original article

Relationship between circulating interleukin-10 and histological features in patients with chronic C hepatitis

Cosimo Marcello Bruno,[a] Maria Valenti,[a] Gaetano Bertino,[a] Annalisa Ardiri,[a] Alfredo Amoroso,[b] Maria Consolo,[b] Clorinda Maria Mazzarino,[b] Sergio Neri[a]

From the [a]Department of Internal Medicine and Systemic Diseases, [b]Department of Biochemical Science and General Pathology, University of Catania, Catania, Italy

Correspondence: Professor C. M. Bruno · Dip. Medicina Interna – Osp. S. Marta via G. Clementi 36, 95124 – Catania · T: +0039 0957435677 · cmbruno@unict.it · Accepted: October 2010

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BACKGROUND AND OBJECTIVES: An imbalance in cytokine production may be involved in the pathogenesis of chronic C hepatitis. The aim of the study was to investigate circulating levels of interleukin-10 (IL-10) in a selected cohort of patients affected by chronic C hepatitis.

DESIGN AND SETTING: Retrospective study based on consecutive hepatitis C virus patients, affected by chronic active hepatitis, attending the general hospital of hepatology unit from June to September 2009

PATIENTS AND METHODS: A total of 49 patients with chronic C hepatitis and 20 healthy control subjects similar in gender and age were examined. Circulating IL-10 was assessed by ELISA commercial kit (R and D Systems) in all investigated subjects.

RESULTS: There was no significant difference in IL-10 values between controls and overall patients ($P>.05$). Nevertheless, among patients, subjects with more severe necroinflammation had higher values than others ($P<.001$). Moreover, a close relationship was found between IL-10 values and serum aspartate aminotransferase ($r=0.61$; $P<.001$).

CONCLUSIONS: These findings suggest that IL-10 may be a useful additional marker to assess necroinflammation and to monitor the evolution of liver damage. They also argue for a potential pathophysiological role for IL-10 in the persistence and progression of hepatitis.

Hepatitis C virus (HCV) infection is largely widespread in Southern Europe and HCV-related chronic hepatitis represents the first cause of cirrhosis in these countries.[1] The reasons for viral persistence and transformation from acute to chronic infection are not clear, but both pathogen and host characteristics can influence the outcome of viral infection. During hepatitis C virus infection, humoral and cell-mediated immune responses play a key role in the host defense. However, the crucial role of T cell response is often unable to control viral replication. In fact, functional T cell exhaustion with impaired proliferative potential and altered cytokine production can occur, leading to persistent viral infection.[2-4]

Cytokines are soluble proteins produced by a wide variety of cells, mainly Th1 and Th2 cells. They can recognize virus-infected cells and, directly or indirectly, regulate the strength of the immunological and inflammatory response. It has been reported that in HCV infection the production of inappropriate levels of cytokines is associated with virus persistence and failure of antiviral therapy.[5] Interleukin-10 (IL-10) is an immunoregulatory cytokine released by macrophages, dendritic cells, B cells, and various subsets of CD4+ and CD8+ T cells.[6,7] This molecule can attenuate the inflammatory response and deregulate cytokine production and T cell proliferation. Because of its immunoregulatory action, it has been postulated that inadequate levels of IL-10 can determine long-term escape of pathogens from immune control and give rise to persistent infections.[8] The aim of the study was to investigate plasma levels of IL-10 in a selected cohort of patients affected by chronic C hepatitis and to correlate its circulating values with biochemical and histopathological features.

PATIENTS AND METHODS

The study was conducted in the same population previously studied,[9] which consisted of 49 neodiagnosed and untreated white patients affected by HCV-related chronic hepatitis (27 men and 22 women; mean [SD] age, 58 [9] years) and 20 white control subjects, comparable in gender and age (11 men and 9 women; mean [SD] age, 56 [8] years). Patients enrolled in the study were selected based on the consecutive patients affected by chronic active C hepatitis, among those attending the internal medicine unit or hepatology center of our department.

Diagnosis in patients was based on clinical (medical history, physical examination), instrumental (ultrasonography, endoscopy, liver biopsy), and laboratory (serum HCV antibodies, HCV-RNA and liver function tests) data. Exclusion criteria were autoimmune diseases, alcohol abuse, and drug-induced liver injury. In addition, patients with evidence of other chronic or acute infective processes (altered white blood cells count, temperature, urinary tract infection, airway infections) were excluded as well as those with suspected hepatocellular carcinoma (HCC) on the basis of ultrasonography, alpha-fetoprotein and carcinoembryonic antigen levels performed during the screening, according to the recommended screening strategy.[10,11] Moreover, to further increase the sensitivity and specificity of screening for HCC, we determined in all patients plasma des-gamma-carboxy prothrombin levels, and those with a serum value greater than 40 mAU were also excluded.[12] Serum des-gamma-carboxy prothrombin levels were determined by sensitive enzyme immunoassay (Eitest PIVKA-II kit; Eisai Laboratory, Tokyo, Japan, cut-off = 40 mAU1/ml) according to the manufacturer's instructions.[13,14] Among control subjects, none had a history of alcohol or drug abuse or clinical signs of infections. Liver function tests and ultrasonography were also performed to exclude liver diseases before inclusion in the IL-10 control group. Informed consent was obtained from all patients and controls and the study conformed to the Helsinki Declaration. While fasting in the morning, a blood sample was withdrawn from all subjects (controls and patients). Samples were centrifuged for 15 minute at 1600*g* and plasma was stored at −80°C until determination. All samples were tested in duplicate.

IL-10 was assessed by ELISA commercial kit (R and D Systems) according to the instructions of the manufacturer and results expressed as pg/mL. Sensitivity of the assay was less than 3.9 pg/mL. Intra- and inter-assay variability was 1.7 and 5.9%, respectively. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and platelet counts were also detected by auto-analyzer in our hospital laboratory, EN ISO 9001:2000 certified. HCV genotype in all patients was 1b. This viral genotype is by far the most widespread in southern and insular Italy[1] and is responsible for almost all HCV infections that we observed.

All sections from liver biopsies were examined by a histopathologist who was unaware of the clinical details. Histological findings were quantified separately with regard to inflammation and fibrosis on the basis of grading and staging score, according to histological activity index.[15] Twenty-six subjects had none or mild fibrosis (staging score 0-1) and 23 had moderate or severe fibrosis (staging score 2-3). According to the grading score, 31 had minimal or mild inflammatory activity (score 1-8) and 18 had moderate or severe inflammatory activity (score 9-18). Analysis of variance and the Kruskall-Wallis test were used to compare mean (standard deviation) between various groups. Relationships between continues variables were investigated by a correlation test. Statistical significance was set at $P<.05$.

Table 1. Demographic characteristics, IL-10 values and liver function tests for different grades and stages of HCV-related chronic hepatitis.

	Controls n=20	All HCV patients (n=49)	Staging 0-1 (n=26)	Staging 2-3 (n=23)	Grading 1-8 (n=31)	Grading 9-18 (n=8)
Gender (M/F)	11/9	27/22	15/11	12/11	17/14	10/8
Mean age (SD) (years)	56 (8)	58 (9)	54 (6)	60 (3)	57 (5)	59 (4)
Mean AST (mU/mL), range	24 (18–33)	93.1 (133-59)	83.2 (66–112)	70.2 (59–83)	88.1 (70–106)	99.9 (84–133)
Mean ALT (mU/mL), range	22 (14–28)	85.4 (91-46)	76.1 (55–88)	71.4 (46–85)	75.3 (69–79)	81.2 (77–91)
Mean IL-10 (SD) (pg/mL)	15.4 (1.9)	16.1 (2.1)	16.6 (2.6)	15.9 (2.3)	15.4 (0.9)[a]	17.2 (2.4)

[a] $P<.001$ for grading 1-8 vs grading 9-18.

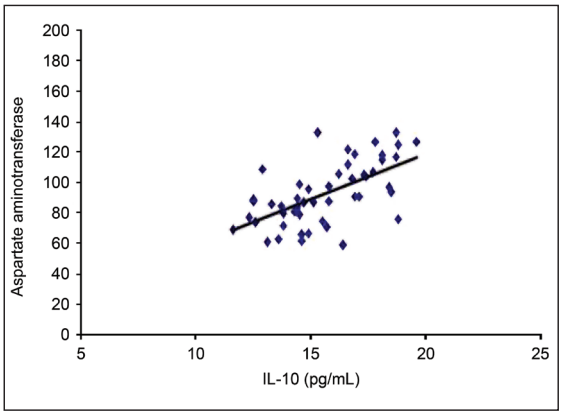

Figure 1. Relationship between serum aspartate aminotransferase and circulating IL-10 in 49 untreated patients with chronic C hepatitis (r = 0.61; *P*<.001).

RESULTS

The mean (SD) IL-10 serum value was 16.1 (2.1) pg/mL in HCV patients and 15.4 (1.9) pg/ml in controls. Statistical analysis showed no significant difference in IL-10 values between controls and overall patients (*P*=.172), but patients with a grading score of 9-18 had higher values than patients with score 1-8 (*P*<.001). No significant difference in IL-10 serum values were found between patients with staging score 0-1 and those with score 2-3 (*P*=.326) (**Table 1**). Mean serum AST and ALT was 93.1 mU/mL (range, 133–59) and 85.4 mU/mL (range 91–46), respectively, in HCV patients.

In addition, in the patient group, linear regression test showed a significant correlation (r = 0.61; *P*<.001) between serum IL-10 and AST values (**Figure 1**). There was no significant correlation between IL-10 and serum ALT (r=0.21; *P*>.05) nor between IL-10 and the AST/platelets ratio index (APRI) (r=0.22; *P*>.05).

DISCUSSION

About 3% of the world population is affected by HCV infection, but spontaneous viral clearance is observed in only 15% to 40% of patients with acute hepatitis, and persistent infection is associated with progression to cirrhosis and hepatocellular carcinoma.[16,17] The pathogenesis of chronic HCV infection is not well understood, but the vigour of T cell response to HCV antigens is one of the most important factors capable of influencing disease outcome. Activated T cells can modulate several immunological effector mechanisms, including synthesis of cytokines.[18] Cytokines are regulatory molecules that play a key role in orchestrating several physiological and pathologic processes during viral infection of the liver.[19] For this reason, it has been postulated that an imbalance in proinflammatory/anti-inflammatory cytokines production, secondary to T cell dysregulation, may be involved in the pathogenesis of chronic C hepatitis.[19,20]

IL-10 is a cytokine, mainly produced by Th2 cells, which inhibits MHC class II expression on monocytes and macrophages and limits the production of proinflammatory cytokines including IL-1, IL-2, IL-6, TNF-a, and IFN-γ. IL-10 can further limit T cells activation and differentiation, leading to suppression of proinflammatory responses in tissues.[8] Therefore, the physiological role of IL-10 during infectious diseases is likely to reduce tissue damage resulting from the unfavorable and excessive effects of inflammation. However, an inappropriate production of IL-10 during a virulent infection may compromise the effectiveness of the immune system, allowing fulminant or persistent infection.

We investigated serum IL-10 values in 49 untreated patients with demonstrated chronic C hepatitis. No significant difference was found in IL-10 levels between patients and controls. However, when we stratified patients on the basis of the grading score, IL-10 was significantly higher in patients with score 9-18 than in those with score 1-8. In addition, we found a close relationship between IL-10 values and serum AST. Even though during hepatitis C infection transaminase levels fluctuate, it is quite certain that an increase of these enzymes, especially AST, is a reliable marker of necroinflammatory activity. Therefore, we can reasonably argue that the increased levels of IL-10 in patients with chronic C hepatitis reflect the degree of necroinflammation.

Other authors have previously investigated IL-10 in subjects with chronic liver disease, including hepatitis C. Most of the authors found an increase of circulating IL-10, but values similar to healthy controls both in subjects with asymptomatic HCV infection and patients with chronic hepatitis have also been described.[21-26] Interestingly, a reduction of circulating IL-10 levels, reflecting the decrease of transaminases in patients treated with interferon or interferon plus ribavirin, especially in responder subjects, has been also reported by some but not all authors.[21-26]

The long-term effects of therapy on our patients are not yet known, but our results show that in untreated subjects, increased IL-10 levels are only detectable in those with a more severe inflammatory activity. Furthermore, the differences between various reports could be due to heterogeneity of investigated populations and prevalence of sustained inflammation in the study series. It is not known whether the increase of IL-10 is only a consequence of persistent inflammation or whether it may play a role in favoring the persistence of

virus infection and chronic disease.

Experimental studies conducted on animal models showed that a IL-10 blockade strategy is capable of ameliorating the outcome of chronic viral infection.[27,28] Moreover, long-term IL-10 treatment in chronic hepatitis C patients appears to decrease various inflammatory parameters, but it has a proviral effect demonstrated by increase of HCV-RNA levels.[29]

Taken together, available studies suggest that IL-10 contributes to reduce the pathogenic effects of infection for the price of a weakening of the immune response aimed at the eradication of the virus, and that timing as well as the relative amounts of IL-10 production are critical for safe resolution of infection. It has also been reported that the heterozygous C/A variant in the IL-10 gene promoter region C592A is associated with a major risk of progression and chronic course of viral hepatitis in a white population.[30] It is therefore likely that an alteration of the host immune system due to genetic predisposition or induced by some proteins of the virus determines the switch in the predominant activation from Th1 to Th2 cells, with a consequent imbalance in the production of immunoregulatory cytokines. Such imbalance could determine a greater increase in IL-10 concentration, leading to impaired viral clearance and persistent necroinflammation.

It has also been postulated that an IL-10 has antifibrotic action.[31] Nevertheless, in our patients, there was no significant relation between serum IL-10 values and staging score nor between serum IL-10 and APRI, an indirect and validated index of liver fibrosis.[32]

In conclusion, our study showed an increase in circulating IL-10 in patients affected by chronic hepatitis C with sustained necroinflammatory activity. In addition, IL-10 levels were related to serum AST.

These findings suggest that IL-10 may be a useful additional marker to assess necroinflammation and to monitor the evolution of liver damage. They also argue for a potential pathophysiological role of IL-10 in the persistence and progression of hepatitis. In the future, a better knowledge of intrinsic mechanisms regulating immunological response and cytokine production could either influence the clinical approach or provide novel therapeutic strategies.

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