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Interleukin-7 augments CD8$^+$ T cells function and promotes viral clearance in chronic hepatitis C virus infection

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ABSTRACT

Interleukin (IL)-7 is a potent proliferation, activation, and survival cytokine for CD8$^+$ T cells to improve viral and tumor specific CD8$^+$ T cell responses. However, the role of IL-7 in regulation of dysfunctional hepatitis C virus (HCV)-specific CD8$^+$ T cells was not fully elucidated. Thus, a total of 53 patients with chronic hepatitis C and 24 healthy individuals were enrolled in the current study. Serum IL-7 and its receptor α chain CD127 expression was measured. The modulatory function of IL-7 to CD8$^+$ T cells was investigated in both direct and indirect contact co-culture with HCVcc-infected Huh7.5 cells. Both serum IL-7 and CD127 expression on CD8$^+$ T cells was significantly reduced in chronic HCV-infected patients, which was negatively correlated with HCV RNA. Stimulation of IL-7 promoted both cytotoxicity and cytokines (interferon-γ, tumor necrosis factor-α, and IL-2) production of CD8$^+$ T cells from patients with chronic hepatitis C. Moreover, IL-7 increased proliferation of CD8$^+$ T cells, while downregulated a critical repressor of cytokine signaling, suppressor of cytokine signaling 3 (SOCS3). The IL-7-mediated enhancement effects to CD8$^+$ T cells were dependent on IL-6 production. The current data suggested that IL-7 induced both cytolytic and noncytolytic functions of CD8$^+$ T cells probably via repression of SOCS3. IL-7 might be considered as one of the therapeutic candidates for treatment of chronic HCV infection.

1. Introduction

Chronic hepatitis C virus (HCV) infection is still an important public health problem, with more than 180 million infections worldwide [1]. Chronic hepatitis C not only results in end-stage liver diseases (*e.g.* decompensated cirrhosis, liver failure, and hepatocellular carcinoma), but also leads to several extrahepatic disorders (*e.g.* essential mixed cryoglobulinemia and porphyria cutanea tarda) [1,2]. Direct-acting antivirals (DAAs) have made chronic hepatitis C a curable condition with short duration and well-tolerance [3], however, there were still new issues existed, such as cardiac adverse reaction, unfavorable treatment characteristics, and immunotolerance [4]. Patients with chronic hepatitis C always exhibit dysfunction of HCV-specific CD4$^+$ and CD8$^+$ T cells activity and fail to generate sufficient antiviral immunity [5,6]. However, the precise mechanism associated with T cells hyporesponsiveness is still not fully elucidated due to host range limitations and lack of animal models.

Interleukin (IL)-7 is well accepted to play vital roles in T cells development, survival, proliferation, and activation. Signaling pathways through IL-7/IL-7 receptor contribute to promotion of T cell homeostasis [7–9]. Prolonged IL-7 signaling induced the proliferation of naïve CD8+ T cells and production of interferon (IFN)-γ, which triggered cell death. In contrast, intermit IL-7 signaling resulted in CD8$^+$ T cell homeostasis to promote cell survival *in vivo* [10]. Moreover, IL-7 has been demonstrated to be an important therapeutic cytokine in chronic viral infection and cancer [11]. Administration of IL-7 improved antiviral T cell function and facilitate viral clearance in lymphocytic choriomeningitis virus (LCMV) clone 13 infected mice, which was along with repression of suppressor of cytokine signaling 3 (SOCS3) and was dependent on endogenous IL-6 secretion [12]. However, a more recent study revealed that IL-7 binding to its receptor on the surface of CD8$^+$ T cells downregulated the expression of CD127 (IL-7 receptor α chain), and induced SOCS2 elevation through signal transducer and activator of transcription-5 (STAT-5) pathway [13]. Controversy remains as to the SOCS regulation by IL-7 signaling pathway in chronic viral infection. Previous study also indicated a

Abbreviations: ALT, alanine aminotransferase; CCK-8, Cell Counting Kit-8; DAAs, direct-acting antivirals; ELISA, enzyme-linked immunosorbent assay; HBV, hepatitis B virus; HCV, hepatitis C virus; HCVcc, HCV in cell culture; HIV, human immunodeficiency virus; IFN, interferon; IL, interleukin; LCMV, lymphocytic choriomeningitis virus; LDH, lactate dehydrogenase; PBMCs, peripheral blood mononuclear cells; PCR, polymerase chain reaction; SIV, simian immunodeficiency virus; SOCS, suppressor of cytokine signaling; STAT, signal transducer and activator of transcription; TNF-α, tumor necrosis factor-α

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remarkable downregulation of serum IL-7 in patients with chronic hepatitis C, while effective antiviral therapy to HCV RNA inhibition led to the elevation of IL-7 expression [14]. Thus, we hypothesized that IL-7 administration also induced viral clearance via regulation of $CD8^+$ T cells function in chronic HCV infection. To test this possibility, $CD8^+$ T cells purified from patients with chronic hepatitis C were stimulated with recombinant human IL-7, and were co-cultured with HCV-infected hepatocytes in direct or in indirect contact system *in vitro*. Both cytolytic and noncytolytic functions of $CD8^+$ T cells purified from chronic hepatitis C patients as well as HCV replication was assessed.

2. Materials and methods

2.1. Subjects

The study protocol was approved the Ethics Committee of Henan Provincial Hospital, and written informed consent form was obtained from each enrolled subject. A total of 53 patients with chronic hepatitis C were enrolled in this study. All patients were treatment-naïve, and hospitalized or followed-up in Department of Infectious Dieseases, Henan Provincial Hospital between July 2016 and December 2016. All patients were positive for both anti-HCV antibody and HCV RNA for more than six month, and negative for other hepatitis viruses, HIV, or bacterial infection. Patients who were co-afflicted with autoimmune diseases or cancers were also excluded for this study. Blood sampling were performed before usage of antiviral agents. For normal controls, twenty-four sex- and age-matched healthy individuals were also enrolled. The clinical characteristics of all enrolled subjects were shown in Table 1.

2.2. Virological assessments

HCV RNA was quantified using Real-time Polymerase Chain Reaction (PCR)-Fluorescence Quantitative Detection Kit (Da'an Gene, Guangzhou, Guangdong Province, China). The limitation of detection for HCV RNA was 2 $\log_{10}$copies/mL. HCV genotype was determined using Second-generation line probe assay (Inno-Lipa II, Innogenetics, Zwijindrecht, Belgium).

2.3. Peripheral blood mononuclear cells (PBMCs) isolation and $CD8^+$ T cells purification

PBMCs were isolated with density gradient centrifugation using Ficoll-Hypaque (Sigma-Aldrich, St. Louis, MO, USA). $CD8^+$ T cells were purified using a commercial Human $CD8^+$ T cells Isolation Kit (Miltenyi Biotech, Bergisch Gladbach, Germany) following instructions from manufacturer. The purity of enriched $CD8^+$ T cells was > 95% using flow cytometry determination.

2.4. Generation of HCV-infected Huh7.5 cells in vitro

Infectious HCV in cell culture (HCVcc) system [15] was obtained from Zhonghaichao Biotech (Kunming, Yunnan Province, China). The HCVcc copy was quantified using real-time PCR. Huh7.5 cells were lentivirally transduced to express HLA-A2, and were seeded into 24-well plates. 107 copies of HCVcc were added for a 5 h incubation. Infectious Huh7.5 cells were cultured for another 48 h, and harvested for further experiments.

Table 1
Clinical characteristics of enrolled subjects.

	Chronic hepatitis C	Healthy individuals
Cases (n)	53	24
Male/Female	30/23	15/9
ALT (IU/L)	32 [23, 74]	17 [11, 25]
HCV RNA ($\log_{10}$copies/mL)	6.17 ± 1.46	Not determined
Genotype (1b/2a/others)	28/17/8	Not determined

2.5. Direct and indirect contact co-culture systems

The direct and indirect contact co-culture systems, which were used to evaluate cytolytic and noncytolytic functions of $CD8^+$ T cells, were initially set up by Phillips et al. [16]. Briefly, 5×10^5 of $CD8^+$ T cells, which were purified from HLA-A2 restricted chronic hepatitis C patients, were co-cultured in direct contact and in indirect contact (effector and target cells were separated by 0.4 μm membrane in Transwell plates [Corning, NY, USA], which allowed the passage of soluble factors only) with 2×10^6 of HCVcc infected Huh7.5 cells in presence of HLA-A2 restricted peptide (HCV NS4B 1793–1801; sequence: SMMAFSAAL; final concentration 10 μg/mL) for 48 h. $CD8^+$ T cells were also treated with recombinant human IL-7 (Peprotech, Rocky Hill, NJ, USA; final concentration 5 ng/mL) in the presence or absence of anti-IL-6-IgG (a neutralizing monoclonal antibody against human IL-6, InvivoGen, San Diego, CA, USA; final concentration 20 ng/mL; Control antibody, mouse IgG, final concentration 20 ng/mL) for 6 h before co-culture. Cells and supernatants were harvested for further experiments.

2.6. Lactate dehydrogenase (LDH) assay

LDH expression in the supernatants of co-cultured systems were measured using LDH Cytotoxicity Assay Kit (Beyotime Biotech, Wuhan, Hubei Province, China) following instructions from manufacturer. The cytotoxicity of HCVcc-infected Huh7.5 cells were calculated as described previously [16]. Briefly, LDH expression in HCVcc-infected Huh7.5 cells were determined as low-level control, while LDH expression in Triton X-100 treated HCVcc-infected Huh7.5 cells were determined as high-level control. The resulting value corresponding to percentage of cell death was calculated in the following equation: (experimental value-low control)/(high control-low control) × 100%.

2.7. Enzyme-linked immunosorbent assay (ELISA)

Concentrations of IL-7, IFN-γ, tumor necrosis factor-α (TNF-α), IL-2, and IL-6 were measured using commercial ELISA Kits (R&D Systems, Minneapolis, MN, USA) following instructions from manufacturer.

2.8. Cellular proliferation assay

Cellular proliferation assay was performed using Cell Counting Kit-8 (CCK-8, Beyotime Biotech) according to instructions from manufacturer. OD_{450nm} reflected the proliferation of tested cells.

2.9. Western blot

$CD8^+$ T cells were lyzsed on ice for 5 min in 2 × SDS buffer containg β-mercaptoethanol, and heated at 95 °C for 10 min. The supernatants were harvested by centrifugation for at 12,000 × *g* for 1 min. Proteins were separated on SDS-PAGE gels followed by eletrotransfer onto a PVDF membrane. The membrane was soaked for 2 h in blocking solution (PBS containing 5% non-fat milk and 0.05% Tween 20), and incubated overnight in the presence of antibodies targeting total STAT5, phosphorylated STAT5, SOCS2, SOCS3, CD127 and GAPDH. All antibodies were purchased from Abcam (Cambridge, MA, USA), and were diluted with 1: 1000 in blocking solution before incubation. Horseradish peroxidase-conjuated antibody IgG (Abcam, 1: 2000 dilution) was added to the membrane for an additional 2 h incubation. Antigen-antibody complexes were observed using enhanced chemiluminescence (Western Blotting Luminol Reagent, Cell Signaling Technology, Danvers, MA, USA).