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Phenotypic and Functional Changes of Cytotoxic CD56^{pos} Natural T Cells Determine Outcome of Acute Hepatitis C Virus Infection$^{\nabla}$

Lucy Golden-Mason,[1,2] Nicole Castelblanco,[1] Cliona O'Farrelly,[3] and Hugo R. Rosen[1,2]*

Division of Gastroenterology and Hepatology, Hepatitis C Center,[1] and Integrated Program in Immunology,[2] University of Colorado at Denver Health Sciences Center and National Jewish Hospital, Denver, Colorado, and Education and Research Centre, St. Vincent's University Hospital, Elm Park, Dublin 4, Ireland[3]

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Innate CD56^{pos} natural killer (NK) and natural T (NT) cells comprise important hepatic antiviral effector lymphocytes whose activity is fine-tuned through surface NK receptors (NKRs). Dysregulation of NKRs in patients with long-standing hepatitis C virus (HCV) infection has been shown, but little is known regarding NKRs in acute infection. Treatment-naïve patients with acute HCV ($n = 22$), including 10 with spontaneous recovery, were prospectively studied. CD56^{pos} NT levels were reduced early in acute HCV infection and did not fluctuate over time. In resolving HCV infection, NT cells with a more activated phenotype (lower CD158A and higher natural cytotoxicity receptor expression) at baseline predated spontaneous recovery. Moreover, NKG2A expression on CD56^{+} NT cells correlated directly with circulating HCV RNA levels. Deficient interleukin-13 (IL-13) production by NT cells and reduced IL-2-activated killing (LAK) at baseline were associated with the ultimate development of persistence. These results indicate a previously unappreciated role for NT cells in acute HCV infection and identify a potential target for pharmacologic manipulation.

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease, with an estimated 1.8% of the American population harboring the virus and 170 million people infected worldwide (1). The most remarkable feature of HCV infection is the high rate of chronicity, with only a minority of exposed individuals (~20%) spontaneously resolving acute infection (7). Protective immune against HCV requires the orchestration of multiple cells and molecules both systemically and in the liver, although the precise mechanisms determining outcome remain incompletely defined (18). Unequivocal data support the central role of the host adaptive immune response to acute HCV infection in determining whether the virus is eliminated or persists (4, 36); however, little is known about the role of the innate immune response in the earliest stages of HCV infection.

Innate CD56^{pos} natural T (NT) cells comprise approximately 5 to 15% of the peripheral T-cell pool, and up to 50% of T cells within the liver environment, the primary site of HCV replication, coexpress CD56 (12). The CD56 antigen is typically expressed by NK cells. CD56^{pos} NT cells are not classical invariant NKT (iNKT) cells but are a broader group of T cells that conform to the original definition of NKT cells, which was "T cells coexpressing NK cell markers" (21). NK receptor (NKR)-positive T cells possess dual innate and adaptive immune functions displaying properties of both T and NK cells capable of both major histocompatibility complex (MHC)-restricted and MHC-unrestricted cytotoxicity and cytokine production. Of note, the human iNKT [CD1d-aGalcer reactive Vα24(JαQ)Vβ11 cells] and other T cells expressing NKR (other than CD56) are found almost exclusively within the CD56^{pos} NT cell population. The human iNKT cell population described is a rare population and, due to the expression of multiple isoforms of CD1 (in contrast to rodents who express only CD1d), is unlikely to be the sole human iNKT cell population (20, 21). We therefore focused on the broader CD56^{+} NT cell population in acute HCV infection to assess the role of NKR^{+} T cells in determining the outcome of acute HCV infection. These innate lymphocyte populations can recognize conserved structures that signal viral invasion, thus providing an important first line of defense against viral infection (3). Recent studies have highlighted important roles for innate lymphocytes in immunity against hepatotrophic viruses including HCV infection (14, 25). In this regard, patients with long-standing chronic HCV infection demonstrate diminished NT cell responses (9, 13, 19).

In addition to stimulation via the TCR, the activation of NT cells is controlled by inhibitory NKRs, which override signals provided by the engagement of activating receptors (23, 27). Although NKRs are classically associated with NK cells, the majority of NKRs are also expressed on a subset of cytotoxic CD56^{pos} T cells (NT cells), which have an activated or effector/memory phenotype (20, 26, 33). Dysregulation of NKR expression has been implicated in chronic viral persistence (10, 16, 17, 28, 29); however, studies of chronic HCV are sparse, and to our knowledge, nothing is known of NKR expression in acute HCV infection. NKRs recognizing classical and nonclassical MHC class I molecules include killer immunoglobulin-like receptors (KIRs) and C-type lectin-like receptors of the CD94/NKG2 family. KIRs recognize classical HLA-A, HLA-B, and HLA-C, primarily mediating inhibition, although some activating isoforms have been described. The binding of inhibitory KIRs to their MHC class I ligands on potential target cells results in the suppression of cytotoxicity and cytokine secretion by KIR-expressing cells (8). The CD94/NKG2A (inhibitory) (32) and NKG2C (activating) (24) receptors recognize non-

* Corresponding author. Mailing address: 4200 East Ninth Ave., Research Bridge, Room 6412, Denver, CO 80220. Phone: (303) 315-2536. Fax: (303) 315-5711. E-mail: Hugo.Rosen@UCHSC.edu.

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TABLE 1. Demographic and clinical details of the acutely HCV-infected group[a]

C/R	Patient ID	Age (yr)	Gender	Ethnicity	Main risk factor	Genotype	Peak ALT level	M6 HCV RNA level (IU/ml)	ΔHCV RNA level (IU/ml) from 0–6 mo
C	HS108	37	F	CA	Sexual/surgery	1a	692	783,610	717,532
C	HS111	20	M	CA	IDU	1a	867	2,526,131	2,473,316
C	HS116	24	M	CA	IDU	2b	603	>7,692,316	>7,520,323
C	HS122	40	M	CA	Unknown	1a	2,056	2,498,935	2,498,042
C	PD106	45	M	CA	IDU	1	247	2,537,333	1,653,673
C	PD108	46	F	CA	Sexual	1a	838	166863	161,683
C	PD109	50	M	CA	Sexual	3a	468	900,963	727,531
C	PD110	42	M	CA	IDU	1a	2,465	152,545	−82,638
C	TN103	26	M	CA	IDU	1a	167	392,303	−6,127,204
C	TN104	47	M	AA	Sexual	1b	201	806,128	198146
C	TN105	30	M	AA	Sexual	1a	208	6147	−317,319
C	TN108	48	M	CA	Unknown	1a	68	1,445,738	300,074
R	HS101	41	M	CA	IDU	1a	222	615	−458
R	HS104	53	F	CA	Needlestick	1b	3,190	615	−5,318,310
R	HS112	24	M	CA	IDU	1a	950	615[c]	−198,799
R	PA101	36	F	CA	Surgery	ST1	1,890	615	0
R	PA103	25	F	CA	IDU	ST1	30	615	0
R	PD102	52	F	CA	Surgery	1a	292	615	0
R	PD103[b]	27	F	CA	IDU	1a	1,461	615	0
R	PD104[b]	27	F	CA	Needlestick	1a	NA	615	0
R	PD105	35	F	CA	IDU	ST1	58	615	−2,743
R	TN106	48	F	CA	IDU	1a	35	615	−5,184,984

[a] C, chronic; R, resolved; F, female; M, male; CA, Caucasian; AA, African American; IDU, intravenous drug use; ST, serotype; ALT, alanine aminotransferase; NA, not available.
[b] Positive RNA at month 2.
[c] Month 4 results.

classical HLA-E, which presents peptides derived from the leader sequence of other MHC class I molecules. The activating NKG2D recognizes class I-related molecules such as MICA/B (23). Natural cytotoxicity receptors (NCRs), including p30, p44, and p46, represent a family of orphan receptors that deliver activatory signals (27).

In the present study, we explore the hypothesis that the activation of peripheral CD56pos NT cell populations is impor-

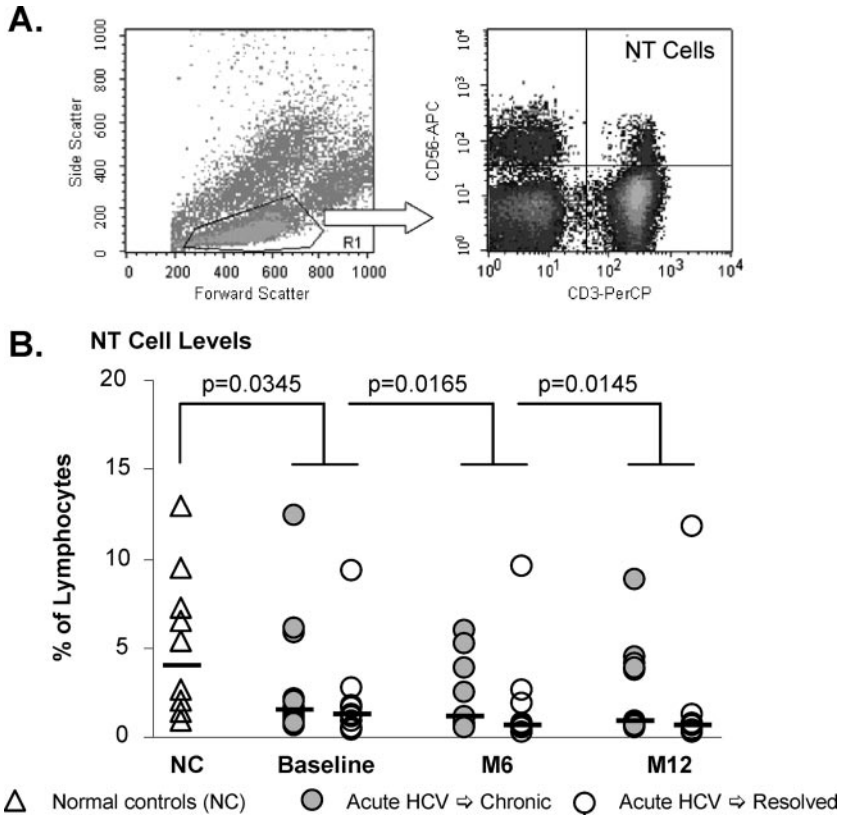

FIG. 1. Levels of CD56pos NT cells. Multiparameter flow cytometric analysis was used to determine if levels of peripheral $CD3^+$ $CD56^+$ NT cells (A) were altered in chronically evolving and/or spontaneously resolving acute HCV infection. An overall reduction of NT cells was observed in acute HCV infection per se compared to healthy controls (NC), which was sustained up to 1 year postenrollment (B). APC, allophycocyanin.

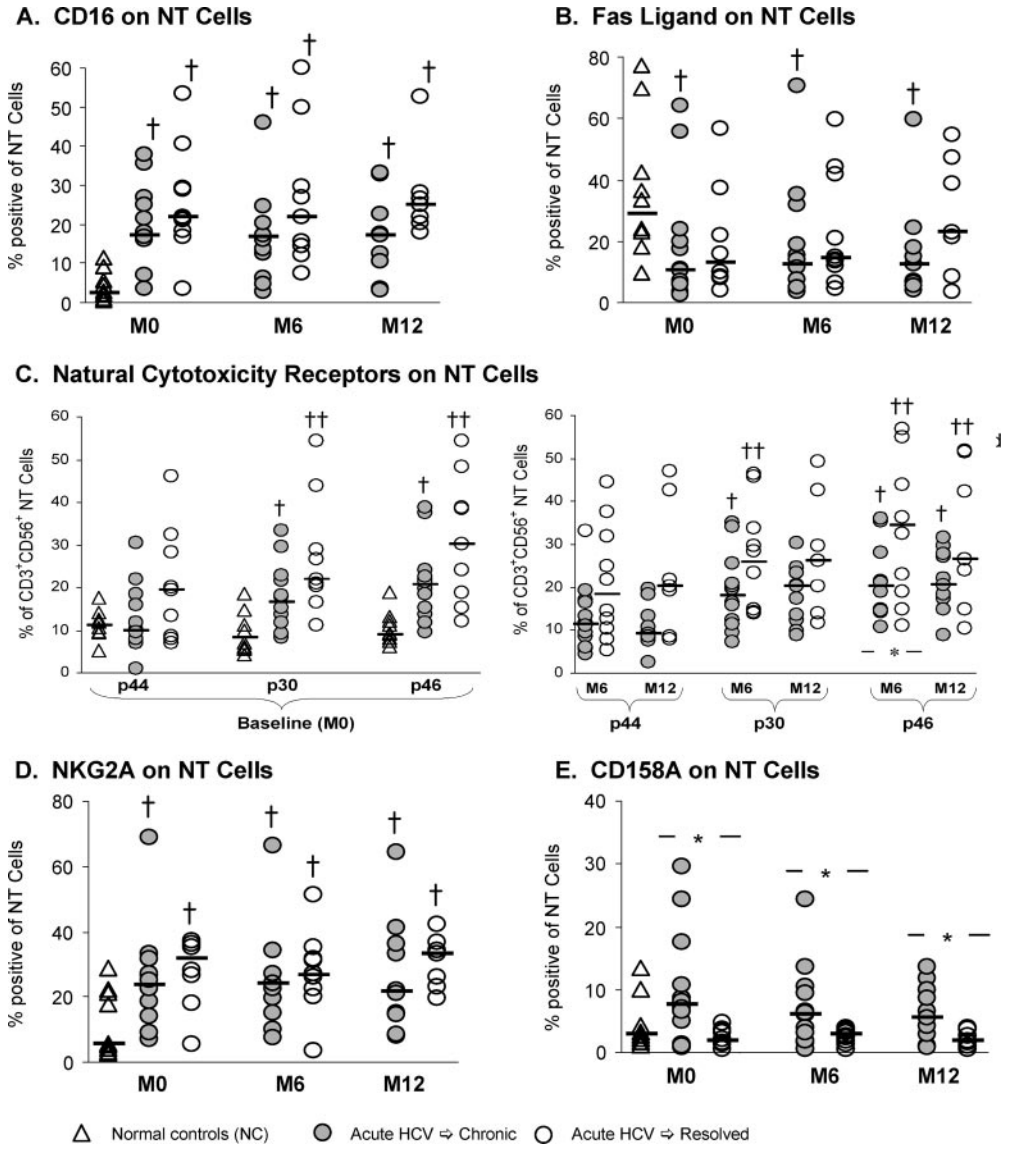

FIG. 2. CD56^{pos} NT cell phenotype in acute HCV infection. Up-regulation of CD16 (A), down-regulation of Fas ligand (B), and higher NCR (C) and NKG2A (D) expression on NTs are seen in acute HCV infection. CD158A is consistently lower in the resolved group (A). *, $P < 0.05$ (chronic versus resolved); †, $P < 0.05$ (versus control).

tant for the early control of HCV and predicts virologic outcome. Using a multiparameter approach, for the first time, we examine the levels, phenotype, and function of CD56^{pos} NT cells in a prospectively tracked cohort of acutely infected patients who developed viral persistence or spontaneous resolution.

MATERIALS AND METHODS

Study population. The study group was comprised of acutely HCV-infected patients recruited from multiple sites. The study protocol was approved by all appropriate institutional review boards. Patients were designated according to site of enrollment: Portland, OR; Harborview Seattle, Seattle, WA; Memphis, TN; and Pennsylvania. Acute HCV was diagnosed based on HCV antibody (Ab) seroconversion in a subject with previously negative HCV testing, seroconversion in a subject with new-onset risk factors and alanine aminotransferase levels 10-fold greater than normal, or HCV RNA positivity with HCV Ab negativity. Twenty-two treatment-naïve patients (12 males and 10 females) (mean age, 37 years) were selected from a larger cohort for the present study. The majority (90.9%) of patients were Caucasian. Spontaneous viral resolution ($n = 10$) and chronicity ($n = 12$) were defined as the absence or presence of HCV RNA at 6 months postenrollment with at least two viral determinations. The median estimated times from exposure to enrollment were 129.5 days (range, 39 to 224) for the chronic group and 125 days (range, 42 to 274) for the resolved group. The demographic details of the acutely infected cohort are described in Table 1. Ten healthy (HCV-unexposed) subjects served as controls. Controls comprised five males and five females; the mean age was 37 years (range, 20 to 53), and 90% were Caucasian. The healthy control group did not differ from the acutely infected cohort with respect to age, gender, or ethnicity.

Sample preparation and storage. Peripheral blood was drawn from the acutely infected cohort at baseline and 6 and 12 months later. Peripheral blood was drawn at a single time point from healthy controls ($n = 10$). Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll (Amersham Biosciences, Piscataway, NJ) density gradient centrifugation and cryopreserved (20% dimethyl sulfoxide in fetal bovine serum) for subsequent analyses.

Flow cytometric analysis of cell surface antigens. Four-color multiparameter flow cytometry was performed using a BD FACSCalibur instrument (BD Bio-

sciences) compensated with single fluorochromes and analyzed using CellQuest software (BD Biosciences). Fluorochrome-labeled (fluorescein isothiocyanate/phycoerythrin [PE]/peridinin-chlorophyll-protein complex (PerCP)/allophycocyanin) monoclonal Abs (MAbs) specific for CD3, CD4, CD8, CD56, CD161, CD94, CD16, CD158a, CD158b, CD158e (NKB1), and NKG2D were obtained from BD Biosciences. Anti-NKG2C-PE and TRAIL-PE MAbs were supplied by R&D Systems (Minneapolis, MN). Anti-NKG2A-PE, NKp30-PE, NKp44-PE, and NKp46-PE were obtained from Immunotech (Beckman Coulter, Fullerton, CA). PBMCs (2.5×10^5 cells) were stained for cell surface antigen expression at 4°C in the dark for 30 min.

Cytokine analysis. Antibodies for measurements of intracellular gamma interferon (IFN-γ), tumor necrosis factor alpha, interleukin-2 (IL-2), IL-10, and IL-13 were supplied by BD Pharmingen. Thawed PBMCs were stimulated with phorbol myristate acetate (10 ng/ml; Sigma-Aldrich) and ionomycin (1 μg/ml; Sigma-Aldrich) for 4 h at 37°C in the presence of brefeldin A (Sigma-Aldrich). After stimulation, cells were stained for surface antigens (as described above), fixed for 30 min at 4°C in 100 μl Fix and Perm medium A (Caltag, Burlingame, CA), permeabilized using 100 μl Fix and Perm medium B (Caltag), and incubated with anticytokine MAbs for 1 h. Cell suspensions were then washed in phosphate-buffered saline–bovine serum albumin–azide, fixed in 200 μl 1% paraformaldehyde, and acquired after 1 h. Cells cultured under the same conditions in the absence of phorbol myristate acetate and ionomycin served as controls.

Cytotoxicity assays. Thawed mononuclear cell suspensions were enriched for NK cells using the NK Isolation Kit II from Miltenyi Biotec (Gladbach, Germany) according to the manufacturer's instructions. Following isolation, the NK cells were cultured with or without IL-2 for 48 h at 37°C and 5% CO_2. Following culture, carboxy fluorescein succinimidyl ester (CFSE)-labeled target cells (K562s) were added to the NK cell populations at an effector-to-target concentration of 10:1 and incubated at 37°C for 4 h. After incubation, cytotoxicity was measured using the flow cytometry-based Total Cytotoxicity & Apoptosis Detection kit from Immunochemistry (Bloomington, MN). Immediately before acquisition, 7-aminoactinomycin D was added to effector/target populations and incubated for 15 min on ice.

Statistical analyses. Results are expressed as medians (ranges). A nonparametric Mann-Whitney U test was used to compare differences between patient groups and over time. Significance was defined as a P value of <0.05. The nonparametric Spearman test was used for correlation analysis. The JMP 6.0 (SAS Institute, Inc., Cary, NC) statistical package was used.

RESULTS AND DISCUSSION

CD56^{pos} NT cells are depleted in acute HCV infection. In the present study, we examined, for the first time, the potential role of CD56^{pos} NT (Fig. 1A) cells in determining the outcome of acute human HCV infection. Alterations in this population are evident even early in infection, many of which are independent of outcome, suggesting an involvement of NT cells in the host response to acute viral infection. In both the chronically evolving and spontaneously resolving patient groups, peripheral NT cell levels are reduced. The NT cell levels remain low in the acutely infected cohort at 6 and 12 months postenrollment, even in the absence of detectable circulating virus (Fig. 1B). This observation is consistent with previous studies demonstrating a reduction of the hepatic NT cell population in chronic HCV infection (9, 19); however, somewhat surprisingly, restoration of this population has not occurred in the resolved patient group up to 1 year postinfection.

NKR phenotype of NT cells in acutely infected patients. Chronic viral infections including HCV can modulate the expression of inhibitory and activating NKRs that fine-tune the activity of CD56$^+$ lymphocytes (6, 10, 11, 15, 17, 28, 29). However, little is known of these receptors in the acute HCV setting; we therefore examined the phenotype of NT cells in our patient cohort to determine whether altered NKR expression was associated with the outcome of acute HCV infection. CD16 was increased in NT cells in acute HCV infection (Fig. 2A), and Fas ligand was reduced (Fig. 2B), although statistical

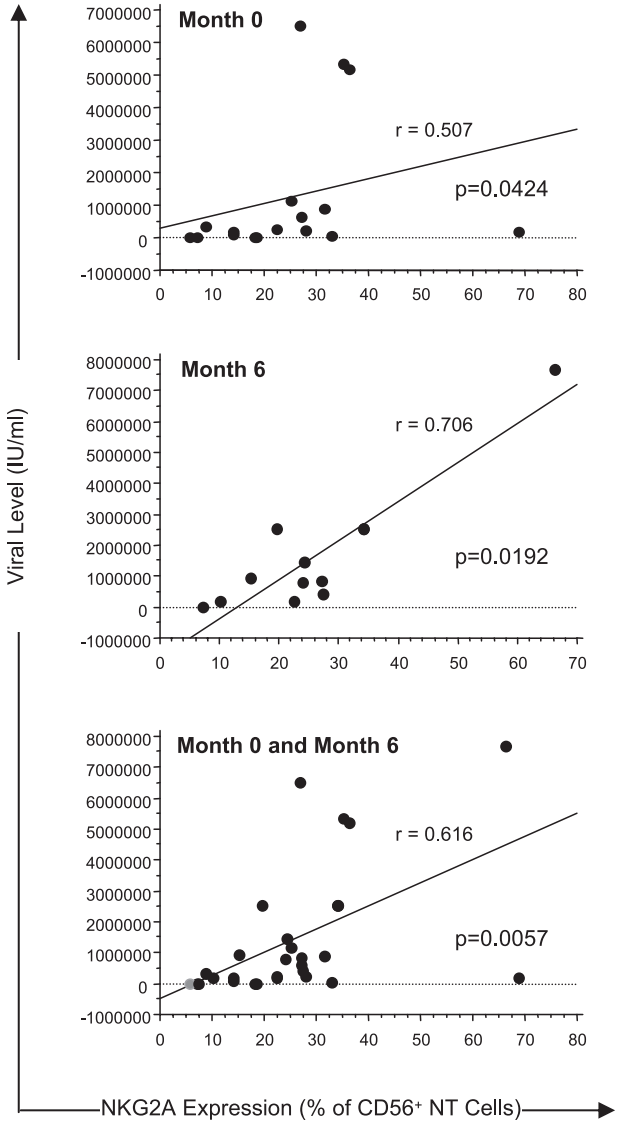

FIG. 3. Viral levels correlate with NKG2A expression on CD56^{pos} NT cells. Increased expression of NKG2A on CD56^{pos} NT cells did not correlate with outcome. However, there is a positive correlation between the proportion of NT cells coexpressing this inhibitory receptor and levels of circulating virus at baseline and 6 months later in patients with detectable HCV RNA. This suggests that HCV is directly involved in the up-regulation of NKG2A on NT cells.

significance was reached only for the chronically evolving patient group when analyzed separately. Expression of CD95 (Fas) was unchanged in acute HCV infection (data not shown). NCRs, p30 and p46, were up-regulated in acute HCV per se, with increased expression particularly evident in the spontaneously resolving group (Fig. 2C). The increased expression of NCRs on NT cells in acute infection contrasts with the previously reported observation that p30 and p46 are decreased on NK cells in chronic HCV infection (29). This may be due to the differential regulation of NCRs on NK and NT cells or, alternatively, to long-term exposure to the virus. However, another recent study demonstrated increased expression of these NCRs on chronically infected NK cells (10). Further studies are

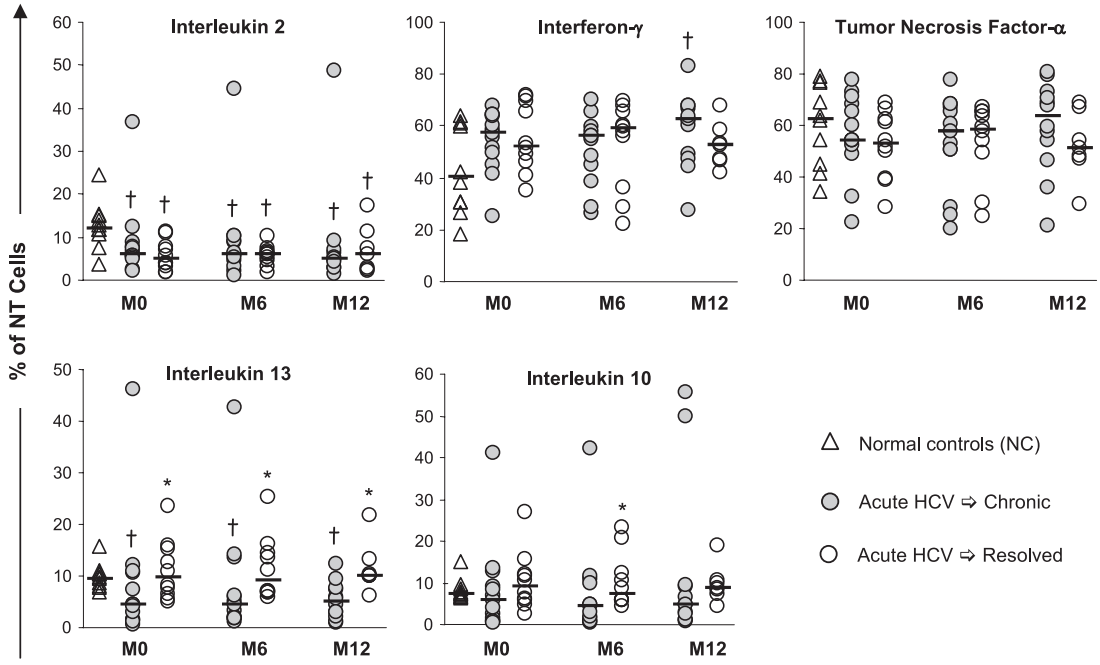

FIG. 4. Cytokine production by CD56^{pos} NT cells. At baseline, a lower percentage of NT cells from HCV-infected patients produces IL-2 than that of NT cells from healthy uninfected controls (NC) irrespective of outcome. The level of IL-2-producing NT cells remains low 6 and 12 months later even in the absence of detectable circulating virus. There is a trend towards increased IFN-γ-producing NT cells in early HCV infection; however, it is not until 1 year after enrollment that elevated numbers of NT cells produce IFN-γ in the chronic group compared to healthy controls, suggesting that longer-term exposure to virus induces IFN-γ production by NT cells. Tumor necrosis factor alpha production at baseline and over time remains unchanged. Of interest, IL-13-producing NT cells correlate with outcome at all time points tested. There is a deficiency in IL-13-producing NTs in the chronic patient group. At the 6-month time point, levels of IL-10-producing NT cells are significantly higher in the resolved patient group, which may reflect resolution of infection.

needed to resolve this question. The pattern of CD16, Fas ligand, and NCR expression on NT cells in both patient groups suggests virus-induced activation independent of the outcome.

As it is the integration of simultaneous activatory and inhibitory signals initiated through NKRs that influences the function of CD56^{pos} lymphocytes, we also looked at the expression of a number of inhibitory NKRs on NT cells in acute HCV infection. Inhibitory NKG2A is upregulated in acute HCV infection per se (Fig. 2D), an observation that has also been described previously for NK cells in chronic HCV infection (17, 29, 30). Of note, expression of stimulatory NKG2C, NKG2D, or CD161 molecules was not affected by HCV infection (data not shown). This may represent virally induced inhibition of NT cells (28, 37) or preferential survival of NT cells expressing NKG2A (6). Higher levels of the CD158A KIR family member, at all time points tested, including baseline, correlated with the development of chronicity (Fig. 2E), whereas the expression of other KIR family members (CD158B/E) was not significantly different than that of healthy controls (data not shown). Although the exact mechanisms governing the expression of NKRs on T cells is unknown, KIRs tend to be stably expressed (35); thus, a genetic predisposition towards increased CD158A expression may be involved in the susceptibility to acquiring HCV. Taken together, the NKR phenotypic profile of NT cells suggests the activation of this population in response to acute HCV infection with a more inhibited phenotype predicting the development of chronicity.

Next, we explored the relationship between NKR expression on CD56^{pos} NT cells and viral load, since the latter may be associated with the subsequent development of persistence or recovery. We therefore correlated the expression of NKRs (percent NT cell positive) with all patients who had a detectable viral level at both month 0 and month 6. With the exception of NKG2A, none of the receptors shown in Fig. 2 correlated, either positively or negatively, with viral load at either time point or taken together (data not shown). This lack of correlation may be due to the relatively small sample size. However, we did find a positive correlation between viral level and NKG2A expression on NT cells at both the 0-month and 6-month time points (Fig. 3). We showed in Fig. 2 that NKG2A was up-regulated on CD56^{pos} NT cells in acute HCV infection per se; thus, the positive correlation with viral load suggests that this increase is specific for HCV and not just a global activation phenomenon.

Functionality of CD56^{pos} NT cells in patients with acute HCV infection. HCV-induced inhibition of NT cell function is also evident in the present study. The preferential loss of IL-2-secreting CD4$^+$ T-helper cells in chronic HCV infection has been reported previously (34); here, we describe reduced numbers of IL-2-producing NT cells in acute HCV infection that are not restored up to 1 year after the disappearance of detectable circulating virus. However, this IL-2 defect does not correlate with the outcome. The only cytokine tested that consistently correlated with subsequent outcome was IL-13, which was impaired in the chronically evolving patient group compared to healthy uninfected controls and the spontaneously

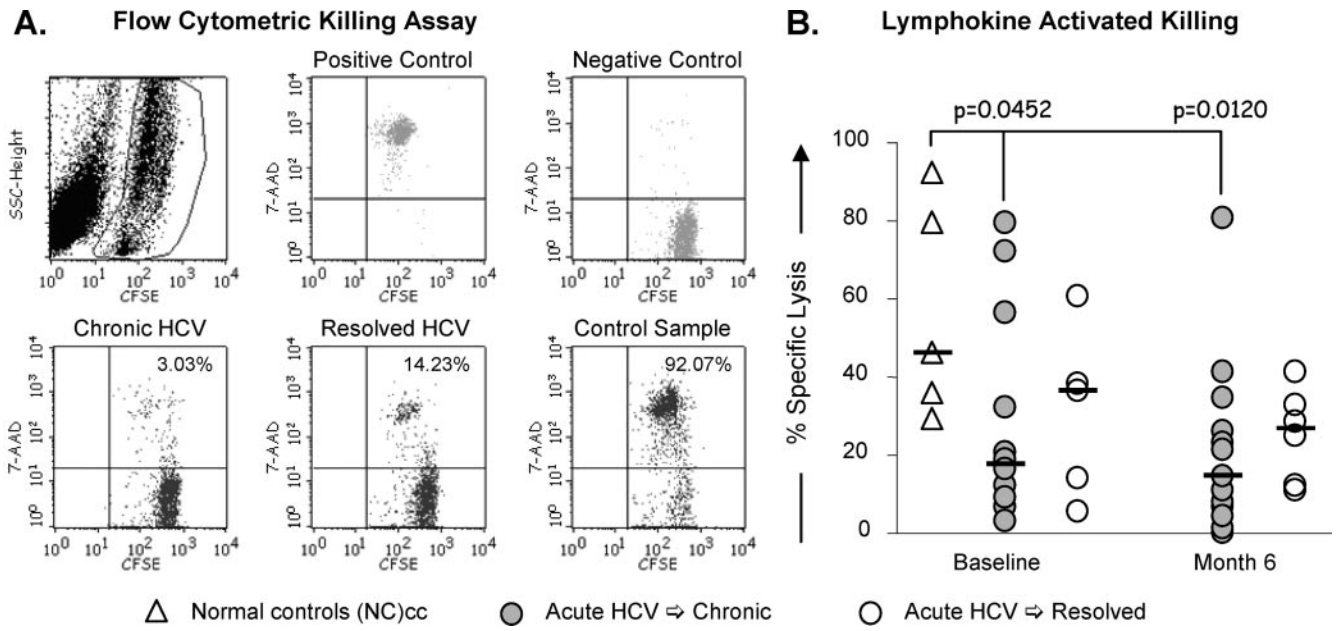

FIG. 5. Cytolytic activity of CD56pos lymphocytes is compromised early in chronically evolving acute HCV infection. The LAK activity of bead-enriched CD56pos lymphocytes after 48 h in culture with IL-2 was assessed against K562 target cells at a 10:1 effector-to-target ratio by flow cytometry. Panel A shows a representative assay including CFSE-labeled K562 target cells, positive and negative controls, and three patient samples, one from each study group. At baseline, depressed LAK activity compared to that of healthy uninfected controls correlated with subsequent chronicity. LAK activity remained significantly reduced in the acute HCV chronically evolving patient group at month 6 postenrollment. There is no statistically significant difference between the resolved and chronic acute HCV-infected patient groups (B). SSC, side scatter; 7-AAD, 7-aminoactinomycin D.

resolving patient group (Fig. 4). IL-13 has important proinflammatory functions including cellular recruitment (22) and has been shown to have direct antiviral effects and to be associated with improved disease status in human immunodeficiency virus infection (2), which may be related to the development of chronicity in acute HCV infection. At the 6-month time point, IL-10-producing NT cells in the resolved patient group were at levels comparable to those of controls and significantly higher than those of the chronic group, possibly reflecting the disappearance of detectable virus. IL-10-producing NT cells may therefore be involved in the resolution of self-limiting viral infection. As IL-10-producing NT cells are not increased in the chronic patient group and their levels do not correlate with viral load (data not shown), it is unlikely that this population is involved in the development of chronicity. While the results presented in Fig. 4 give us some insight into the levels of NT cells producing a range of cytokines, it should be noted that the methodology employed in this study does not quantify the amount of cytokine being produced but rather measures the frequency of cytokine-producing cells. It is conceivable that a smaller percentage of NT cells may produce higher quantities, and therefore, it would be of interest in future studies to quantify cytokine production by this population.

In addition to the secretion of cytokines, CD56pos T cells have potent lymphokine-activated MHC-unrestricted cytolytic effector functions (31, 33), likely important for early control of the virus (20). In support of this premise, our study demonstrated impaired LAK activity at baseline in the chronically evolving, but not the spontaneously resolving, patient group compared to healthy uninfected controls (Fig. 5). At 6 months postenrollment, depressed LAK activity was still evident in the chronically evolving patient group. Deficient IL-2-induced LAK activity has been associated with human immunodeficiency virus progression (5, 38), but its involvement in determining the outcome of HCV infection has not previously been reported. Impaired IL-2-mediated LAK predates the development of chronicity, indicating that the cytolytic activity of CD56pos NT cells may be an important host antiviral effector function for the resolution of HCV in the acute setting. In addition to lower-than-normal levels of IL-2-producing CD56pos NT cells in acute HCV infection per se, we demonstrate a reduced sensitivity, in the chronically evolving patient group, to the cytolytic activating properties of IL-2. It is reasonable to speculate that this would result in a profound cytolytic defect in vivo that promotes viral persistence and the development of chronicity. There is a trend toward lower LAK activity and higher viral load at baseline ($P = 0.0582$) but not at month 6 postenrollment (data not shown). This suggests that intact NT cell LAK activity early in infection is important for viral eradication or control; however, once the virus is replicating efficiently, NT cell LAK activity has little influence on viral level.

Collectively, our data indicate a previously unappreciated role for cytotoxic NT cells in the earliest stages of acute human HCV infection that determines the ultimate outcome. These results evoke additional interesting questions, which remain to be answered by future studies. Do specific HCV proteins mediate alterations in phenotype, function, or survival of NT cells? Does the NT cell population normalize in long-term

resolved patients and remain inhibited in long-term chronic patients? How do NT cells respond to interferon-based antiviral treatment? Because early inhibition of $CD56^{pos}$ NT cells predisposes individuals to the development of chronicity, this population represents a realistic therapeutic target that warrants further investigation and manipulation in HCV infection.

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We declare that we have no competing interests.

REFERENCES

1. **Alter, M. J.** 1997. Epidemiology of hepatitis C. Hepatology **26:**62S–65S.
2. **Bailer, R. T., A. Holloway, J. Sun, J. B. Margolick, M. Martin, J. Kostman, and L. J. Montaner.** 1999. IL-13 and IFN-gamma secretion by activated T cells in HIV-1 infection associated with viral suppression and a lack of disease progression. J. Immunol. **162:**7534–7542.
3. **Biron, C. A., K. B. Nguyen, and G. C. Pien.** 2002. Innate immune responses to LCMV infections: natural killer cells and cytokines. Curr. Top. Microbiol. Immunol. **263:**7–27.
4. **Bowen, D. G., and C. M. Walker.** 2005. Adaptive immune responses in acute and chronic hepatitis C virus infection. Nature **436:**946–952.
5. **Brenner, B. G., M. Gornitsky, and M. A. Wainberg.** 1994. Interleukin-2-inducible natural immune (lymphokine-activated killer cell) responses as a functional correlate of progression to AIDS. Clin. Diagn. Lab. Immunol. **1:**538–544.
6. **Byers, A. M., N. P. Andrews, and A. E. Lukacher.** 2006. CD94/NKG2A expression is associated with proliferative potential of CD8 T cells during persistent polyoma virus infection. J. Immunol. **176:**6121–6129.
7. **Cohen, J.** 1999. The scientific challenge of hepatitis C. Science **285:**26–30.
8. **Colonna, M., C. Dohring, J. Samaridis, D. Scheidegger, M. Dessing, M. Cella, and K. S. Campbell.** 1996. Human killer inhibitory receptors: specificity for HLA-class I molecules and mechanisms of signal transduction. Transpl. Proc. **28:**3035.
9. **Deignan, T., M. P. Curry, D. G. Doherty, L. Golden-Mason, Y. Volkov, S. Norris, N. Nolan, O. Traynor, G. McEntee, J. E. Hegarty, and C. O'Farrelly.** 2002. Decrease in hepatic CD56(+) T cells and V alpha 24(+) natural killer T cells in chronic hepatitis C viral infection. J. Hepatol. **37:**101–108.
10. **De Maria, A., M. Fogli, S. Mazza, M. Basso, A. Picciotto, P. Costa, S. Congia, M. C. Mingari, and L. Moretta.** 2007. Increased natural cytotoxicity receptor expression and relevant IL-10 production in NK cells from chronically infected viremic HCV patients. Eur. J. Immunol. **37:**445–455.
11. **De Maria, A., M. Fogli, P. Costa, G. Murdaca, F. Puppo, D. Mavilio, A. Moretta, and L. Moretta.** 2003. The impaired NK cell cytolytic function in viremic HIV-1 infection is associated with a reduced surface expression of natural cytotoxicity receptors (NKp46, NKp30 and NKp44). Eur. J. Immunol. **33:**2410–2418.
12. **Doherty, D. G., and C. O'Farrelly.** 2000. Innate and adaptive lymphoid cells in the human liver. Immunol. Rev. **174:**5–20.
13. **Durante-Mangoni, E., R. Wang, A. Shaulov, Q. He, I. Nasser, N. Afdhal, M. J. Koziel, and M. A. Exley.** 2004. Hepatic CD1d expression in hepatitis C virus infection and recognition by resident proinflammatory CD1d-reactive T cells. J. Immunol. **173:**2159–2166.
14. **Golden-Mason, L., and H. R. Rosen.** 2006. Natural killer cells: primary target for hepatitis C virus immune evasion strategies? Liver Transpl. **12:**363–372.
15. **Guma, M., C. Cabrera, I. Erkizia, M. Bofill, B. Clotet, L. Ruiz, and M. Lopez-Botet.** 2006. Human cytomegalovirus infection is associated with increased proportions of NK cells that express the CD94/NKG2C receptor in aviremic HIV-1-positive patients. J. Infect. Dis. **194:**38–41.
16. **Jacobs, R., K. Weber, K. Wendt, H. Heiken, and R. E. Schmidt.** 2004. Altered coexpression of lectin-like receptors CD94 and CD161 on NK and T cells in HIV patients. J. Clin. Immunol. **24:**281–286.
17. **Jinushi, M., T. Takehara, T. Tatsumi, T. Kanto, T. Miyagi, T. Suzuki, Y. Kanazawa, N. Hiramatsu, and N. Hayashi.** 2004. Negative regulation of NK cell activities by inhibitory receptor CD94/NKG2A leads to altered NK cell-induced modulation of dendritic cell functions in chronic hepatitis C virus infection. J. Immunol. **173:**6072–6081.
18. **Kanto, T., and N. Hayashi.** 2006. Immunopathogenesis of hepatitis C virus infection: multifaceted strategies subverting innate and adaptive immunity. Intern. Med. **45:**183–191.
19. **Kawarabayashi, N., S. Seki, K. Hatsuse, T. Ohkawa, Y. Koike, T. Aihara, Y. Habu, R. Nakagawa, K. Ami, H. Hiraide, and H. Mochizuki.** 2000. Decrease of CD56(+)T cells and natural killer cells in cirrhotic livers with hepatitis C may be involved in their susceptibility to hepatocellular carcinoma. Hepatology **32:**962–969.
20. **Kelly-Rogers, J., L. Madrigal-Estebas, T. O'Connor, and D. G. Doherty.** 2006. Activation-induced expression of CD56 by T cells is associated with a reprogramming of cytolytic activity and cytokine secretion profile in vitro. Hum. Immunol. **67:**863–873.
21. **Kronenberg, M.** 2005. Toward an understanding of NKT cell biology: progress and paradoxes. Annu. Rev. Immunol. **23:**877–900.
22. **Lama, V. N., H. Harada, L. N. Badri, A. Flint, C. M. Hogaboam, A. McKenzie, F. J. Martinez, G. B. Toews, B. B. Moore, and D. J. Pinsky.** 2006. Obligatory role for interleukin-13 in obstructive lesion development in airway allografts. Am. J. Pathol. **169:**47–60.
23. **Lanier, L. L.** 2005. NK cell recognition. Annu. Rev. Immunol. **23:**225–274.
24. **Lanier, L. L., B. Corliss, J. Wu, and J. H. Phillips.** 1998. Association of DAP12 with activating CD94/NKG2C NK cell receptors. Immunity **8:**693–701.
25. **Li, Y., T. Zhang, C. Ho, J. S. Orange, S. D. Douglas, and W. Z. Ho.** 2004. Natural killer cells inhibit hepatitis C virus expression. J. Leukoc. Biol. **76:**1171–1179.
26. **Mingari, M. C., G. Pietra, and L. Moretta.** 2005. Human cytolytic T lymphocytes expressing HLA class-I-specific inhibitory receptors. Curr. Opin. Immunol. **17:**312–319.
27. **Moretta, A., C. Bottino, M. Vitale, D. Pende, C. Cantoni, M. C. Mingari, R. Biassoni, and L. Moretta.** 2001. Activating receptors and coreceptors involved in human natural killer cell-mediated cytolysis. Annu. Rev. Immunol. **19:**197–223.
28. **Moser, J. M., J. Gibbs, P. E. Jensen, and A. E. Lukacher.** 2002. CD94-NKG2A receptors regulate antiviral CD8(+) T cell responses. Nat. Immunol. **3:**189–195.
29. **Nattermann, J., G. Feldmann, G. Ahlenstiel, B. Langhans, T. Sauerbruch, and U. Spengler.** 2006. Surface expression and cytolytic function of natural killer cell receptors is altered in chronic hepatitis C. Gut **55:**869–877.
30. **Nattermann, J., H. D. Nischalke, V. Hofmeister, G. Ahlenstiel, H. Zimmermann, L. Leifeld, E. H. Weiss, T. Sauerbruch, and U. Spengler.** 2005. The HLA-A2 restricted T cell epitope HCV core 35-44 stabilizes HLA-E expression and inhibits cytolysis mediated by natural killer cells. Am. J. Pathol. **166:**443–453.
31. **Ortaldo, J. R., R. T. Winkler-Pickett, H. Yagita, and H. A. Young.** 1991. Comparative studies of CD3− and CD3+ CD56+ cells: examination of morphology, functions, T cell receptor rearrangement, and pore-forming protein expression. Cell. Immunol. **136:**486–495.
32. **Palmieri, G., V. Tullio, A. Zingoni, M. Piccoli, L. Frati, M. Lopez-Botet, and A. Santoni.** 1999. CD94/NKG2-A inhibitory complex blocks CD16-triggered Syk and extracellular regulated kinase activation, leading to cytotoxic function of human NK cells. J. Immunol. **162:**7181–7188.
33. **Pittet, M. J., D. E. Speiser, D. Valmori, J. C. Cerottini, and P. Romero.** 2000. Cutting edge: cytolytic effector function in human circulating CD8+ T cells closely correlates with CD56 surface expression. J. Immunol. **164:**1148–1152.
34. **Semmo, N., C. L. Day, S. M. Ward, M. Lucas, G. Harcourt, A. Loughry, and P. Klenerman.** 2005. Preferential loss of IL-2-secreting CD4+ T helper cells in chronic HCV infection. Hepatology **41:**1019–1028.
35. **Shilling, H. G., N. Young, L. A. Guethlein, N. W. Cheng, C. M. Gardiner, D. Tyan, and P. Parham.** 2002. Genetic control of human NK cell repertoire. J. Immunol. **169:**239–247.
36. **Smyk-Pearson, S., I. A. Tester, D. Lezotte, A. W. Sasaki, D. M. Lewinsohn, and H. R. Rosen.** 2006. Differential antigenic hierarchy associated with spontaneous recovery from hepatitis C virus infection: implications for vaccine design. J. Infect. Dis. **194:**454–463.
37. **Takehara, T., and N. Hayashi.** 2005. Natural killer cells in hepatitis C virus infection: from innate immunity to adaptive immunity. Clin. Gastroenterol. Hepatol. **3:**S78–S81.
38. **Ullum, H., A. C. Lepri, H. Aladdin, T. Katzenstein, J. Victor, A. N. Phillips, J. Gerstoft, P. Skinhoj, and B. K. Pedersen.** 1999. Natural immunity and HIV disease progression. AIDS **13:**557–563.