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Longitudinal analysis of peripheral and intrahepatic NK cells in chronic HCV patients during antiviral therapy

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ABSTRACT

Introduction: A strong immune response is integral to the clearance of HCV infection. NK cells are specialized cells that are able to inhibit replication of HCV in infected hepatocytes. Previous studies have correlated therapy outcome in HCV to the expression of various markers on NK cells. However, the effect of viral load reduction on NK cell function during therapy is still largely unknown, particularly in the liver. Therefore we investigated NK cell phenotype and effector function in both the peripheral and intrahepatic compartments during the course of antiviral therapy in chronic HCV patients.
Methods: Chronic HCV patients were treated for 24 or 48 weeks with triple therapy consisting of telaprevir, pegIFN-α and ribavirin. Blood and fine needle aspiration (FNA) biopsies of the liver were collected at start and 6 h, 1 week and 12 weeks during therapy. Flowcytometry was performed for expression of different markers (NKG2A, NKG2D, NKp46, and CD69).
Results: Our results demonstrate a highly activated phenotype of NK cells in liver compared to blood in chronic HCV patients. Six hours after start of triple therapy, no activation of intrahepatic NK cells was observed in the liver as compared to baseline. At 1 week after start of triple therapy, the frequency of NK cells with the activating receptor NKp46 was increased in blood, whereas at week 12, the frequencies of the inhibitory receptor NKG2A was increased. No alterations were observed during therapy in liver NK cell phenotype.
Conclusion: IFN-based therapy for chronic HCV affects NK cell phenotype in peripheral blood more than in the liver.

1. Introduction

Natural killer (NK) cells are crucial in the control and elimination of virally infected cells. Activated NK cells are able to kill virus-infected cells via cytotoxic molecules, such as perforin or granzymes. In addition, cytokines produced by NK cells (e.g. IFN-γ, TNF) lead to suppression of viral replication as well as activation of subsequent adaptive immune responses. The activation of NK cells results from expression of various activating and inhibitory receptors. NK cell receptors include C-type lectins for inhibitory (NKG2A) and activation (NKG2C/D) signals, as well as the natural cytotoxicity receptors (NCRs) NKp30 and NKp46 that deliver activation signals.

During chronic viral infections in human, such as hepatitis C virus (HCV) infection, blood NK cells have been shown to be altered as compared to NK cells from healthy individuals. These modulations may hamper efficient antiviral immune responses capable of eliminating the virus, and consequently may lead to viral persistence. NK cells from chronic HCV patients display a mildly augmented cytotoxic potential as compared to healthy individuals, whereas the ability of NK cells to produce IFN-γ is not or only weakly affected (Ahlenstiel et al., 2010; Oliviero et al., 2009). Oliviero demonstrated that NK cells from HCV patients display a more activated phenotype, and therefore possibly contribute to the immune responses involved to control viral persistence. However, compared to NK cells from healthy individuals, NK cells from chronic HCV patients show higher expression of the inhibitory receptor CD94/NKG2A and produce higher levels of the immunosuppressive cytokines IL-10 and TGF-β when cultured with hepatic cells (Jinushi et al., 2004), suggesting an inhibitory role.

Abbreviations: FNA, fine needle aspiration biopsy; CHCV, chronic hepatitis C virus; HIV, human immunodeficiency virus; NK, natural killer; AHCV, acute hepatitis virus; IFN, interferons; NCR, natural cytotoxicity receptor; pegIFN-2α, pegylated-interferon-alpha; PBMC, peripheral blood mononuclear cells.

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Table 1
Individual patient information.

	HCV-RNA (IU/ml)			ALT (IU/ml) baseline	Treatment response	Treatment history	Fibrosis
	Baseline	Week 4	Week 12				
E008	$1.5 * 10^6$	15	$1.0 * 10^6$	35	Non-response	Experienced	F0–F1
E010	$1.2 * 10^6$	15	15	42	Non-response	Experienced	F0–F1
E011	$3.2 * 10^6$	$2.9 * 10^4$	15	251	Non-response	Experienced	F0–F1
E014	$0.7 * 10^6$	15	15	39	SVR	Experienced	F0–F1
E016	$0.9 * 10^6$	15	15	51	SVR	Experienced	F4
E017	$5.0 * 10^6$	15	15	32	SVR	Naive	F2
E018	$3.8 * 10^6$	15	15	201	SVR	Experienced	F4
E019	$2.3 * 10^6$	15	15	85	SVR	Experienced	F0–F1
E020	$1.9 * 10^6$	$1.0 * 10^4$	–	40	Non-response	Naive	F0–F1
E022	$0.7 * 10^6$	15	15	131	SVR	Naive	F4

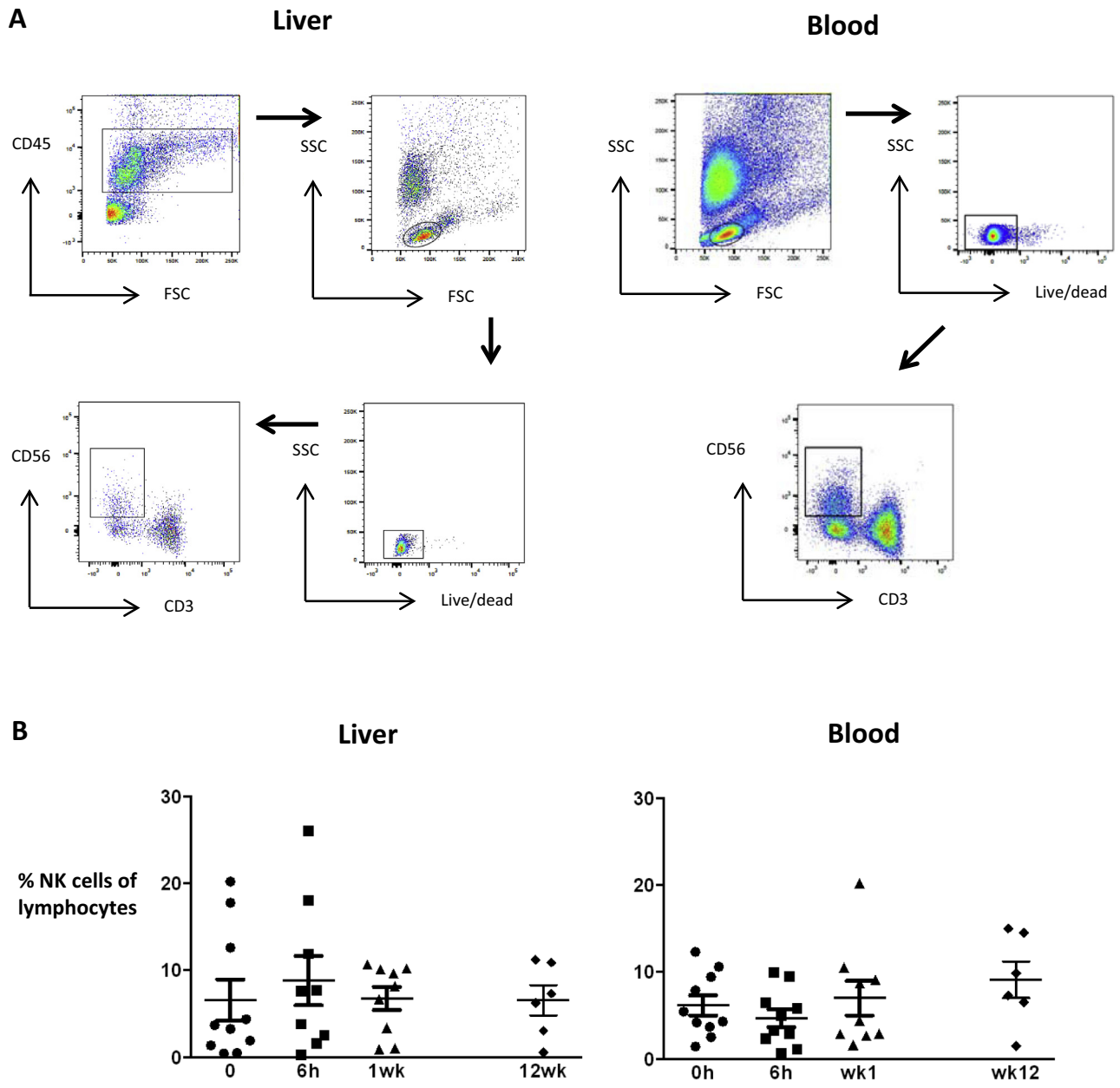

Fig. 1. The frequency of NK cells in the liver and blood of chronic HCV patients is not altered during triple therapy. The frequency of $CD56^+CD3^-$ NK cells was determined by flow cytometry within the population of $CD45^+$ leukocytes from fine needle aspirate biopsies of 10 patients. (A) Representative dot plots of liver and blood samples showing the gating strategy are depicted. (B) Data show the percentage of NK cells in each individual patient and the mean within the total lymphocyte population in liver and whole blood during triple therapy.