Antigen-Specific Natural Killer Cell Responses in Chronic Hepatitis C Virus Infection

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Abstract

Hepatitis C virus has infected 170 million people worldwide and is a leading cause of chronic viral hepatitis, liver cirrhosis and hepatocellular carcinoma. Chronic HCV infection results in an inflammatory liverdisease leading to fibrosis and cirrhosis. The progression of liver disease is thought to be immune-mediated because HCV itself is noncytopathic.

Given that HCV-specific T cells are diminished in number and functionally exhausted in chronic HCV infection, it remains unclear which cell population drives disease pathogenesis. Here, we investigated the function of natural killer (NK) cells, the major innate immune cell population in the liver. The NK cell population increases further in the setting of chronic hepatitis C infection. We investigated whether NK cells could respond to HCV in an antigen-specific manner. PBMCs from 39 patients with chronic HCV infection (genotype 1) not recently on antiviral medication (>2 years) were stimulated for 8 hours in a whole blood activation assay with pools of overlapping 18-mer peptides comprising HCV structural (E1, E2) and nonstructural (NS3) proteins. Cytokine production by NK cells and T cells was assessed by multicolor flow cytometry. Controls included stimulation of blood from HCV-uninfected individuals with HCV peptides and stimulation of blood from HCV-infected individuals with a pool of hepatitis D virus (HDV) peptides.

We found that the frequency of IFN-γ+ NK cells was 5-fold greater than the frequency of IFN-γ+ T cells. A minority of IFN-γ+ NK cells coproduced TNF-α. Frequencies of IFNγ+ NK cells stimulated with HCV E2 peptides ($p < 0.01$) and HCV NS3 peptides ($p < 0.05$) were statistically greater than the frequency of IFNγ+ NK cells stimulated with the HDV control peptide pool. NK cell responses to HCV peptides varied between subjects, but did not correlate with T cell responses or viremia.

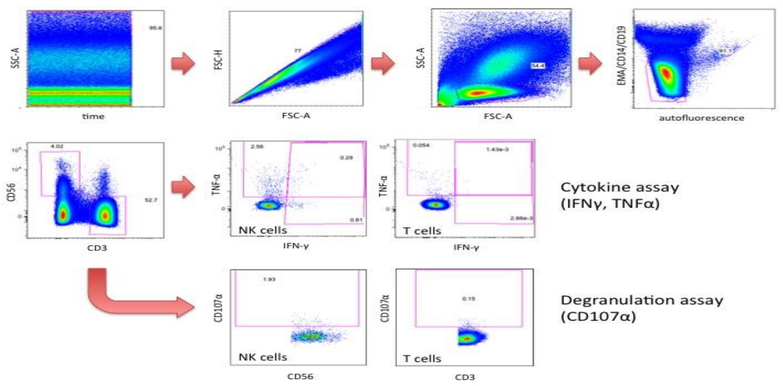
This study demonstrates that NK cells are activated in an antigen-specific manner in chronic HCV infection and respond to both structural and nonstructural HCV proteins. The frequency of cytokine-producing NK cells was greater than the frequency of cytokine-producing T cells in response to peptide stimulation of whole blood. The mechanism of antigen-specific NK cell activation requires further investigation.

Methods

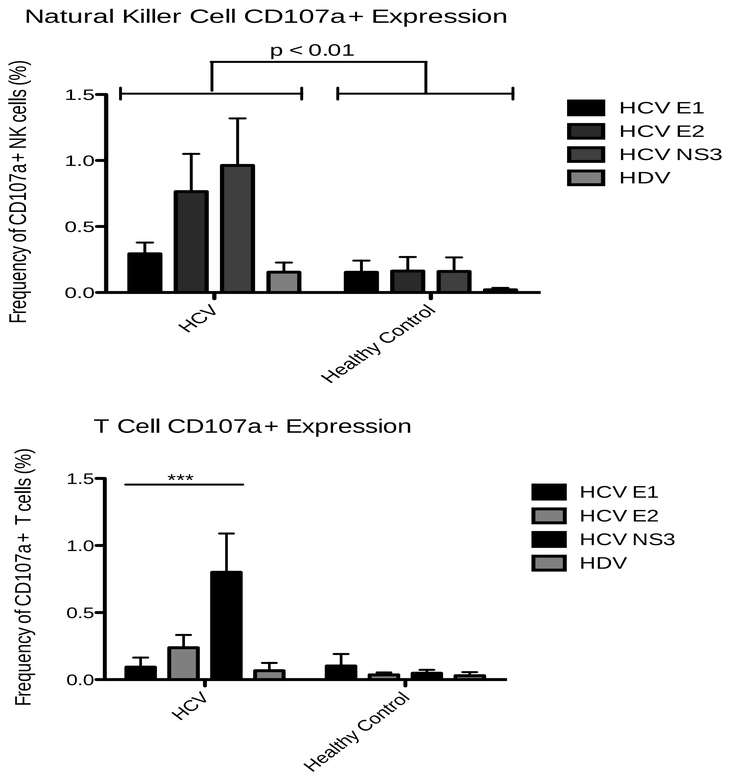
NK cell cytokine production and degranulaton were assessed using flow cytometry following 8 hour stimulations of whole blood from chronicaly HCV-infected individuals (genotype 1a or 1b) and from healthy blood donors.

•Heparinized whole blood was stimulated with pooled overlapping peptides (OLPs) comprising either E1, E2, or NS3 HCV peptides, HDV peptides, or no peptide. Cells were costimulated with CD28, CD49d, and brefeldin A. Monensin and CD107a APC were added in degranulation assays.

•After incubation, red cells were lysed and cells were surface stained with EMA, CD14, CD19, CD3, and CD56, followed by intracellular staining for IFNγ and TNFα (cytokine assay).



Degranulation of NK and T Cells after Stimulation with HCV E1, E2 and NS3 Peptides



CD107α expression was assessed by flow cytometry in NK and T cells of HCV-infected patients (n=10) and healthy blood donors (n=10). The frequency of CD107α+ NK cells was increased in HCV-infected samples over healthy volunteers after stimulation with structural (E1, E2) and nonstructural peptides (NS3), as shown by the significant variance ($p < 0.01$) between CD107a+ NK cell frequency in HCV+ samples and healthy controls. CD107α+ NK cell responses to HCV E1 and E2 peptide stimulation were larger than the corresponding CD107α+ T cell responses. NS3 stimulation resulted in the greatest degree of NK cell degranulation, although differential response to the individual HCV antigens varied among subjects (2 way ANOVA, Bonferroni posttest). The magnitude of the NK cell response did not correlate to ALT, viral load, or total serum IgG levels.

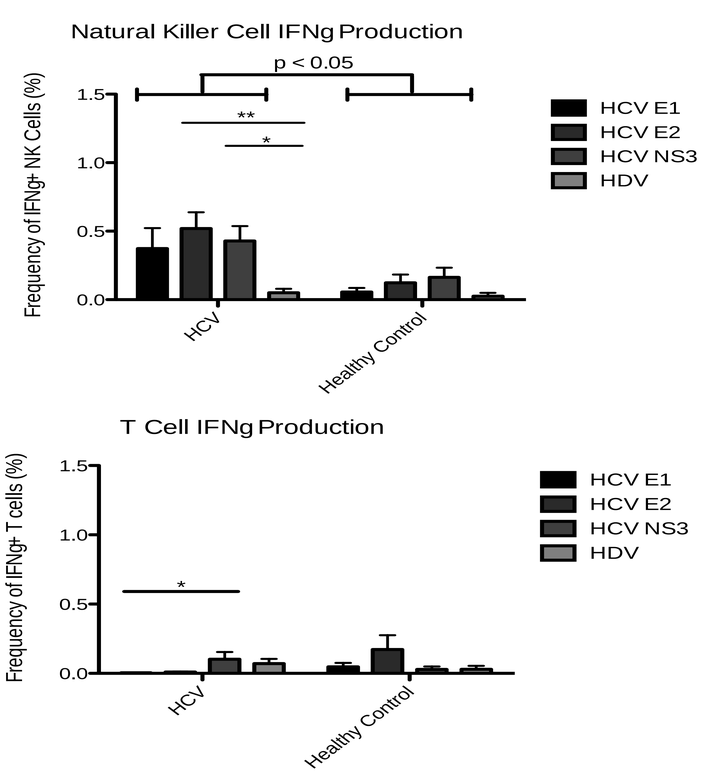
Patient Demographics

	Patients with chronic HCV
Subjects	39
Age (median years)	59
Gender (male : female)	28:11
HCV RNA (median copies/mL)	15,417,000 (869,400-67,770,000)
ALT (median IU/mL)	84 (15-610)
Serum IgG (median mg/dL)	1700 (918-2700)

Inclusion criteria:

- Chronic infection with HCV genotypes 1a or 1b
- No current or recent (>2 years) treatment for hepatitis C

Cytokine production of NK and T Cells after Stimulation with HCV E1, E2 and NS3 Peptides



IFN-γ and TNF-α expression was assessed by flow cytometry in NK and T cells of HCV-infected patients (n=27) and healthy blood donors (n=10). The frequencies of IFNγ+ NK cells stimulated with HCV E2 peptide ($p < 0.01$) and HCV NS3 peptide ($p < 0.05$) were statistically greater than the frequency of IFNγ+ NK cells stimulated with HDV peptides, suggesting antigen specificity of the NK cell response. The variance between IFNγ+ NK cell responses of HCV-infected individuals and healthy controls was also significant ($p< 0.05$), demonstrating that IFNγ production by HCV peptide-stimulated NK cells from HCV-infected patients is stronger than IFNγ production by NK cells from healthy controls.

Summary & Conclusions

1. Natural killer cells degranulate and produce cytokines in an antigen-specific manner in chronic HCV infection.
2. The NK cell response is greater than the corresponding T cell response and targeted against structural and nonstructural HCV antigens.

The results demonstrate that

- NK cells produce a robust and antigen-specific immune response in chronic hepatitis C infection.

1. Thobakgale CF, et al. 2012. Frequent and Strong Antibody-mediated Natural Killer Cell Activation in Response to HIV-I Env in Individuals with Chronic HIV-1 Infection. J. Virol. 86: 6986-6993.
2. Smalls-Mantey A, et al. 2012. Antibody-Dependent Cellular Cytotoxicity against Primary HIV-Infected CD4+ T cells Is Directly Associated with the Magnitude of Surface IgG Binding. J. Virol. 86: 8672-8680.