NKp46) and killer cell immunoglobulin-like receptor (KIRs).[10,11] During viral infection, the balance shifts from inhibition to activation after a critical threshold of activation signals exceeds those of inhibition.[12] In addition to the regulation of the function of NK cells by these cell surface receptors, differentiation from $CD56^{bright}$ to $CD56^{dim}$ cells has also been shown to contribute to functional and phenotypic heterogeneity observed during the lifespan of NK cells. Many details of this differentiation process remain undefined, but the dynamics and outcome are likely affected by viral infections. During the differentiation process, NK cells lose expression of NKG2A, and subsequently acquire KIR and CD57 expression, ultimately resulting in a more terminally differentiated phenotype, that display reduced IFN-γ-producing and proliferative capacities.[13,14]

Besides differentiation of NK cells, in recent years, development of NK cells into memory-like cells has also been reported in both mice and humans, suggesting more similarities between NK cells and CD8+ T cells than originally thought.[15,16] In humans, a long-lived memory-like NK cell population was described at higher frequencies in CMV-seropositive individuals than CMV-seronegative individuals.[17,18] These cells were found to express high levels of the of C-type lectin NKG2C, in combination with maturation marker CD57.[17] A memory phenotype for NK cells has also been suggested in *Mycobacterium tuberculosis* and hantavirus,[19,20] but little information is available in HCV infections. This would, however, be highly relevant as in high-risk populations like injecting drug users (IDUs), reinfection is frequently observed in patients who would have previously spontaneously cleared an HCV infection. The clinical observation that primary HCV infections are cleared in only a minority of individuals, while spontaneous clearance of an HCV reinfection occurs in approximately 80% of cases,[21,22] strongly suggests that immunity acquired during the primary infection may play a role in the protection against HCV reinfection, and that some sort of immunological memory to HCV has developed.

With limited information available on the development and persistence of memory-like NK cells during and after HCV infection, we chose to characterize and compare the NK cell compartments of individuals who either resolved an acute HCV infection and individuals that developed a chronic infection. The aim of this study was to determine whether memory-like NK cells are present following the resolution of a primary HCV infection, as well as whether the phenotypic and functional parameters associated with the NK cell differentiation process differ between the resolved patients and those chronically infected with HCV. By utilizing the unique cohort of self-clearing IDUs and characterizing the effector and memory NK cell compartments in these individuals, we hope to provide immunological insight on how and to what extent NK cells might enhance immune-based antiviral strategies, such as vaccines and immunomodulators, in HCV infection.

2 | MATERIALS AND METHODS

2.1 | Patient selection and characteristics

Isolated peripheral blood mononuclear cells (PBMC) of 32 individuals were selected from archived samples collected in the Amsterdam Cohort Studies (ACS), which were in 1984 to investigate the epidemiological, behavioural and psychosocial aspects of HIV/AIDS among homosexual men and drug users.[23-25] In these studies, participants were requested to visit the centre every 6 months for HIV/HCV screening and were evaluated for at-risk behaviour and various related psychosocial determinants. Blood was collected and stored for future studies. HCV RNA and antibody tests were performed retrospectively for all participants until 2003 and prospectively from October 2007 until 2010 for HIV-negative participants.[26] The characteristics of the selected patients are presented in Table 1. All individuals selected were HIV negative/unexposed, at-risk IDUs who were age- and ethnically matched individuals who displayed similar risk behaviours and who were reinfection-naïve. The individuals were categorized into three groups for cross-sectional analysis: (i) at-risk individuals who were never exposed to HCV infection and did have serological evidence of HCV infection at the time of sampling or at a later time point (n=10); (ii) at-risk individuals who contracted a self-limiting acute HCV infection (viral clearance within first 6 months of infection) (n=11); and (iii) at-risk individuals that eventually developed a chronic infection (viral persistence 6 months postinfection) (n=11). Using the virologic data provided by the bi-yearly visits of participants in the ACS, an approximate time of infection was determined for the cleared and chronic patients (±6 months) and samples were selected approximately 3 years postinfection. Additionally, when available, samples were selected from the same self-clearing and chronically infected individuals approximately 1 year postinfection for longitudinal analysis. The study was approved by the local ethics committee, and all patients and controls in the study gave informed consent before blood donation.

2.2 | Expression of cell surface and intracellular molecules by flow cytometry

To determine the frequency and phenotype of peripheral blood NK cells, multicolour flow cytometry was performed on PBMC with anti-CD3-Alexa-Fluor700 (OKT-3, Beckman), anti-CD56-APC-eFluor780 (CMSSB, Beckman), anti-CD57-APC (HCD57, BioLegend), anti-KIR2D-Biotin (NKVFS1, Miltenyi biotech), anti-KIR3DL1/DL2-Biotin (5133, Miltenyi biotech), Streptavidin-eFluor450 (eBioscience), anti-NKG2A-PE (Z199, Beckman), anti-NKG2C-Alexa-488 (134591, R&D), anti-NKG2D-PerCP-Cy5.5 (1D11; BD), anti-NKp30-PE (Z25, Beckman), anti-NKp44-Biotin (P44-8, BioLegend), anti-NKp46-PE-Cy5 (BAB281, Beckman), anti-CD69-PacificBlue (FN50; BioLegend) and Live/Dead Aqua (Life Technologies). For intracellular expression of cytotoxic markers, PBMC ($0.5x10^6$ cells/200 μL) were fixed with 2% formaldehyde and permeabilized for intracellular staining with anti-granzyme B-PE (GB11, eBioscience), anti-perforin-PerCP-eFluor710 (G9, eBioscience) and anti-TRAIL-Alexa488 (75402, R&D). Cells were

TABLE 1 Participant characteristics at time of sample collection (approximately 3 y postacute HCV infection[a])

		Uninfected	Cleared HCV	Chronic HCV
Numbers		10	11	11
Sex	M/F	8/2	4/7	4/7
Age (y)		32.3 (25-59)	30.9 (24-42)	34.7 (24-47)
Ethnicity	Caucasian	10	8	9
	Asian	–	2	–
	Other	–	1	2
Time since infection (y)[a]		–	2.9 (2.4-5.0)	2.9 (2.0-3.8)
HCV bDNA (IU/mL)		–	<615	$4.9x10^5$ ($6.3x10^3$-$1.9x10^6$)
Anti-HCV		negative	positive	positive
HCV genotype	genotype 1	–	1	6
	genotype 3		4	4
	genotype 4		1	1
	unknown		5	–

[a]Estimated as time between first positive HCV bDNA test (with negative test at previous visit) and time of sample collection.

again assessed by flow cytometry (FACS Canto II, BD) and analysed using FlowJo version 10.1 (Tree Star Inc). Quadrants were set on negative expression of markers on $CD3^-CD56^-$ cells. Approximately 10^5 viable lymphocytes were collected for each individual sample.

2.3 | Stimulation and intracellular cytokine analysis of NK cells

Peripheral blood mononuclear cells were stimulated with IL-12 (0.25 ng/mL) and IL-18 (10 ng/mL) overnight for surface marker expression and intracellular cytokine production. After 18 hours, Brefeldin A (10 μg/mL, Sigma) was added to the cultures and the cells were incubated for an additional 3 hours. Cellular activation and surface markers were measured using anti-CD3-PacificBlue (OKT3, eBioscience, San Diego, CA, USA), anti-CD56-PE (MY31, BD) and anti-CD69-APC (L78, BD), followed by fixation with 2% formaldehyde, and permeabilization for intracellular staining with anti-IFN-γ-FITC (25723.11, BD). Cytokine-producing cells were detected by flow cytometry (FACS Canto II, BD) and analysed using FlowJo version 10.1 (Tree Star Inc, Ashland, OR, USA). Quadrants were set on negative expression of CD69 on monocytes and the absence of IFN-γ production by B cells.

2.4 | Statistical analysis

Data are expressed as the mean value ± SEM, unless indicated otherwise. The data were analysed with Prism software, version 5.0 (GraphPad Software Inc., La Jolla, CA, USA) using the Mann-Whitney U test to compare the variables between independent groups and the Spearman rank correlation coefficient test for nonparametric correlations. In all analyses, a two-tailed $P<.05$ (95% confidence interval) was considered statistically significant.

3 | RESULTS

3.1 | NK cells of patients with a chronic, but not self-limited, HCV infection have an increased $CD56^{bright}$/$CD56^{dim}$ ratio and expression of granzyme B and TRAIL

Previous studies have implicated a role for NK cells in the innate immune response to acute HCV infection.[27,28] To further dissect and understand the characteristics of NK cells following self-resolution of acute HCV infection or during progression to chronicity, we chose to perform a cross-sectional analysis of NK cell phenotype and function in uninfected individuals, patients with a self-limited HCV infection (cleared) and patients who developed a chronic infection. As a first step, using flow cytometric analysis, we evaluated the composition of PBMC, and showed that the percentage of total NK cells (defined by $CD56^+CD3^-$ expression) within the lymphocytes did not differ in the peripheral blood of any of the three groups (Figure 1A). However, an increase in the frequency of $CD56^{bright}$ and reduction of $CD56^{dim}$-expressing NK cells was observed in chronic patients as compared to both the uninfected and self-clearing individuals.

Concurrently with the compartmental analysis, the cytotoxic potential of NK cells from the uninfected, cleared and chronic groups was assessed by intracellular analysis of the cytolytic mediators perforin, granzyme B and TNF-related apoptosis-inducing ligand (TRAIL) (Figure 1B). In contrast to the frequency of perforin-expressing NK cells, which remained stable across all groups, the frequencies of granzyme B and TRAIL-expressing NK cells were significantly higher in chronic HCV patients compared to uninfected individuals or patients who had cleared the infection. Collectively, these results show an increase in $CD56^{bright}$ expression and cytolytic potential of NK cells from chronic HCV patients, but not self-limiting patients, when compared to uninfected individuals.

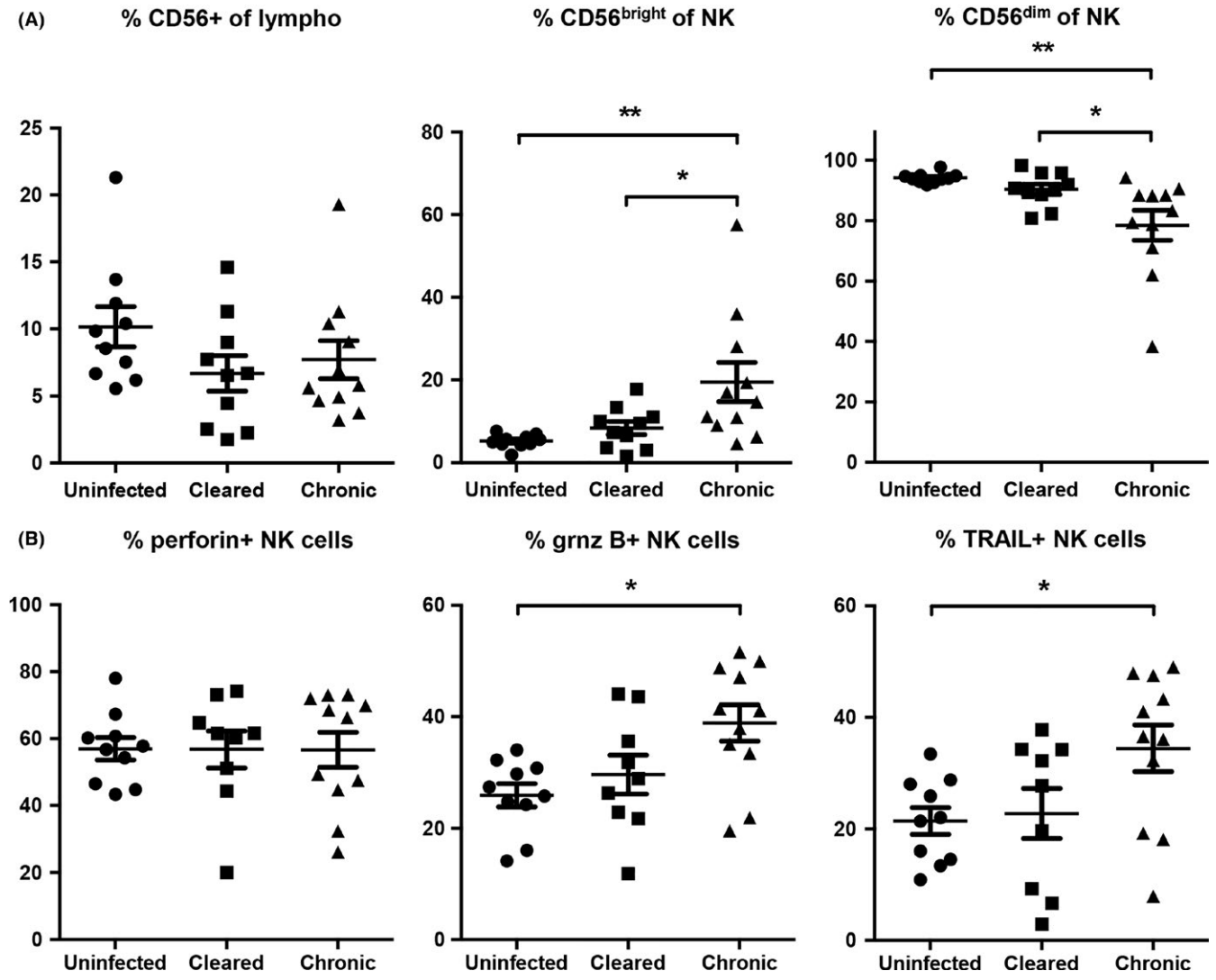

FIGURE 1 Natural killer (NK) cells of patients with a chronic, but not self-limited, HCV infection have an increased $CD56^{bright}/CD56^{dim}$ ratio and expression of granzyme B and TRAIL. (A) Flow cytometric analysis was performed on the peripheral blood mononuclear cells of uninfected, self-clearing and chronic HCV patients to determine the frequency of total $CD56^{+}CD3^{-}$ NK cells in lymphocytes, and the frequency of $CD56^{bright}$ and $CD56^{dim}$ of these NK cells. (B) Intracellular flow cytometric analysis of perforin, granzyme B and TRAIL was performed on the NK cells of the aforementioned patient groups. Data are shown as mean±SEM. * denotes $P<.05$ and ** $P<.01$ (Mann-Whitney U test)

3.2 | The frequency of cytokine-induced IFN-γ production is unaltered in NK cells of self-limited and chronic HCV patients compared to uninfected individuals

Differential capacities for particular effector functions have been described for the $CD56^{bright}$ and the $CD56^{dim}$ NK cell subsets.[29-31] To explore whether the observed differences in NK cell composition translated to differential NK cell effector function, we investigated the cytokine-producing capacity of these NK cells. PBMC from uninfected individuals, self-limited HCV patients or chronic HCV were cultured in the presence of IL-12 and IL-18, and the surface expression of activation marker CD69 and intracellular expression of IFN-γ were determined (Figure 2A). The percentage of CD69-expressing NK cells after IL-12 plus IL-18 stimulation was consistent across all three groups (Figure 2A-B). Albeit not significant, a trend towards, reduced frequencies of IFN-γ-producing NK cells were observed in patients with self-limited HCV relative to chronically infected patients. Neither the percentage of IFN-γ producing NK cells of self-clearing patients nor chronic HCV patients were significantly altered compared to uninfected individuals.

3.3 | Expression of natural cytotoxicity receptors unaltered on NK cells of self-clearing HCV patients

NK cell activity is tightly regulated by a balance of activating and inhibitory receptors, and during viral infection, the balance shifts from inhibition, the steady-state condition, towards activation.[10,11] NCRs (eg, NKp30, NKp44, NKp46) and C-type lectin-like receptors (eg, NKG2D) are two of main classes of activating NK receptors, and we therefore

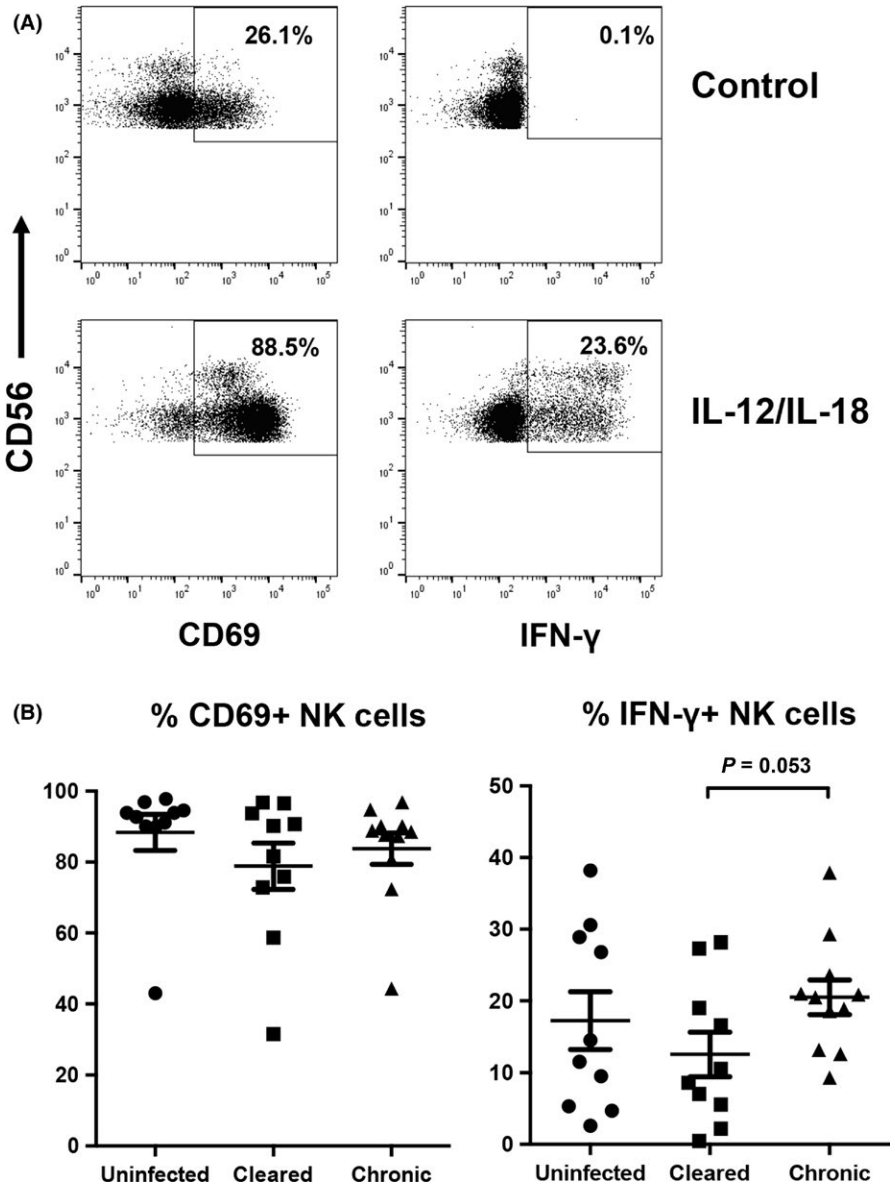

FIGURE 2 Frequency of IL-12/IL-18-induced IFN-γ production is unaltered in Natural killer cells of self-limited and chronic HCV patients compared to uninfected individuals. (A) Peripheral blood mononuclear cells from uninfected, self-clearing and chronic HCV patients were either left unstimulated (control) or stimulated with IL-12/IL-18 overnight, and surface/intracellular flow cytometric analysis was performed for the expression of CD69 and IFN-γ. (B) Collective results for multiple donors of CD69- and IFN-γ-positive cells after IL-12 and IL-18 stimulation. Data are shown as mean±SEM. Mann-Whitney U test was used for statistical testing of significance

chose to investigate the expression of these receptors on NK cells in a self-limited and chronic HCV setting. Flow cytometric analysis showed no differential expression of NKp30, NKp44 or NKG2D on NK cells between the uninfected, cleared and chronic groups (Figure 3). However, the frequency of NKp46-expressing NK cells was significantly higher in chronic HCV patients compared to uninfected controls and showed a trend towards higher frequencies as compared to the NK cells of self-limited patients. Collectively, this data show no difference in the expression of particular NCR and other activating receptors in self-clearing HCV patients compared to uninfected individuals, whereas NKp46 expression was elevated in chronic patients.

3.4 | NK cell memory phenotype is not associated with self-clearance or chronic disease in HCV infection

The long-standing dogma of NK cells as a purely innate immune cell population has been challenged in recent years, with increasing reports describing qualities of NK cells that have commonly been associated with immunological memory in response to pathogens.[15,16] We therefore decided to investigate and characterize this unique NK cell population in HCV infection, and to determine its association with the resolution of acute HCV infections. First, the expression of maturation marker CD57 and the activating CD94-NKG2C receptor was assessed by flow cytometry in uninfected individuals, as well as self-cleared and chronic HCV patients (Figure 4A). CD57 expression, but not NKG2C, was significantly greater on NK cells of self-limited HCV patients compared to uninfected controls, whereas in chronic patients, CD57 and NKG2C expression remained unaltered.

Next, we examined the frequency of $CD57^{+}NKG2C^{++}$-expressing NK cells, as this population has been reported to represent the long-lived NK cell population in humans.[17,32,33] Using flow cytometry, we were able to identify this population of NK cells, but did not observe a significant difference in the percentage of $CD57^{+}NKG2C^{++}$-expressing NK cells between uninfected individuals or either HCV patient group

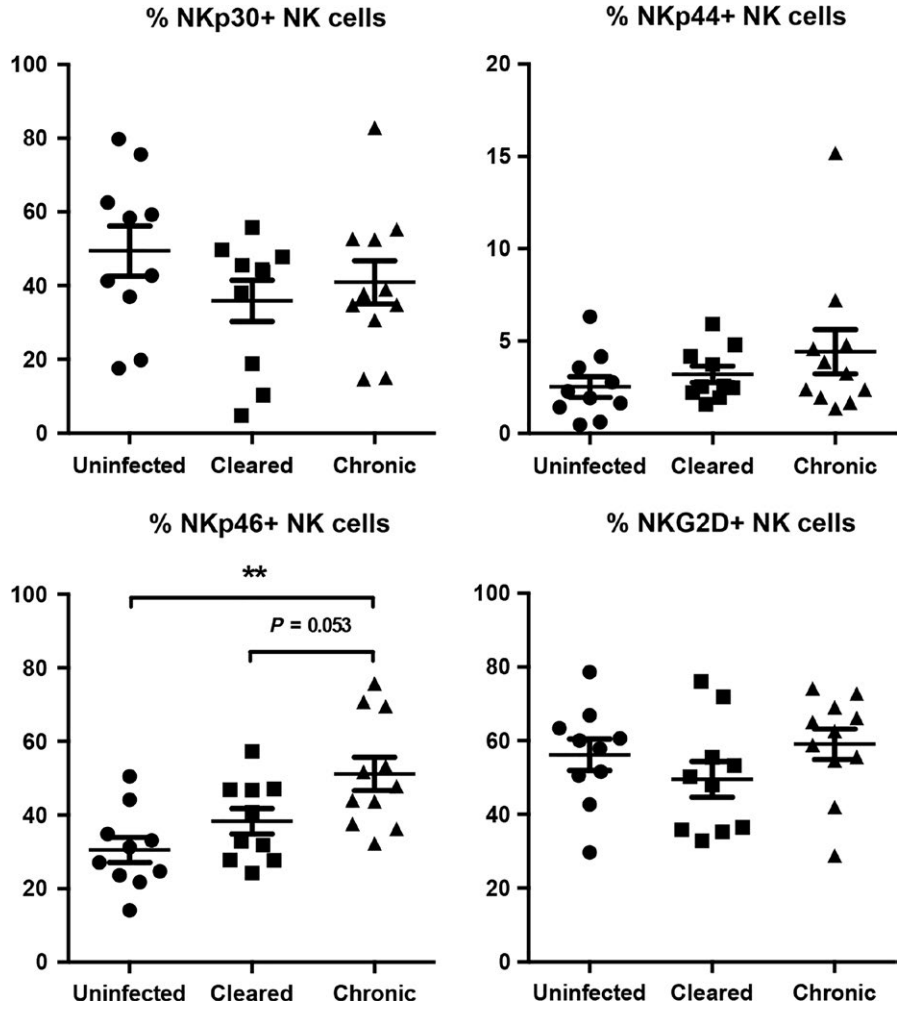

FIGURE 3 Expression of NKp30, NKp44, NKp46 and NKG2D is unaltered on Natural killer (NK) cells of self-clearing HCV patients, whereas the frequency of NKp46^{+} NK cells is enhanced in patients with chronic HCV. Flow cytometric analysis was performed on the peripheral blood mononuclear cells of uninfected, self-clearing and chronic HCV patients to determine the expression of NKp30, NKp44 and NKp46, as well as NKG2D on total NK cells. Data are shown as mean±SEM. ** *P*<.01 (Mann-Whitney U test)

(Figure 4B). The expression of CD57^{+}NKG2C^{++} NK cells remained stable from 1 to 3 years postcontraction of acute HCV infection in the cleared patients, and in chronic patients (Figure 4C), suggesting that this population is not diminished after viral clearance or during prolonged chronic infection. As a whole, these results show that despite an increased expression of CD57 on NK cells of self-limited HCV patients, this did not translate to differential frequencies of memory-associated NK cell populations in either self-cleared or chronic HCV patients.

3.5 | Self-limited HCV patient have more highly differentiated NK cell repertoires than uninfected and chronically infected individuals

The maturation marker CD57 expression has not only been linked to memory in NK cells, but it has also been implicated, in combination with inhibiting CD94-NKG2A receptor and KIRs, as defining markers in the differentiation of CD56^{dim} NK cell repertoires.[13,14] To determine the differentiation status among NK cell populations and the role it may play in the clearance of HCV infection, we assessed the expression and co-expression of NKG2A, CD57 and KIRs. NK cells of self-limited HCV patients expressed significantly reduced levels of NKG2A compared to both uninfected and chronically infected individuals (Figure 5A). This reduction of NKG2A expression was only observed in CD57^{-} NK cells, but not CD57^{+} NK cells. KIR expression in the total NK cell compartment of cleared patients resembled that of uninfected individuals, whereas chronic patients expressed reduced levels of these receptors (Figure 5B). KIR expression in self-limited HCV patients appeared to be mainly attributed to the CD57^{+} NK cells and, similarly to the chronic patients, was significantly reduced on the CD57^{-} NK population. NKG2A expression on NK cells was inversely correlated with both KIRs and CD57 expression only on the NK cells of the cleared group, whereas KIRs and CD57 were positively correlated in this subset of patients. Collectively this data suggest an enrichment of more highly differentiated NKG2A^{-}CD57^{+}KIR^{+} cells in patients with a self-limiting HCV infection compared to uninfected and chronically infected individuals.

4 | DISCUSSION

By making use of a unique cohort of monitored, at-risk intravenous drug users in this study, we were able to dissect the phenotypic and functional parameters associated with NK cell differentiation and NK

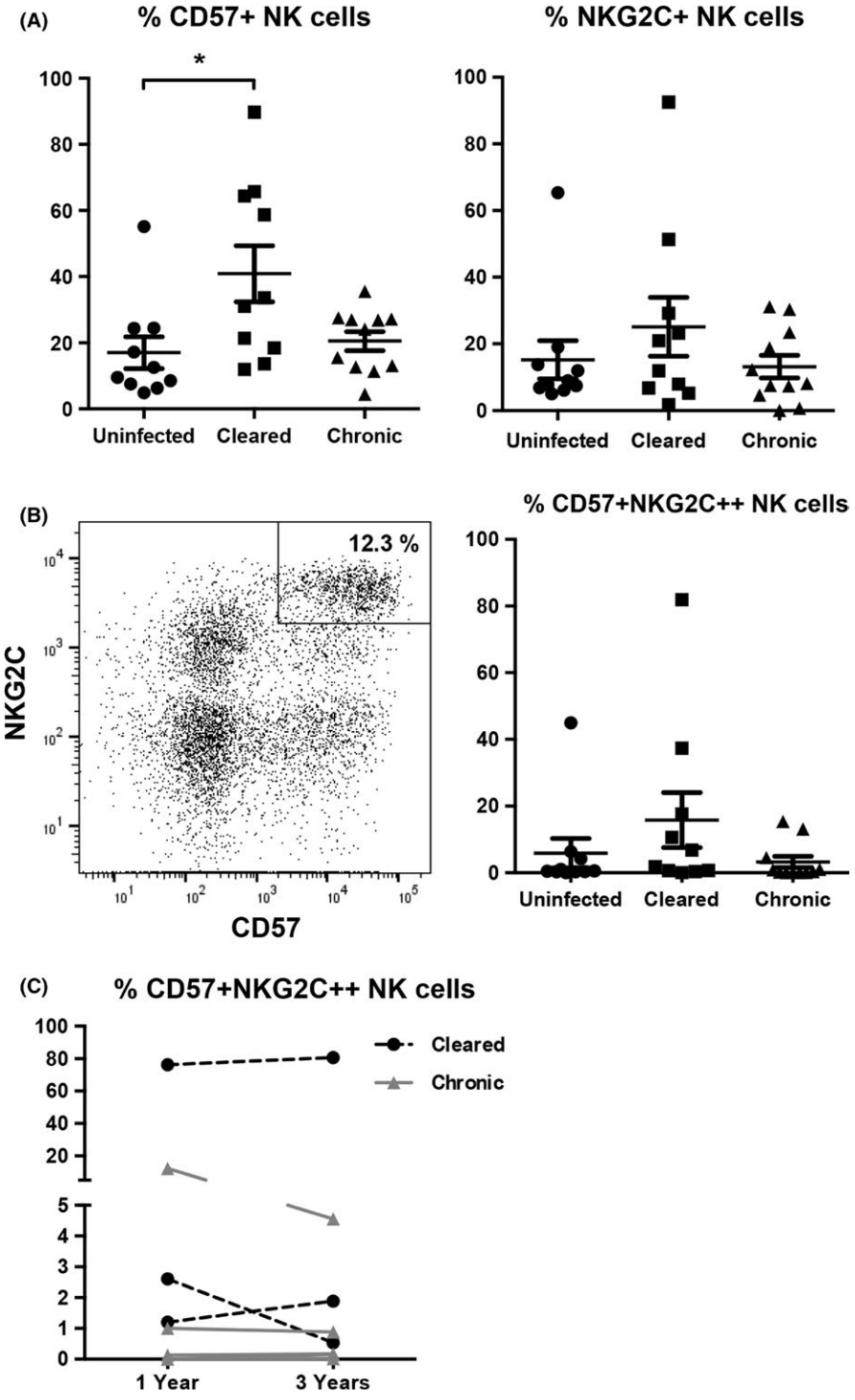

FIGURE 4 Natural killer (NK) cell memory-associated phenotype is not associated with self-clearance or chronic disease in HCV infection. (A) NK cells in peripheral blood mononuclear cells from uninfected, self-clearing and chronic HCV patients were analysed using flow cytometry for the expression of the NK cell maturation markers CD57 and NKG2C. (B) NK cells with a memory-associated phenotype were defined based on the expression of $CD57^{+}$ and $NKG2C^{++}$. Collective results for multiple donors of the frequency of these $CD57^{+}NKG2C^{++}$ NK cells. (C) Paired time points for patients at either 1 year or 3 years postinfection. Data are shown as mean±SEM. * denotes $P<.05$ (Mann-Whitney U test)

cell memory in patients 3 years after acute HCV infection and either the subsequent self-clearance of infection or progression to chronicity. Increased frequencies of NK cells expressing $CD56^{bright}$ and cytolytic mediators (granzyme B and TRAIL) were observed in chronically infected patients, but not those that cleared the acute infection, suggesting an overall more naïve and effector NK cell repertoire during chronic infection. NK cell repertoires of patients with a self-limited infection displayed a more highly differentiated phenotype, characterized by an increased CD57 and KIR expression, as well as reduced NKG2A expression, but did not exhibit a memory-like phenotype. Lastly, cytokine-induced activation, as seen by CD69, was comparable in all groups, whereas IFN-γ-expressing NK cells showed a trend, but nonsignificant, decrease in self-limited patients as compared to the chronic group.

A key finding of this study was that, although we were able to identify the memory-like NK cell population based on $CD57^{+}NKG2C^{++}$ expression in a subset of individuals from all groups in this study, no significant differences were observed in the total percentages of this

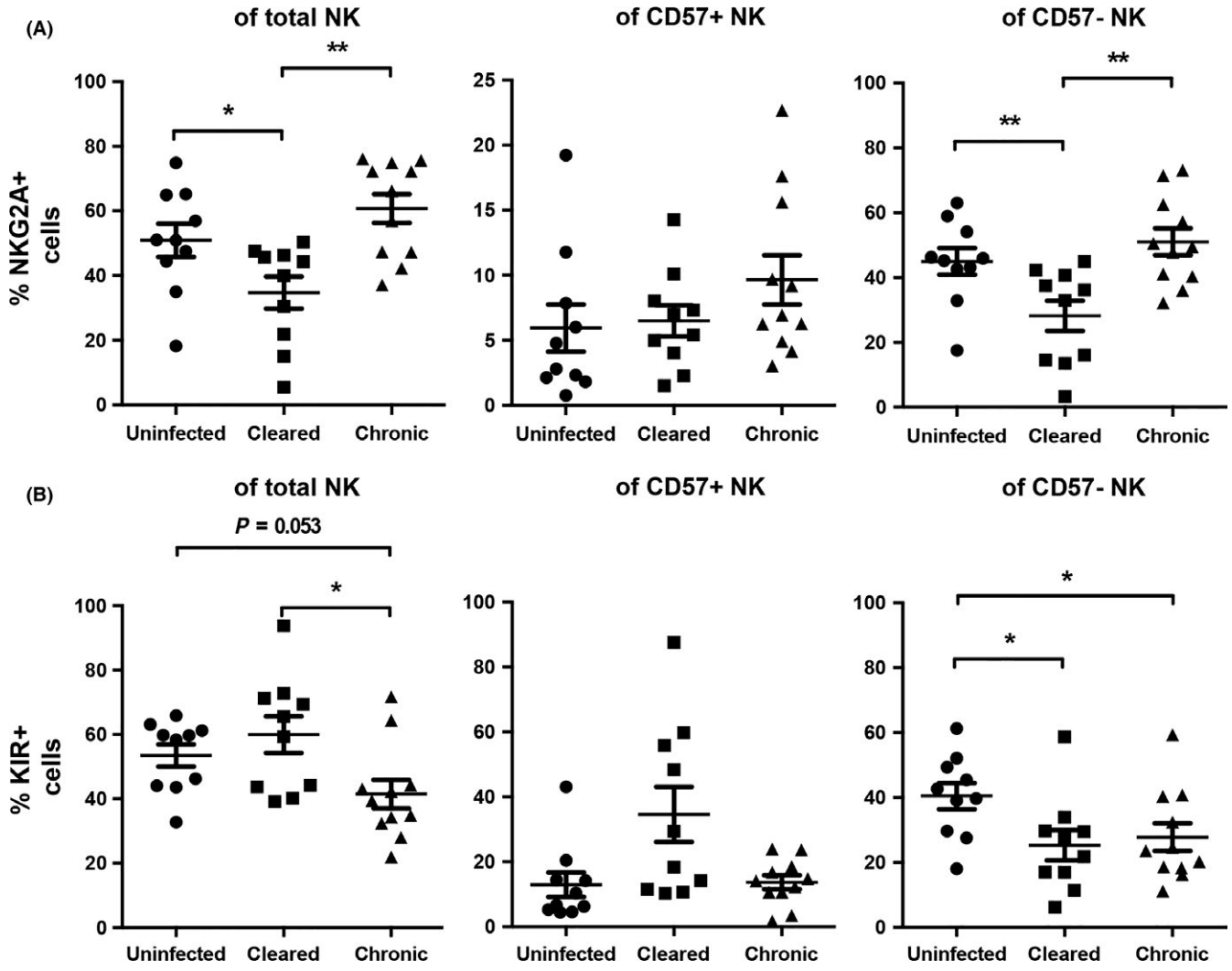

FIGURE 5 Self-limited HCV patients have more highly differentiated Natural killer (NK) cell repertoires than uninfected and chronically infected individuals. NK cells in peripheral blood mononuclear cells from uninfected, self-clearing and chronic HCV patients were analysed using flow cytometry for the expression of NKG2A and KIRs (KIR2D, KIR3DL1 and KIR3DL2). (A) NKG2A expression on total NK cells, and $CD57^+$ or $CD57^-$ NK cells. (B) KIR expression on total NK cells, and $CD57^+$ or $CD57^-$ NK cells. Data are shown as mean ± Natural killer SEM. * denotes $P<.05$ and ** $P<.01$ (Mann-Whitney U test)

specific NK cell repertoire. Additionally, the longitudinal analysis of both chronic and self-resolving patients from 1 to 3 years after estimated time of infection demonstrated a stable and unaltered expression and composition of this memory-like NK cell compartment. Our findings argue against the hypothesis that $CD57^+NKG2C^{++}$ NK cells observed after resolution of HCV infection are genuine memory NK cells induced by HCV exposure. Additionally, the protective features attributed to expanded expression of these populations upon re-exposure previously described in other viral infections cannot justify the increased resistance to reinfection described in acute-resolved HCV patients.

Although we did not observe differences with respect to memory-like characteristics of NK cells, the phenotype and function of cells observed for acute-resolved patients did still drastically differ when compared to that of chronic patients and the uninfected group. Distinct alterations were observed between self-clearing patients and both the chronic and uninfected groups in respect of their expression of maturation and differentiation-associated markers on NK cells. The expression of CD57 and KIRs was positively correlated (Fig. S1), and the expression of both these receptors was inversely correlated to that of NKG2A, suggesting a tight link between the regulation and expression of these receptors on the surface of NK cells of acute-resolved patients. This phenotypic alteration and regulation of markers resemble those previously described for NK cell differentiation.[23,24] During this differentiation process, NK cells lose expression of NKG2A, and subsequently acquire KIRs and CD57, ultimately resulting in a more terminally differentiated phenotype that display reduced IFN-γ production and proliferative capacity. Our data indicate that NK cells of acute self-limited HCV patients persist in a more highly differentiated state, even years after the clearance of infection, although it is unclear whether this is the cause or result of the resolution of the acute infection. However, the reduced proliferative and effector functionality

described for terminally differentiated NK cells makes it difficult to attribute the immunological resistance to reinfection in self-limited HCV patients to this NK cell repertoire.

In line with previous studies,[34,35] we observed an altered distribution of CD56$^{bright/dim}$ NK cell subsets in chronic HCV patients, but did not observe an overall decrease in the levels of NK cells as described by other studies.[34,36-38] The NK cells of these chronic patients also expressed significantly lower levels of CD16 (data not shown) and increased levels of NKp46 as compared to not only the uninfected group but also the resolved patients. Increased NKp46 expression in this group is of particular interest, as it has been shown to be one of the principle avenues of NCR-mediated killing and correlated with the cytolytic activity of NK cells in HCV patients.[39-41] This also supports skewing towards cytotoxicity, with increased frequencies of granzyme B- and TRAIL-expressing cells, observed in the chronic patients group. Interestingly, the frequencies of perforin-expressing NK cells remained constant. This differential regulation of perforin- versus granzyme B-expressing NK cells has been described before by others, who showed distinct induction after triggering with IL-2, IL-4, PMA/ionomycin and IFN-α.[42,43]

Interestingly, however, some of these studies report depressed IFN-γ in chronic patients, whereas no differences were observed in the frequency of IFN-γ-producing NK cells of chronic HCV patients in our study, but instead a trend towards reduction in the resolving HCV patients. This could be attributed to the highly differentiated phenotype of NK cells in this particular group, previously described to be impaired in their cytokine-producing and proliferative capacities.[13] Overall, our data, in combination with that previously described in literature, depict NK cells in chronic patients as phenotypically naïve, with increased expression of NCRs and cytolytic mediators, reflecting the activation response to a persistent viral stimulation.

Collectively, our findings depict an altered, more extensively differentiated, but not memory-like, NK cell compartment in patients after resolving an acute infection, as compared to both uninfected individuals and patients with ongoing infections. This is in contrast to NK cells of chronic HCV patients that displayed a more naïve and effector phenotype with elevated cytotoxic capabilities. Although the highly differentiated NK cell repertoire in acute-resolved patients may not describe the resistance to reinfection observed in self-clearing acute HCV patients, the identification of these distinct NK cell repertoires may shed light on the role NK cells play in determining the outcome of secondary HCV infections, and the underlying immunological defects that lead to chronic infection.

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SUPPORTING INFORMATION

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