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Original Article

Comparison between interleukine-17 levels by NK-T cells in patients with chronic hepatitis C and healthy individuals

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Abstract

Background and Aims: Natural killer T (NKT) cells have been suggested to play critical roles in a wide range of immune responses especially against hepatotropic pathogens such as hepatitis C virus (HCV). In the present study, we investigated the status of NKT cells by producing interleukin-17 cytokine in peripheral blood.

Materials and Methods: As case-control study, 15 patients with chronic HCV infection and healthy individuals were enrolled in the study. We determined the serum and peripheral blood IL-17 levels after activating by based on Galactosylceramid and IL-2 then the IL-17 level was measured by Enzyme-linked immunosorbentassey (ELISA) methods.

Results: Plasma level of IL-17 in patients with chronic infection did not differ compared to the control group (5.5±2.4 vs. 6.2±1.2 pg/ml; p=0.5). The level of supernatant IL-17 was significantly higher in both Galactosylceramid-IL-2-stimulated PBMCs than control group and the ratio IL-17 after 72 h was higher than the other times. The plasma level of IL-17 in HCV 3a-infected patients was higher than 1a-type patients but the difference was not significant (6±2.5 vs. 4.8±2.5 p=0.45).

Conclusion: As the recent data, the roles of NKT cells in human liver injury and fibrosis remain unknown. However, the precise role of NKT cells in HCV patients as this play a role in innate immune responses in the liver in higher samples was performed.

Keywords: NKT cells, hepatitis C virus, Th17 cells

Introduction

Hepatitis C virus (HCV) is a hepatotropic positive-strand RNA virus that has chronically infected 170 million people of the world population (1-4). A significant excess of infected patients develop chronic hepatitis, cirrhosis, and liver functional defects (5). Approximately 80% of the patients fail to control the infection and develops a chronic infection (6). Interplay between immune response with HCV leads to disease outcomes such as fibrosis progression; therefore, characteristics of the immune response to HCV for identifying precise mechanism of HCV disease is crucial (7, 8).

Liver cells are major sites for HCV infection and distinctive immune organ with approximately dominant innate immunity; therefore, immune cells related to these cells could be interplay with this virus (9). Innate immune cells such as natural killer (NK) and natural killer T (NKT) cells are enriched in the liver and thymus, constitute around 30–50% and 5–20% of intrahepatic and peripheral blood lymphocytes, respectively (10, 11). These cells and related cytokines with those as biological mediators at different stages of liver disease could potentially play a role in HCV

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control and the pathogenesis of HCV-induced inflammatory liver disease (12, 13). They are usually expanded in chronic viral infections that NKT cells are restricted by CD1d and are TH-1-biased in HCV-infected livers. To better understand the potential role of NKT cells in the pathogenesis of HCV-induced hepatitis, must be identifying produced cytokines and chemokines by NKT cells (14, 15), which may exert direct antiviral effector functions and modulating the adaptive immune responses .

In addition to, involvement of the cytokines of these cells in pathogenesis of infectious disease, recent studies have shown that there are new subsets of NKT cells that can produce interleukin-17 (IL-17) and enriched in liver (16). Some studies have indicated the increased IL-17 mediator correlated with the degree of liver fibrosis. IL-17 is also associated with other inflammatory diseases such as bladder cancer, rheumatoid arthritis, and anti-tumor responses (17-19). Recent studies demonstrated that IL-17 acts as an interface between inflammatory responses and cell mediated immunity in cancer and infectious diseases. IL-17 by enhancing of inflammatory cytokines such as IL-6 and TNF-α and major role in recruitment of inflammatory cells such as neutrophil can promote the inflammatory reactions and fibrosis progression (19, 20). The control of inflammatory reactions and disease by NKT cells direct to production of the pro-inflammatory cytokines (21); IL-17, after the exposing of NKT cells with lipoid antigens such as α-Galactosylceramid, and, also direct death of infectious cells (22, 23). However, it is not clear whereas the number of data indicated the main role of NKT cells in liver fibrosis but other data, supporting role of these cells for preventing of liver inflammatory reactions .

According to contrasting data in previous studies for major role of NKT, as innate immunity major cells, in defense against viral infections and also chronic HCV infection outcomes, therefore, in line with previous studies, for the purpose of the present study, the culture supernatants was examined for plasma concentration of IL-17 in patients with chronic HCV infection and healthy individuals.

Methods

Study Population

Blood samples were taken from 15 patients with chronic HCV infection and 6 healthy individuals without history of liver disease. Written informed consent forms were obtained from all study subjects in the study that was approved by the Ethics Committee of Yazd University of Medical Sciences were signed by the patients between September 2012 and February 2013. The chronic HCV was diagnosed by elevated serum transaminase levels for at least six months and consistently detectable serum HCV RNA. All patients were anti-HCV positive and were negative for serological markers of HBV surface antigen (HBs-Ag) and anti-Human Immunodeficiency Virus (HIV-1,2). Unused of immune suppressing and HCV antiviral drugs was including criteria. Genotyping of HCV and HCV RNA titer was performed according to previous studies .(٢٤)

Isolation of PBMCs and serum separation

Five mL of peripheral blood was obtained in heparinated-containing tubes and PBMCs were isolated from buffy coats using Ficoll-Hypaque density gradient centrifugation (Pharmacia, Uppsala, Sweden). Samples were centrifuged at 3500 rpm for 20 minutes at 20°C without brake, and washed three times at 1800g for 5 min at 20°C with phosphate-buffered saline (pH = 7.3±0.1). The cell viability was examined by trepan-blue and cultured at the density of 1×105 cells/well. Four experimental groups were considered as follows: 1) PBMCs stimulated with IL-2 (5 ng/ml), 2) PBMCs stimulated with Galactosylceramid (100 ng/ml), 3) PBMCs stimulated with Galactosylceramid and IL-2, and 4) plasma alone. PBMCs suspensions and supernatant were harvested after 48 and 72 hours, and stored at −70°C for further analysis. In addition, 2 mL peripheral blood was collected for plasma separation, which were stored at -70°C until use for cytokine measurement .

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Enzyme-linked immunosorbent assay (ELISA)

The cytokine levels of IL-17 in culture supernatants and plasma were measured and quantitated by ELISA kits according to the manufacture's instruction (Mabtech,). Sensitivity for IL-17 was 2 pg/mL. The standard stocks were serially diluted in reagent diluents to generate seven points for the standard curves. The optical density of each well was immediately determined using a microplate reader set to 450 nm. The IL-17 levels were expressed in pg/mL.

Statistical Analysis

Data were analyzed using nonparametric Mann-Whitney U tests by SPSS software v. 15 (SPSS, Chicago, IL, 173 USA). The mean ± SD were determined, a T-test for comparison of means of different parameters was used. $p<0.05$ was regarded as significant in all statistical analysis.

Results

Table 1 demonstrates the distributionindicates the demographic, clinical, and laboratory characterization of patients and controls regarding different clinical and laboratory data criteria. The mean age was 32.1 ± 6 years, ranging between 21 to 43 years.

IL-17 cytokine level

The plasma level of IL-17 in patients with chronic HCV and normal controls was examined using ELISA method. Plasma level of IL-17 in patients with chronic infection did not differ compared to the control group (5.5±2.4 vs. 6.2±1.2 pg/ml; p=0.5). The effect of IL-2 on the synthesis of IL-17 by activated PBMCs of patients with chronic HCV and healthy controls was assessed by ELISA analysis of supernatants from PBMCs co-cultured with IL-2 and Galactosylceramid after 48 and 72 h (Table 2). The level of supernatant IL-17 was increased in the IL-2-treated PBMCs than control group but this difference was not statistically significant. On other hand, tThe supernatant IL-17 level in Galactosylceramid-stimulated PBMCs in chronic HCV patients produced more compared to healthy group that although the difference was not significant. Of interest, the level of supernatant IL-17 in supernatant was significantly increased in both Galactosylceramid- and IL-2-stimulated PBMCs in patients than control group that ratio IL-17 after 72 h was higher than other times . The plasma level of IL-17 in HCV 3a -infected patients was higher than 1a- type patients that this difference was not significant (6±2.5 vs. 4.8±2.5 p=0.45).

Table 1: Clinical and laboratory parameters in hepatitis C patients and control group.

Variables	Chronic Hepatitis C (n=15)	Control (n=6)	*p*
Sex			
Male	13	5	0.5
Female	2	1	
Viral titer (IU/mL)	1.4×10^6	-	
Genotypes			
1a	6	-	
3a	9	-	

Discussion

In this study, plasma and PBMCs levels of IL-17 in patients chronic HCV and healthy individuals were analyzed. In addition to previous studies, NKT cells activates by α-GalCer that has been shown to induce rapidly NKT cell activation and enhance the anti-tumor activity of NKT cells (22, 23). NKT cells are shown to play a role in chronic liver injury, inflammation, and fibrosis (25). Note the case, which liver is particularly enriched in NKT cells and in which are activated by hepatotropic viruses such as hepatitis C virus (HCV). It appears that the activation of NKT cells play an essential role in recruiting virus-specific T cells and in inducing antiviral immunity in liver (14, 21). Therefore the mediators related to NKT cells such as IL-17 could be involved with hepatitis infections (16, 18). Based on our findings the levels of plasma IL-17 in chronic HCV patients were higher than healthy individuals although the difference was not significant (17, 26-28). Of course this data was in accordant with previous

Table 2: Plasma and supernatant levels of IL-17 in chronic HCV patients and control group.

Variables	Chronic hepatitis C		Control	P value
IL-17 plasma level Mean±SEM	5.5±2.4		6.2±1.2	0.5
	48 h	72 h		
IL-17 Supernatant level stimulated with IL-2 Mean±SEM	0.27±0.1	0.3±0.15	0.1±0.18	0.24
IL-17 Supernatant level stimulated with Gal * Mean±SEM	0.15±0.03	0.4±0.14	0.11±0.08	0.16
IL-17 Supernatant level stimulated with both Gal *+IL-2 Mean±SEM	0.34±0.12	0.66±0.15	0.16±0.07	0.05

* Galactosylceramid

survey but in contrast to other studies. The limitations of recent results may be due to the low number of samples .

In addition to the effect of the IL-17 level in blood and also low half life for cytokine therefore the levels of the IL-17 indicated not better geed data. The activation of lymphocytes by the specific cytokines such as IL-2 may have contributed to the responses induced by lymphocytes because it appears that NKT cell responses may support T cell responses via their effect on antigen-presenting cells. Also acceleration of the NKT cells by α-GalCer with a specific effect on NKT cell could be shown the role of these cells in liver injury. Progression of liver fibrosis by increased liver injury may dominate over the effect of α-GalCer on liver fibrosis, leading to stimulatory effects of a single α-GalCer injection on liver fibrosis induced by NKT cells (16, 25). Little data is available on the role of NKT cells-related mediators in chronic HCV patients in Iran. The present study showed the increasing effect of α-GalCer along with IL-2 rather than alone conditions. This increasing level was found in chronic HCV patients than healthy individuals. This data demonstrate that the especially activation of NKT cells in HCV patients due to the involvement of these cells with secreting IL-17.

Conclusion

Our findings suggest that NKT cells may play a detrimental role in chronic HCV patients depending on the degree of NKT cell activation. During chronic liver injury, NKT cells may be playing a role in activating the early response with secreted IL-17 but for determine the role of the liver fibrosis is better the patients with disease different stage was include. Of course, reported that NKT cells increase in chronically infected livers and produce cytokines such as IFN-γ, IL-4 and IL-13, suggesting that NKT cells may contribute to the progression of liver fibrosis in patients with chronic hepatitis viral infection. Our study did not rule out the role of NKT cells in human liver injury and fibrosis, and further investigations are warranted regarding the role of NKT cells in patients infected with HCV.

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