Research Article

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Reduced frequencies of NKp30+NKp46+, CD161+, and NKG2D+ NK cells in acute HCV infection may predict viral clearance

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Background & Aims: While the majority of HCV-infected patients progress to chronic hepatitis, a small fraction of individuals are able to clear the virus. Resolution of infection occurs within the first few weeks to months of infection, suggesting that innate immune functions may be critical for early control. Epidemiologic data support a role for particular NK cell receptor bearing populations in this control, yet the mechanism by which NK cells respond to HCV early in infection is unknown.

Methods: Changes in the phenotype and function of NK cells were investigated in a cohort of 43 individuals identified during various stages of HCV infection with different clinical outcomes.

Results: Acute, chronic, and resolved HCV infections were characterized by an expansion of $CD56^{neg}$ NK cells. Furthermore, increased levels of HLA-C-binding KIR^+ NK cells were observed in HCV resolvers, while all stages of HCV infection were associated with reduced percentages of $NKG2D^+$, $NKp30^+$, and $NKp46^+$ NK cells, and a slight increase in the ability of NK cells to respond to target cells bearing the ligands for these receptors. In contrast, $NKG2A^+$ and $CD94^+$ NK cells were elevated in acute and chronic HCV infection, but not in resolved infection. Most importantly, in acute infection, lower frequencies of $NKp30^+$, $NKp46^+$, $CD161^+$, and $NKG2D^+$ NK cells were observed in patients who were subsequently able to clear HCV infection than in those becoming chronically infected.

Conclusions: These data implicate particular populations of NK cells in the early control and clearance of HCV infection.

Keywords: Hepatitis C virus; Natural killer cells; Innate immunity; CD161; NKp30; NKp46; Resolution of HCV infection; Acute infection.

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Abbreviations: HCV, hepatitis C virus; NK, natural killer; IFN-γ, interferon-gamma; KIR, killer cell immunoglobulin-like receptor; HLA, human leukocyte antigen; MHC, major histocompatibility complex; NCR, natural cytotoxicity receptor; DC, dendritic cell; RT-qPCR, quantitative reverse transcription polymerase chain reaction; PBMC, peripheral blood mononuclear cell; ADCC, antibody-dependent cellular cytotoxicity.

Introduction

Roughly 170 million people worldwide suffer from chronic hepatitis C virus (HCV) infection, a condition that results in liver cirrhosis or hepatocellular carcinoma in approximately 10–20% and 1–5% of cases, respectively [1]. One-fifth of *de novo* HCV infections are cleared during the acute phase; thus progression to chronic disease occurs in the majority of infected individuals [2]. Viral clearance is thought to rely largely on a broad, potent, and prolonged host cellular immune response [3–5]. Accordingly, defective T cell immunity is strongly associated with viral persistence [6].

In individuals that are able to clear HCV infection, viral control occurs within the first few months of infection, at a time when the adaptive immune response is just developing. Before the onset of the adaptive immune response, it is thought that innate immune effector cells, such as natural killer (NK) and NKT cells release interferon-gamma (IFN-γ), which is directly responsible for the non-cytopathic inhibition of HCV replication [7]. Besides producing inflammatory cytokines with antiviral activity, NK cells are also capable of eliminating infected cells without the need for prior antigen sensitization. Furthermore, epidemiological data suggest that particular NK cell receptor–ligand combinations are associated with the clearance of HCV infection, directly implicating these cells in the early control of HCV infection [8].

NK cell activation is tightly regulated by a balancing act of activating and inhibitory signals that are integrated in a complex network of receptors expressed on the cell surface. NK receptors include members of (i) the killer cell immunoglobulin-like receptor (KIR) superfamily that primarily recognizes certain allotypes of human leukocyte antigens (HLA)-A, -B, -C, and -G, (ii) the C-type lectin superfamily including the lectin-like heterodimers CD94–NKG2 recognizing the non-classical major histocompatibility complex class I (MHC-I) molecule

Table 1. Characteristics of HCV patients and healthy controls.

HCV stage	N	Age	Sex (M:F)	ALT	Viral load IU/ml	GT	Days PI	Risk	Outcome
A		25	F	148	16726	1b	65	surgery	R
A		58	M	53	UD	ND	80	surgery	R
A		44	M	22	UD	3 (sero)	230	sex	R
A		40	F	14	UD	1 (sero)	115	sex	R
A		13	M	125	5067	1a	70	ND	R
A		26	M	144	7553	1 (sero)	90	IDU	R
A		38	M	30	<615	ND	60	intranasal	R
A		29	F	175	UD	1	120	ND	R
A		31	M	1621	500000	1a	ND	IDU	R
A		30	M	336	363000	1a	200	IDU	C
A		34	F	39	2745	1	55	sex	C
A		54	M	1446	>700000	1	30	stick	C
A		20	M	39	280	1a	130	IDU	C
A		20	M	32	11500	1b	150	IDU	C
A		26	M	441	335000	1b	100	ND	C
A		62	F	91	850001	1a	60	Transfusion	C
A		54	F	733	5050	2	90	stick	C
A		38	M	253	130111	4	ND	IDU	C
A		26	M	957	627000	4a	ND	Shared razor	C
A		28	F	82	2680000	3	60	ND	C
Acute	20								
Median (range)		31 (13-62)	13:7	135 (14-1621)	9527 (0-2680000)		90 (30-230)		
Resolved	12								
Median (range)		46 (37-55)	4:5	22 (8-94)	0				
Chronic	11								
Median (range)		38 (24-76)	7:4	44 (15-79)	500000 (0-2900000)				
Negative	14								
Median (range)		26 (22-57)	4:10	ND	0				

A, acute; R, resolved; C, chronic; N, number of patients; M, male; F, female; ALT, alanine aminotransferase; IU, international units; UD, undetectable; GT, genotype; PI, post-infection; ND, not determined; IDU, injection drug user.

HLA-E and the activating NKG2D recognizing the MHC-I-related molecules MICA and MICB [9], and (iii) the natural cytotoxicity receptors (NCRs) that interact with particular viral proteins but whose cellular ligands remain largely undefined [10–12]. In addition, nearly 90% of peripheral NK cells also express the FcγRIIIa (CD16) receptor, involved in the recognition and lysis of antibody-coated cells [13]. NK cells can also modulate the quality of the adaptive immune response, mainly via their interaction with dendritic cells (DCs) [14]. Thus, NK cells play a critical role during the acute response to infection, including the early direct containment of viral replication and the initiation and maintenance of an effective adaptive immune response.

Given the pivotal role of NK cells in the host's immune response to viral infections and the fact that these cells are dramatically enriched in the liver compared to other tissues [15], numerous studies have investigated their importance in chronic HCV infection. One mechanism by which HCV establishes chronicity could involve the alteration of some important functions of NK cells very early in the course of the infection. This hypothesis is supported by mounting evidence demonstrating that patients with chronic HCV infection have altered NK cell subset distribution and/or NK cell receptor expression [16–25]; it is nevertheless less well understood whether altered NK cell phenotypic changes correlate with impaired NK cell function [16–27]. However, chronic HCV infection is associated with an increased number of NK cells bearing the inhibitory receptor CD94/NKG2A, a feature that has been proposed to result in NK cell dysfunction and impaired DC activation, potentially leading to viral persistence [20,23,25]. Whether alterations occur in the frequency of NCR-bearing NK cells in the context of chronic HCV infection is still a matter of debate [16,23].

Given that control of the infection occurs within the first weeks to months of infection, we hypothesized that distinct cellular immune responses are elicited during acute infection in individuals that go on to clear the infection. While the phenotypic and functional changes of NKT cells in acute HCV infection have been studied in detail [25], little is known regarding changes in the NK cell receptor expression profiles in the acute phase of the infection. In order to assess the importance of NK cell activation in acute HCV infection and to determine if altered patterns of NK cell receptor expression in the early stages of the infection is associated with the outcome of the disease, we performed a comprehensive phenotypic and functional analysis, including the extensive characterization of the repertoire of NK cell receptors in the context of acute, chronic, and resolved HCV infections. Our data show that acute HCV infection is associated with an expansion of NK cells with an altered phenotype but preserved