Natural Killer Cells Are Polarized Toward Cytotoxicity in Chronic Hepatitis C in an Interferon-Alfa–Dependent Manner

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BACKGROUND & AIMS: Patients with chronic hepatitis C virus (HCV) infection display great variability in disease activity and progression. Although virus-specific adaptive immune responses have been characterized extensively and found to be impaired in chronic hepatitis C, the role of innate immune responses in disease activity and progression of chronic hepatitis C is not well understood. **METHODS:** We studied 42 HCV-infected patients and 12 healthy uninfected controls. **RESULTS:** We found an increased frequency of natural killer (NK) cells expressing tumor necrosis factor–related apoptosis-inducing ligand (TRAIL), NKp44, NKG2C, and CD122 in chronic hepatitis C as compared with healthy controls ($P < .05$ for all markers) and stronger activation of NK cells in the liver than in the blood ($P < .05$). This NK cell phenotype was associated with polarization of NK cell function toward CD107a expression as a marker of degranulation, but with not increased interferon (IFN)-γ production of $CD56^{dim}$ NK cells. The polarized NK cell phenotype correlated with alanine aminotransferase levels ($r^2 = 0.312$, $P = .03$). To investigate whether in vivo exposure of NK cells to HCV-induced type I IFN was causing this NK cell phenotype, peripheral blood mononuclear cells from 10 healthy controls and 8 HCV-infected patients were stimulated in the presence of IFN-alfa, which resulted in increased NK cell expression of TRAIL and CD107a ($P < .001$), but not IFN-γ. **CONCLUSIONS: Collectively, these results describe a polarized NK cell phenotype induced by chronic exposure to HCV-induced IFN-alfa. This phenotype may contribute to liver injury through TRAIL expression and cytotoxicity, whereas the lacking increase in IFN-γ production may facilitate the inability to clear HCV.**

Infection with hepatitis C virus (HCV) results in viral persistence in about 70%–80% of cases, and is associated with chronic liver inflammation and an increased risk for cirrhosis and hepatocellular carcinoma. Liver injury and disease progression are thought to be driven by host immune responses.[1] However, the relative contributions of the adaptive and innate host immune responses and their respective effector functions have not been well defined.

Most studies on the immunology of hepatitis C have focused on the adaptive immune response. In acute self-limited hepatitis C, the HCV-specific CD4 and CD8 T-cell response can be vigorous, with more than 10% of all peripheral blood lymphocytes recognizing HCV antigens.[2,3] The recruitment of HCV-specific CD4 and CD8 T cells to the liver coincides with the onset of liver injury (as determined by increased alanine aminotransferase [ALT] levels, the decrease in HCV titer, and, ultimately, with HCV clearance. In chronic hepatitis C, HCV-specific T cells are described as ineffective and functionally impaired. First, HCV-specific T cells are present at very low frequency in both blood and liver of chronically HCV-infected patients, typically comprising less than 0.05% of all peripheral blood lymphocytes.[4–6] Second, they are terminally differentiated and do not proliferate well.[6] Third, they are impaired in their effector functions owing to up-regulation of inhibitory molecules, such as programmed death-1 and cytotoxic T-lymphocyte antigen 4.[7] This impaired T-cell phenotype is even more pronounced in the liver, the site of HCV replication, than in the blood. Fourth, HCV actively escapes from recognition of the few remaining, functional, HCV-specific T cells via mutations and quasispecies shifts.[8] These findings suggest that the adaptive immune response is not the sole or even the primary contributor to disease progression.

In contrast to T cells, the role of innate immune cells has not been studied extensively. This is a significant omission because the liver is selectively enriched in key components of the innate immune system. Natural killer (NK) cells, for example, represent only 5%–10% of peripheral blood lymphocytes, but about 30% of lymphocytes in the healthy human liver,[9] and an increase in the NK cell

Abbreviations used in this paper: IFN, interferon; IL, interleukin; IQR, interquartile range; KIR, killer cell immunoglobulin-like receptor; NK, natural killer; PBMCs, peripheral blood mononuclear cells; TRAIL, tumor necrosis factor–related apoptosis-inducing ligand.

0016-5085/10/$36.00
doi:10.1053/j.gastro.2009.08.066

frequency in the liver has been observed in mouse models of viral hepatitis.[10]

As opposed to T cells, NK cells do not require antigen-specific priming to recognize virus-infected cells. Once activated, NK cells exert cytotoxicity and produce antiviral cytokines, such as interferon (IFN)-γ and tumor necrosis factor-α, and chemokines, such as macrophage inflammatory protein 1 α and macrophage inflammatory protein 1 β. Thus, NK cells are not only in the right location but also are able to mount immediate effector responses to hepatotropic viruses. Indeed, this has been shown for inflammatory flares in patients with chronic hepatitis B virus infection, where NK cells contribute to liver injury via a tumor necrosis factor–related apoptosis-inducing ligand (TRAIL)-dependent mechanism.[11]

The overall role of NK cells in HCV infection, however, is not well understood. Immunogenetic studies suggest that distinct haplotypes of killer cell immunoglobulin-like receptors (KIRs) and their HLA ligands influence the outcome of acute and chronic HCV infection. Homozygosity for KIR2DL3 and HLA-C group 1 alleles, for example, is associated with an increased likelihood of HCV clearance,[12] whereas KIR2DS3 is associated with an increased risk of liver injury in chronic HCV infection.[13] These associations are probably the result of differential NK cell activation and function owing to KIR/HLA interaction. As shown in vitro, NK cells from subjects with KIR2DL3 and HLA-C group 1 compound genotype respond faster and more efficiently to influenza A virus infection than NK cells from subjects with less favorable genotypes.[14] Importantly, activated NK cells also have been shown to recognize and lyse HCV replicon-containing hepatoma cells in vitro[15] and therefore should be able to recognize and kill HCV-infected hepatocytes in vivo. However, published data are controversial in regards to up-regulation or down-regulation of specific NK cell markers and NK cell effector functions in HCV infection.[16–20] Furthermore, the mechanisms underlying these phenotypic and/or functional changes remain unknown. Although it has been proposed that individual HCV proteins alter NK cell functions based on in vitro assays,[21,22] it should be noted that HCV-infected hepatocytes do not secrete soluble viral proteins and that HCV particles with either genotype 1a or 2a envelope proteins do not directly modulate NK cell function.[23]

The current study describes the phenotype and function of NK cells in liver and blood of patients with chronic HCV infection. NK cells were activated in chronic hepatitis, but displayed a polarized phenotype with increased degranulation and expression of TRAIL, but not IFN-γ. This polarized phenotype correlated with markers of liver injury and was induced by exposure to IFN-alfa. Based on these results, we propose a model in which polarization of NK cell function by HCV-induced IFN-alfa contributes to both the progression of liver disease and the failure to clear HCV.

Materials and Methods

Study Cohort

All subjects gave written informed consent for research testing under protocols approved by the National Institute for Diabetes and Digestive and Kidney Diseases Institutional Review Board. Blood samples were drawn in citrate dextrose tubes from 42 patients with chronic HCV infection and from 12 healthy blood donors. Peripheral blood mononuclear cells (PBMCs) were separated on Ficoll-Histopaque (Mediatech, Manassas, VA) density gradients, washed 3 times with phosphate-buffered saline (PBS; Mediatech), and cryopreserved. Paired analysis of liver and blood NK cells was performed for 10 patients. After sufficient tissue was submitted for pathologic evaluation, a 5-mm segment was homogenized, washed in PBS, and resuspended in RPMI 1640 supplemented with 10% fetal bovine serum (US BioTechnologies, Pottstown, PA), 10 mmol/L HEPES, 100 IU/mL penicillin, 100 μg/mL streptomycin, and 2 mmol/L $_L$-glutamine (Mediatech) (complete medium). Liver-infiltrating lymphocytes were stained directly with the antibodies indicated later.

NK Cell Analysis

Frequency and phenotype. PBMCs and liver-infiltrating lymphocytes were stained with ethidium monoazide, anti–CD19-PeCy5 (BD Biosciences, San Jose, CA), anti–CD14-PeCy5 (Serotec, Raleigh, NC), and anti–CD3-AlexaFluor700 (BD Biosciences) to exclude dead cells, B cells, monocytes, and T cells, respectively, and with anti–CD56-PeCy7 (BD Biosciences) and anti–CD16-PacificBlue (BD Biosciences) to identify NK cells. Cells additionally were stained with either (1) anti–TRAIL-PE (BD Biosciences), (2) anti–CD122-fluorescein isothiocyanate and anti–NKG2C-PE (R&D Systems, Minneapolis, MN), (3) anti–NKp44-PE, (4) anti–NKp46-PE, (5) anti–NKG2A-PE, or (6) anti–NKG2D-PE (all from Beckman Coulter Inc, Miami, FL).

Degranulation. NK cell degranulation (ie, an increase in cell surface CD107a expression),[24] was assessed in response to major histocompatibility complex class I–negative K562 cells (ATCC, Manassas, VA). Specifically, cryopreserved PBMCs were thawed and cultured at 4×10^6 cells/mL at 37°C in complete medium without any exogenously added cytokines. After 14 hours, PBMCs were washed, resuspended at 10^6 cells/mL, and stimulated in the presence of anti-CD107a (15 μL/mL) with either (1) K562 cells at an effector to target ratio of 1:1, (2) interleukin (IL)-12 (0.5 ng/mL; R&D Systems) and IL-15 (20 ng/mL; R&D Systems), (3) K562 cells at an effector to target ratio of 1:1 in the presence of IL-12 (0.5 ng/mL; R&D Systems) and IL-15 (20 ng/mL; R&D Systems),[25] or (4) in complete medium without K562 and cytokines. After 6 hours at 37°C, PBMCs were washed, stained with the same ethidium monoazide/antibody