Cytokine Patterns Correlate with Liver Damage in Patients with Chronic Hepatitis B and C

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Abstract. T-cell immunoregulatory cytokines influence the persistence of hepatitis C virus (HCV) chronic infection and the extent of liver damage. Th1 cytokines positively correlate with hepatic inflammation in chronic hepatitis B virus (HBV) infection. The pro-inflammatory cytokines IL-6 and IL-18, are involved in viral clearance and in metabolic and viral hepatic diseases, respectively. The aim of this study was to evaluate the profile of Th1/Th2 cytokines in HCV and HBV hepatitis. HBV-infected patients showed higher plasma IFN-γ levels than the HCV+ patients or the control group (p <0.0001). Plasma TNF-α and IL-2 were higher in HBV+ in comparison to HCV+ patients (p <0.001) or the control group (p <0.005). Plasma IL-6 and IL-18 were higher in both groups of patients compared to the control group (p <0.04). In HCV+ and HBV+ groups, IL-6 was positively correlated with the duration of the illness (p <0.01 and <0.001, respectively) and viral load (p <0.001 and <0.001, respectively), while IL-18 was positively correlated with serum ALT activity (p <0.01 and <0.001, respectively) and serum AST activity (p <0.01 and <0.001, respectively). We found that in HCV+ and HBV+ patients there are higher levels of Th1 cytokines, particularly in the course of chronic hepatitis B, and that IL-18 and IL-6 levels may have important roles as markers of both inflammation and hepatic injury, particularly in the course of hepatitis C.

Keywords: HBV, HCV, cytokines, IL-6, IL-18, Th1/Th2, hepatitis

Introduction

Hepatitis C virus (HCV) and B virus (HBV) are the major causes of chronic liver disease throughout the world, with 55-80% of patients developing chronic hepatitis after infection with the viruses [1,2]. The outcomes of chronic HCV and HBV infections are extremely variable. The majority of cases are associated with insidious and progressive liver disease that may eventually lead to cirrhosis and hepatocellular carcinoma. However, the pathogenesis of liver damage during chronic HCV and HBV infections is poorly understood [3]. There is suggestive evidence that T-cell immunoregulatory cytokines may play a key role in influencing the persistence of HCV infection and the extent of liver damage [4-8]. In the course of HBV-related hepatitis, some Th1 phenotype cytokines are positively correlated with hepatic inflammatory activity [9].

Activated CD4+ T cells can be divided into 2 subsets based on their cytokine secretion profiles [10-12]. The T helper type 1 (Th1) subset produces interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), and interleukin (IL)-2, and participates in cell-mediated immune responses [13,14]; the T helper type 2 (Th2) subset produces IL-4 and IL-10, and mediates humoral immune responses [15] as well as anti-parasitic [16-18] and allergic responses [19,20]. The Th1/Th2 cytokine balance is likely important in determining the rate of HCV infection chronicity and HCV-induced liver injury [3,21,22]. In fact, some authors have suggested that a preferential shift towards either Th1 or Th2 response may influence the clinical outcome and disease progression [23-27]. This issue
has not been fully clarified, particularly in HBV and HCV-related hepatitis [28,29].

IL-6 and IL-18 are defined as pro-inflammatory cytokines, particularly as IL-6 plays a role in immune responses that may lead to viral clearance [30,31], and as IL-18 levels are correlated with metabolic and viral hepatic diseases [32].

The viral infections and some chronic injuries are known to suppress the immune system [33-35]. While the pathogenesis of chronic HCV or HBV infection has not been clearly defined yet, many researchers believe that cytokines play important roles in both immunoregulation and immune impairment [36-38].

The aims of the present study were (a) to evaluate the Th1/Th2 cytokine profiles and pro-inflammatory cytokine levels in plasma of HCV-positive patients compared to HBV-positive patients, and (b) to clarify the relationships of IL-18 and IL-6 to the degree of inflammation in viral liver disease by comparing their plasma levels to various markers of liver disease.

Materials and Methods

Twenty Caucasian patients with chronic hepatitis C virus (HCV) infection and 20 subjects with chronic hepatitis B virus (HBV) infection were recruited on their first examination at the Infectious Diseases Division. Twenty uninfected healthy subjects, matched for ethnicity, sex, and age, were recruited as a control group. All subjects gave written informed consent; the study was approved by the Medical Ethics Committee of “G. D’Annunzio” University Medical School.

All patients underwent a complete medical and laboratory evaluation including a liver ultrasound scan and biopsy, the results of which were used to divide the patients into 2 groups on the basis of the presence of HBV infection (14 males and 6 females, age 52.3 ± 12.2 yr) or HCV infection (15 males and 5 females, age 46.4 ± 10.2 yr).

The liver biopsies were ≥15 mm in length. The slides were stained with H&E. Liver biopsies were read by a single liver pathologist who was unaware of the patients’ clinical and laboratory data. Biopsies from patients with chronic hepatitis C and B were graded by hepatitis activity index scores according to Knodell [39]. The patients were all afflicted with mild or moderate degrees of chronic hepatitis C or B. The diagnosis of HCV or HBV infection was defined by typical biochemical and histological data and by detection of anti-HCV antibodies or HBV markers (Abbott Axsym HCV-3 and Ortho HCV 3.0 ELISA). All HBV+ patients were HbsAg positive, anti-HBs negative, and HbeAg positive (ELISA). Serum HCV-RNA and HBV-DNA were determined by the polymerase chain reaction (PCR) (AmpliCor method, Roche Molecular Diagnostics, Milan, Italy), with detection limits >600 HCV-RNA IU/ml of plasma and >300 HBV-DNA copies/ml of plasma.

The entire study population was negative for other forms of viral hepatitis and for human immunodeficiency virus infection (HIV). Other conditions known to cause liver dysfunction were excluded on the basis of clinical evaluation. None of the patients were taking steatogenic or antiviral drugs, nor had they done so for ≥6 mo. None showed clinical or biochemical signs of advanced liver disease (their plasma prothrombin times and serum albumin and total bilirubin levels were within normal ranges).

Fasting blood samples were drawn to test serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (γ-GT), alkaline phosphatase, glucose, total cholesterol, and triglycerides (enzymatic method, Ortho Clinical Diagnostics, Rochester, NY, USA), α1-fetoprotein (Liaison AFP kit, DiaSorin, Vercelli, Italy), and ferritin (Liaison ferritin kit, DiaSorin).

Cytokine patterns. At the time of liver biopsy, blood was withdrawn in sterile heparinized tubes, transported on ice to the laboratory, centrifuged at 6°C, and the plasma stored at −70°C until assayed. Plasma cytokine levels were measured in duplicate in all patients. The cytokines IFN-γ, TNF-α, IL-2, IL-4, IL-10, and IL-6 were evaluated by cytokometric bead array (CBA) assays (human Th1/Th2 cytokine kit, BD Biosciences, San Diego, CA, USA). For this assay, soluble cytokines are captured on microparticles and measured using a fluorescence-based detection system and flow cytometric analysis, as previously described [40]. A series of 10 dilutions of cytokine standards was run in each assay for the generation of standard curves. Samples were analyzed in a FACSCaliber flow cytometer using the BD CBA analysis software.

Plasma interleukin-18 (IL-18) levels were measured by enzyme-linked immunosorbent assay (IL-18 ELISA, R&D Systems, Minneapolis, MN, USA). The minimum detection limit estimated by serial dilution was 12.5 pg/ml, since the mean ±2 SD of the 6.25 pg/ml standard was lower than the mean −2 SD of the 12.5 pg/ml standard.

Statistics. The data are reported as mean ± SD. Statistical significance was assessed by t test for unpaired data. A p value <0.05 was required. Spearman’s correlation coefficients between plasma IL-18 and IL-6 levels and disease duration, viral load, serum AST, and serum ALT were computed.

Results

The 3 groups of patients (HCV, HBV, and controls) were well matched for age and sex. In patients with HCV+, we observed shorter disease duration than in the group HBV+ (3.5 ± 2.7 vs 6.7 ± 1.3 yr, p = 0.001). Positive determination of viral load was documented in all patients in the HCV+ and HBV+ groups. Clinical parameters are listed in Table 1.

Regarding Th1 assessment, the patients with HBV infection showed higher values of plasma IFN-γ levels than the HCV+ or the control group.
Table 1. Parameters and clinical laboratory measurements (mean ± SD) in the study populations.

<table>
<thead>
<tr>
<th>Parameter (and units)</th>
<th>HCV-infected patients (n = 20)</th>
<th>HBV-infected patients (n = 20)</th>
<th>Healthy control subjects (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>14/6</td>
<td>15/5</td>
<td>12/8</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>52.3 ± 12.2</td>
<td>46.4 ± 10.2</td>
<td>49.5 ± 7.8</td>
</tr>
<tr>
<td>Disease duration (yr)</td>
<td>3.5 ± 2.7</td>
<td>6.7 ± 1.3 **</td>
<td>-</td>
</tr>
<tr>
<td>Viral load (IU/ml, copies/ml)</td>
<td>382 ± 362 (x10^3) a</td>
<td>73 ± 120 (x10^3) b</td>
<td>-</td>
</tr>
<tr>
<td>Serum AST activity (U/L)</td>
<td>95.6 ± 117.9</td>
<td>51.9 ± 39.3</td>
<td>31.9 ± 2.8 † † $$$</td>
</tr>
<tr>
<td>Serum ALT activity (U/L)</td>
<td>125.4 ± 166.6</td>
<td>65.9 ± 48.7</td>
<td>35.1 ± 6.8 † † $$$</td>
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<tr>
<td>Serum γ-GT activity (U/L)</td>
<td>76.1 ± 57.0</td>
<td>54.4 ± 67.7</td>
<td>47.4 ± 4.5 † †</td>
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<tr>
<td>Serum alkaline phosphatase (U/L)</td>
<td>107.0 ± 56.7</td>
<td>97.2 ± 83.4</td>
<td>46.0 ± 5.1 † † $$$</td>
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<td>Serum ferritin (µg/L)</td>
<td>295.4 ± 216.6</td>
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</tbody>
</table>

HCV: hepatitis C virus; HBV: hepatitis B virus; AST: aspartate aminotransferase; ALT: alanine aminotransferase; γ-GT: gamma-glutamyltranspeptidase.

HCV patients vs HBV patients: ***p <0.001; *p <0.05
Controls vs HCV patients: †††p <0.005; ††p <0.01; †p <0.05
Controls vs HBV patients: §§§p <0.005; §§p <0.01; § p <0.05

Table 2. Th1/Th2 cytokine patterns in plasma samples from the study populations.

<table>
<thead>
<tr>
<th>Parameter (and normal range)</th>
<th>HCV-infected patients (n = 20)</th>
<th>HBV-infected patients (n = 20)</th>
<th>Healthy control subjects (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Th1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN-γ (0-5.7 pg/ml)</td>
<td>4.2 ± 6.4</td>
<td>32.5 ± 31.0***</td>
<td>1.9 ± 1.3 §§</td>
</tr>
<tr>
<td>TNF-α (0-4.4 pg/ml)</td>
<td>0.4 ± 0.8</td>
<td>2.5 ± 3.0**</td>
<td>0.5 ± 0.8 §§§</td>
</tr>
<tr>
<td>IL-2 (0-3.4 pg/ml)</td>
<td>0.3 ± 0.2</td>
<td>5.6 ± 7.8**</td>
<td>0.5 ± 0.5 §§§</td>
</tr>
<tr>
<td>Th2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-4 (0-4.6 pg/ml)</td>
<td>0.5 ± 0.3</td>
<td>4.3 ± 2.7****</td>
<td>0.3 ± 0.7 §§§</td>
</tr>
<tr>
<td>IL-10 (0-4 pg/ml)</td>
<td>0.7 ± 1.5</td>
<td>3.7 ± 5.9*</td>
<td>0.2 ± 0.2 † §</td>
</tr>
</tbody>
</table>

IFN-γ: interferon-gamma; TNF-α: tumor necrosis factor-alpha; IL: interleukin

HCV patients vs HBV patients: ***p <0.0001; *p <0.01; +p <0.04
Controls vs HCV patients: †p <0.05
Controls vs HBV patients: §§§p <0.005; §§p <0.01; § p <0.05

Table 3. Spearman’s rank correlation coefficients between some variables in HCV+ and HBV+ patients.

<table>
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<tr>
<th>Parameter</th>
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<th>HBV-infected patients</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IL-6</td>
<td>IL-18</td>
<td>IL-6</td>
</tr>
<tr>
<td>Disease duration (yr)</td>
<td>0.65 **</td>
<td>-</td>
<td>0.88 ***</td>
</tr>
<tr>
<td>Viral load (IU/ml)</td>
<td>0.83 ***</td>
<td>-</td>
<td>0.88 ***</td>
</tr>
<tr>
<td>Alanine aminotransferase (ALT)</td>
<td>-</td>
<td>0.63 **</td>
<td>-</td>
</tr>
<tr>
<td>Aspartate aminotransferase (AST)</td>
<td>-</td>
<td>0.57 **</td>
<td>-</td>
</tr>
</tbody>
</table>

** p<0.01, *** p<0.001
1 HCV-RNA (IU/ml) for patients HCV+ and HBV-DNA (copies/ml) for patients HBV+.
TNF-α and IL-2 levels were higher in patients with HBV+ compared to the HCV+ patients (p < 0.001) or the control group (p < 0.005). Only in HBV infection did the IFN-γ and IL-2 levels exceed the normal ranges. The Th2 cytokine pattern demonstrated that IL-4 and IL-10 levels were higher in HBV patients than in HCV patients (p < 0.0001 and < 0.04 respectively) or the control group (p < 0.01), but the levels were always within normal range (Table 2).

In regard to the inflammatory cytokines, plasma IL-6 was higher in the HCV+ group than the controls (12.3 ± 19.1 vs 3.9 ± 11.5 pg/ml, p < 0.02) and in the HBV+ group than the controls (8.4 ± 11.7 vs 3.9 ± 11.5 pg/ml, p < 0.04). Moreover, plasma IL-18 levels were higher in patients with HCV+ (518.5 ± 251.9 pg/ml) than in those with HBV+ (369.8 ± 166.0 pg/ml) (p < 0.01) or in the controls (132.8 ± 66.9 pg/ml) (p < 0.0001). Plasma IL-18 levels were higher in HBV+ patients than in the control group (369.8 ± 166.0 vs 132.8 ± 66.9 pg/ml, p < 0.0001) (Fig. 1).

Strong correlations were found between high plasma levels of IL-6 and IL-18 and the hepatic index of disease. In both the HCV+ and HBV+ groups, correlation analysis documented that IL-6 was positively correlated with illness duration (p < 0.01 and p < 0.001, respectively) and viral load (p < 0.001 and p < 0.001, respectively). Plasma IL-18 levels in HCV+ and HBV+ groups were positively correlated with serum ALT activity (p < 0.01 and < 0.001, respectively) and serum AST activity (p < 0.01 and < 0.001, respectively) (Table 3).

Discussion

Cytokines are mediators of various biological processes including inflammation, apoptosis, necrosis, and fibrosis [41-45]. Paradoxically, they are also involved in the regeneration of liver tissue after injury. Experimental evidence suggests that immune response factors, especially the pro-inflammatory cytokines, play an important role in liver injury induced by HBV and HCV [46-48]. Recent studies suggest that treatment outcomes may depend on the development of type Th1 and Th2 cell responses [49]. Specifically, activation of Th1 immunity may play contribute to successful treatment of hepatitis B and C [26,50-55].

Several studies showed that hepatitis HBV+ is characterized by type Th1 cytokines that play an important role. In fact, IL-4 and IFN-γ are effective in the chronic progression of hepatitis, while TGF-β is effective in the development of fibrosis. Serum cytokine levels may be effective tools in the estimation of chronic progression and fibrosis development [56,57]. Other studies demonstrate that Th2 cells may be associated with the persistence of HBV infection [9,58]. Moreover, there is evidence that HCV+ patients present a Th1 type immune response with over-production of IFN-γ [24-26]. In contrast, Fan et al [58] documented a Th2 type immune response during chronic active hepatitis C infection.

Our data show elevated levels of IFN-γ, TNF-α, and IL-2 in HBV+ patients in comparison to HCV+ patients and healthy subjects. Therefore our
patients with chronic HBV infection demonstrate a Th1 type cytokine profile. The patients with HCV related hepatitis showed a trend towards the Th1 type response, with an increase of IFN-γ, although these data were not statistically significant.

The IL-6 cytokine is a major mediator of inflammation and acute phase responses of the liver and serves to block apoptosis during the inflammatory process [60,61] and its activity may affect chronic disease progression [62]. As IL-18 promotes the differentiation of naïve T cells into Th1 cells, it may also have a negative role in the immunopathogenesis of chronic hepatitis C [63]. Nevertheless, its different levels in separate compartments of the body should be dynamically evaluated in order to clarify Th1/Th2 balance in HCV infection [26,46,50-52].

Our results documented higher plasma levels of IL-6 and IL-18 in patients with hepatitis HCV+ and HBV+ compared to controls; moreover the IL-18 and IL-6 levels were higher in HCV-infected patients than in HBV+ patients. Our data show that during the course of HBV infection there is an increase of Th1 type cytokines while in the course of chronic HCV hepatitis the increase of pro-inflammatory cytokines is typically more profusely represented. In addition, we observed strong positive correlations between plasma IL-18 levels and indices of inflammation and necrosis in patients with HCV and HBV. In regard to IL-6, we have highlighted associations with illness duration and HCV-RNA or HBV-DNA. We showed that in HCV and HBV patients the plasma levels of IL-18 and IL-6 could have important roles of markers of both inflammation and hepatic injury.

In conclusion, our data, supported by other studies [26,46,50,58,61,62], implicate an important pathogenic role of humoral immunity in liver injury in HCV+ patients, and a role of cell-mediated immunity in patients with chronic hepatitis from HBV. In addition, our data demonstrate, for the first time, positive correlations between plasma IL-18 and IL-6 levels and indices of hepatic injury. Our data support the roles of IL-18 and IL-6 as markers of global liver injury. However, relatively little is presently known about these cytokines in HCV and HBV infections and further studies are needed.

References

Cytokine levels in HBV- and HCV-related hepatitis


39. Knodell RG, Ishak KG, Black WC, Craig R, Kaplowitz N, Kiernan TW, Wollman J. Formulation and application of a