Serum Profile of T Helper 1 and T Helper 2 Cytokines in Hepatitis C Virus Infected Patients

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ABSTRACT

Background: T-helper (Th) lymphocyte cytokine production may be important in the immune pathogenesis of hepatitis C virus (HCV) infections. Th1 cytokines such as; interleukin-2 (IL-2), and interferon gamma (IFN-gamma) are necessary for host antiviral immune responses, while Th2 cytokines (IL-4, IL-10) can inhibit the development of these effector mechanisms.

Objectives: The aim of the present study was to assess the serum profile of Th1 and Th2 cytokines in treated and non-treated HCV infected individuals.

Patients and Methods: This study was carried out in 63 HCV infected patients (31 under treatment and 32 untreated) and 32 matched HCV-sero negative healthy subjects. Serum samples were checked with an enzyme-linked immune sorbent assay (ELISA) for IL-2, IL-4, IL-10 and IFN-gamma.

Results: Levels of circulating IL-2, IL-4, IL-10 and IFN-gamma were significantly elevated in HCV patients versus normal controls (2 822.6 ± 1 259.9 pg/mL; 1 987 ± 900.6 pg/mL; 1 688.5 ± 1 405.1 pg/mL and 1 501.9 ± 1 298 vs. 950.8 ± 286.9 pg/mL; 895.9 ± 332.3 pg/mL; 519.0 ± 177.6 pg/mL and 501.9 ± 1 298 vs. 654.66 ± 71.59 pg/mL, respectively; P < 0.001). The serum levels of all cytokines were significantly lower in the patients under treatment than those of the untreated patients (P < 0.001).

Conclusions: On the basis of our data, the simultaneous increase of Th1 and Th2 related cytokines may indicate that both Th1 and HCV infected patients are involved in the pathogenesis of HCV infections. Moreover, this activated T-cell response in HCV infected patients may be regulated by treatment.

Implication for health policy/practice/research/medical education:
On the basis of our data, the simultaneous increase of Th1 and Th2 related cytokines may indicate that both Th1 and Th2 cytokines are involved in the pathogenesis of HCV infections.

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1. Background

The hepatitis C virus (HCV) is an etiologic agent responsible for parenterally transmitted hepatitis, infecting approximately 1% of the general population worldwide (1). The clinical course of a HCV infection is highly variable, from chronic infection in a majority of cases, to self-limited infection with loss of HCV-RNA in a minority of patients (2, 3). Although the mechanism of HCV infection outcomes is not well defined, it is believed that immunological mechanisms such as cytokine production are involved in HCV pathogenesis (4, 5). Cytokines serve as the immune response molecules which have various physiological functions and regulate the immunological, inflammatory and reparative host responses, and these are mainly secreted by lymphocytes and monocytes. T cell derived cytokines are important in the host immune response.

Activated T lymphocytes are divided into two functional subsets, Th1 and Th2 cells, on the basis of the cytokines that they produce (6). Th1 cytokines, including interleukin-2 (IL-2) and interferon-gamma (IFN-gamma), promote a cell-mediated immunity (CMI) response whereas Th2 cytokines including IL-4 and IL-10 are involved in antibody-mediated immunity. Th1 and Th2 responses have been shown to interact in a HCV infection (7, 8) and the imbalance between Th1 and Th2 responses favors humoral immune responses and down regulates cell mediated immunity, which is important for host defense against viral infections (9). Recent studies have demonstrated conflicting results on the levels of Th1 and Th2 cytokines in HCV infections (10-14). While some reports have demonstrated elevated levels of IL-2, IFN-gamma (11, 15), IL-4 and IL-10 (14, 16), others have reported no increase in the levels of Th1 (13, 17) and/or Th2 cytokines (15). Viral Therapy may be regulating an activated T-cell response in HCV infected patients and this creates a decreased viral load (11).

The most effective standard treatment in patients with chronic hepatitis C is a combination of pegylated interferon and ribavirin (18). The exact mechanisms by which interferon therapy alters the course of HCV disease have not been fully described. Atsukawa et al. (19) reported that interferon therapy polarizes the Th cell balance toward Th1 dominance and results in the reduction of Th2 cytokines, mainly IL-10. Th1 cytokines are required to eliminate HCV infected cells and impairment of these cytokines and increased levels of Th2 cytokines may be responsible for the chronicity of HCV infections (19). Therefore, the outcome of a HCV infection is related to the replication rate of the virus and the interactions between the virus and the host’s immune system (10). In addition, recent experimental studies have supported the role of immune response mechanisms in terminating HCV infections (13, 19). By further understanding the immunopathogenesis of HCV therapy, future strategies can be designed for improved HCV infection outcomes.

2. Objectives

The aim of the present study was to assess the serum profile of Th1 and Th2 cytokines (IL-2, IL-4, IL-10 and IFN-gamma) in treated and non-treated HCV infected individuals.

3. Patients and Methods

In this study, all 63 HCV infected patients who were referred to private clinics and hospitals of a central Iranian city, Arak, from January 2010 to January 2011, and 31 matched (age and sex) healthy subjects from the Arak Blood Transfusion Center, were enrolled. The 31 HCV infected patients were receiving combined pegylated interferon and ribavirin, and 32 cases did not receive any treatment. Cases and controls with the hepatitis B virus (HBV) and/or human immunodeficiency virus (HIV) infection were excluded from the study. Informed consent was obtained from all patients. A questionnaire was used to gather clinical and paraclinical data; alanine aminotransferase (ALT), viral load, and HCV genotype, and this was completed by clinicians. The project was approved by the Arak University of Medical Sciences’ Ethical Committee. Anti-HCV was tested by an enzyme-linked immune sorbent assay (ELISA) with a commercial enzyme immunoassay kit (Bio-Rad, Segrate, Italy). A recombinant immunoblot assay (RIBA Innogenetics, Gent, Belgium) was employed to confirm anti-HCV reactivity. All subjects were tested for IL-2, IL-4, IL-10 and IFN-gamma with an ELISA (Wuhan Boster Biological Technology, Ltd., Wuhan, China). The specificity of all kits was 100%. The sensitivity of IL-2, IL-4, IL-10 and IFN-gamma kits were < 1, < 1.5, < 0.5 and < 2 pg/ml respectively. Sampling and all assay protocols, cut-offs, and result interpretations were carried out according to the manufacturers’ instructions.

3.1. Statistical Analysis

The chi-square and T2 tests were calculated with the SPSS 16 package program for statistical analysis (Chicago, IL, USA). Multiple comparisons were carried out using an analysis of variance (ANOVA) test. The Spearman rank test was used for correlation. The significance level was set at P < 0.05. Data are presented as mean ± SD or, when indicated, as an absolute number and percentage. Unfortunately, some immunological indicators are not normally distributed, and this can minimally affect the results.

4. Results

A total of 63 HCV infected patients and 32 matched HCV-seronegative healthy subjects were enrolled in this study. The subjects included 31 HCV infected patients who were receiving treatment and 32 cases who did not receive any treatment. The mean age of the treated and untreated group was 33.47 ± 7.06 and 34.55 ± 8.9 years, respectively.

The possible routes of HCV transmission were inject-


Table 1. Comparison of Serum Cytokines in the Three Groups

| Group                      | IFN-gamma (pg/ml) | IL-2 (pg/ml) | IL-4 (pg/ml) | IL-10 (pg/ml) | P value |
|----------------------------|-------------------|--------------|--------------|---------------|---------|
| HCV treated patients, Mean ± SD | 784.81 ± 679.49   | 2026.9 ± 706.84 | 1512 ± 321.06 | 884.45 ± 119.93 | < 0.001 |
| HCV untreated patients, Mean ± SD  | 2196.5 ± 1382.21 | 3593.4 ± 1201.98 | 2447.2 ± 1038.5 | 2467.5 ± 1209.12 | < 0.001 |
| Controls, Mean ± SD            | 264.66 ± 71.59    | 950.81 ± 286.94 | 895.91 ± 332.33 | 519.03 ± 177.64 | < 0.001 |

Abbreviation: IFN, interferon; IL, interleukin; HCV, hepatitis C virus

Serum Cytokines in HCV Infected Patients

Sofian M et al.

Many studies have been conducted on the importance of viral immune responses, while Th2 cytokines can inhibit HCV infections. Th1 cytokines are necessary for host anti-infected patients. T-helper lymphocyte cytokine production may be important in the immune pathogenesis of HCV infections. Th1 cytokines are necessary for host antiviral immune responses, while Th2 cytokines can inhibit the development of these effector mechanisms (20). Many studies have been conducted on the importance of Th1/Th2 cytokine profiles in chronic HCV infections (1, 13, 15, 17, 21). There are conflicting data from these studies regarding the levels of Th1/Th2 cytokines in a HCV infection. Although in some surveys serum levels of Th1 cytokines, including IFN-gamma and IL-2 have been reported to be elevated in HCV infections (11), some others have shown low levels of IFN-gamma in patients with HCV infections (10). Napoli et al. (22) found that IFN-gamma and IL-2 mRNA were increased in the livers of patients with chronic HCV. They suggested that the role of Th1 cytokines is in mediating hepatocellular damage. Osna et al. (13) showed lower IFN-gamma and higher IL-10 levels in chronic HCV patients, than in healthy controls. Abayli et al. (10) also revealed an enhanced Th2 response during chronic HCV infections. A study by Chen et al. (1) reported that serum levels of IL-4 and IL-10 were significantly higher in HCV patients than in the controls. Another survey by Fan et al. (12) showed that IL-2, IL-4 and IL-10 levels were significantly increased in HCV infected hosts when compared to normal controls, but the production of Th2 cytokines was more predominant. Reiser et al. (21) demonstrated elevated serum IL-4 and IL-10 levels in patients with chronic HCV infection. Cacciarelli et al. (11) showed that levels of circulating IL-2, IL-4, IL-10, and IFN-gamma were significantly elevated in HCV patients versus normal controls and that treatment with IFN-alpha decreased the levels of IL-4, and IL-10. Another study also showed that the levels of Th2 cytokines (IL-4 and IL-10) were significantly increased in chronic HCV infected patients, compared with normal controls (23). The other investigation reported that cytokine levels in HCV patients were similar to levels observed in healthy volunteers. During IFN-alpha and ribavirin therapy no statistically significant changes in cytokine levels were observed in patients who achieved a sustained virological response, compared to unsuccessfully treated patients (24). The discrepancy between these studies may be due to epidemiological and geographic variations such as; small sample sizes, ethnic differences, comorbid conditions and composition of the study populations. Our survey showed elevated levels of Th1 and Th2 cytokines among HCV infected individuals. We did not find a Th1 to Th2 shift in these patients. Our results are in agreement with studies by Cacciarelli et al. (11) and Fan et al. (12). In the present study, decreased cytokine levels were demonstrated in patients under treatment. Cacciarelli et al. (11) also showed a trend toward decreased levels of cytokines during therapy. The limitations of our
study are the small sample size and conducting a cross-sectional study instead of a longitudinal study. We also acknowledge the lack of detailed clinical histories and pathological records as a limitation. In conclusion, on the basis of our data, the simultaneous increase of Th1 and Th2 related cytokines may indicate that both Th1 and Th2 cytokines are involved in the pathogenesis of HCV infections. In addition, this activated T-cell response in HCV infected patients could be regulated by treatment. Our data provides some additional evidence for the involvement of an immune cellular immune response in terminating HCV infections. However, further studies, including longitudinal studies as well as a larger population sample, are necessary to confirm our findings.

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Authors’ Contribution
All authors were contributed in writing the manuscript.

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References
1. Chen TY, Hsieh YS, Wu TT, Yang SF, Wu CJ, Tsay GJ, et al. Impact of serum levels and gene polymorphism of cytokines on chronic hepatitis C infection. Transf Res. 2007;150(2):216-21.
2. Barrett S, Gohl J, Coughlan R, Ryan E, Stewart S, Cockram A, et al. The natural course of hepatitis C virus infection after 22 years in a unique homogeneous cohort: spontaneous viral clearance and chronic HCV infection. Gut. 2000;49(3):423-30.
3. Zucker SD. Whomsoever ignores the natural history of the hepatitis C virus is doomed to treat it. Gastroenterology. 2002;122(2):578-9.
4. Powell EE, Edwards-Smith CJ, Hay JL, Clouston AD, Crawford DH, Shorthouse C, et al. Host genetic factors influence disease progression in chronic hepatitis C. Hepatology. 2000;31(4):828-33.
5. Tsutsumi T, Suzuki T, Shimoike T, Suzuki R, Moriya K, Shintani Y, et al. Interaction of hepatitis C virus core protein with retinoid X receptor alpha modulates its transcriptional activity. Hepatology. 2002;35(4):937-46.
6. Koziel MJ, Dudley D, Afshah N, Grakoui A, Rice CM, Choo QL, et al. HLA class I-restricted cytotoxic T lymphocytes specific for hepatitis C virus. Identification of multiple epitopes and characterization of patterns of cytokine release. J Clin Invest. 1999;104(5):2311-21.
7. Fishman MA, Perelson AS. Th1/Th2 cross regulation. J Theor Biol. 1994;170(4):25-56.
8. Pernis A, Gupta S, Gollob KJ, Garfein E, Coffman RL, Schindler C, et al. Lack of interferon gamma receptor beta chain and the prevention of interferon gamma signaling in TH1 cells. Science. 1995;269(5221):245-7.
9. Sher A, Gazzinelli RT, Oswald IP, Clerici M, Kullberg BG, Pearce EF, et al. Role of T-cell derived cytokines in the downregulation of immune responses in parasitic and retroviral infection. Immunol Rev. 1992;127:193-204.
10. Abayli B, Canataroglu A, Akkiz H. Serum profile of T helper 1 and T helper 2 cytokines in patients with chronic hepatitis C virus infection. Turk J Gastroenterol. 2003;4(3):7-11.
11. Cacciarelli TV, Martinez OM, Gish RG, Villanueva JC, Krams SM. Immunoregulatory cytokines in chronic hepatitis C virus infection: pre- and posttreatment with interferon alfa. Hepatology. 1996;24(1):6-9.
12. Fan XG, Liu WE, Li CZ, Wang ZC, Luo LX, Tan DM, et al. Circulating Th1 and Th2 cytokines in patients with hepatitis C virus infection. Mediators Inflamm. 1998;7(4):295-7.
13. Osna N, Silinova G, Vilgert N, Hagina E, Ruse V, Giedraitis V, et al. Chronic hepatitis C: Thelper1/T-helper2 imbalance could cause virus persistence in peripheral blood. Scand J Clin Lab Invest. 1997;57(8):703-40.
14. Shapiro S, Gershstein V, Elias N, Zuckerman E, Saliman N, Lahat N. mRNA cytokine profile in peripheral blood cells from chronic hepatitis C virus (HCV)-infected patients: effects of interferon-alpha (IFN-alpha) treatment. Clin Exp Immunol. 1998;114(3):55-60.
15. Cribier B, Schmitt C, Rey D, Lang JM, Kinn A, Stoll-Keller F. Production of cytokines in patients infected by hepatitis C virus. J Med Virol. 1998;55(3):389-91.
16. Lucey DR, Clerici M, Shearer GM. Type 1 and type 2 cytokine dysregulation in human infectious, neoplastic, and inflammatory diseases. Clin Microbiol Rev. 1996;9(4):342-62.
17. Malaguarnera M, Di Fazio I, Laurino A, Pistone G, Restuccia S, Trovato BA. Decrease of interferon gamma serum levels in patients with chronic hepatitis C. Gastroenterology. 2000;120(4):828-33.
18. Dogra G, Chakravarti A, Kar P, Chawla YK. Polymorphism of tumor necrosis factor-alpha and interleukin-10 gene promoter region in chronic hepatitis C virus patients and their effect on pegylated interferon-alpha therapy response. Hum Immunol. 2001;72(5):395-9.
19. Atsukawa M, Nakatsu K, Kobayashi T, Shimizu M, Tamura H, Harimoto H, et al. Ribavirin downmodulates inducible co-stimulator on CD4+ T cells and their interleukin-10 secretion to assist in hepatitis C virus clearance. J Gastroenterol Hepatol. 2012;27(4):829-31.
20. Ferrari C, Urbani S, Penna A, Cavalli A, Valli A, Lamonaca V, et al. Immunopathogenesis of hepatitis C virus infection. J Hepatol. 1999;31(Suppl 1):S3-8.
21. Reiser M, Marousis CG, Nelson DR, Lauer G, Gonzalez-Peralta RP, Davis GL, et al. Serum interleukin 4 and interleukin 10 levels in patients with chronic hepatitis C virus infection. J Hepatol. 1997;26(3):478-81.
22. Napoli J, Bishop GA, McQuiness P, et al. Increased intrahepatic TH1 cytokine mRNA levels correlate with tissue damage in chronic hepatitis C. Hepatology. 1995;22:2222.
23. Fan X, Liu W, Li C, Fan X, Liu W, Li C. Determination of serum cytokines in individuals with HCV infection. Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi. 2000;14(2):145-7.
24. Inglot M, Gladysz A, Rymer W, Molin I, Zalewska M, Machaj A. Cytokine assessment in untreated hepatitis C virus infected patients and during interferon alpha +ribavirin therapy. Wind Lek. 2006;61(3):318-8.