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Modulation of the anti-inflammatory interleukin 10 and of proapoptotic IL-18 in patients with chronic hepatitis C treated with interferon alpha and ribavirin

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SUMMARY. The aim of this work was to analyse apoptosis rate, measured by the serum levels of proapoptotic interleukin (IL)-18 and of soluble Fas (sFas), as well as of anti-inflammatory IL-10, in patients with chronic hepatitis C, at baseline and after treatment with interferon alpha and ribavirin. Twenty-seven patients with biopsy-proven chronic hepatitis C were studied, at baseline and after treatment with interferon alpha (21 cases) or pegylated interferon (6 cases) plus ribavirin. A group of 15 healthy sex- and age-matched individuals was selected as control. Serum concentrations of sFas, IL-10 and IL-18 were determined by ELISA in sandwich. The relationship of these molecules to necro-inflammatory and fibrotic activity was evaluated. Evolution of the serum concentrations of these molecules was analysed after treatment. Significantly increased serum concentrations of sFas were detected in patients with chronic hepatitis, compared with controls. Levels of this molecule were significantly correlated with necroinflammatory activity. Likewise, concentrations of IL-10 were significantly increased in the group of patients, compared with controls. Treatment with interferon and ribavirin induced a significant decrease of IL-18 concentration independently of the viral response. In contrast, levels of sFas decreased only in those patients with sustained response to therapy. Finally, baseline levels of IL-10 were significantly increased in patients without response to treatment, compared with those with sustained response, but the concentration did not change with the treatment. Increased serum levels of IL-10 are a negative prognostic marker of response to hepatitis C treatment. A significant decrease of apoptotic rate, as determined by sFas, can be expected in patients with a response to therapy.

Keywords: apoptosis, chronic hepatitis C, interferon alpha, interleukin 10, interleukin 18, ribavirin.

INTRODUCTION

The exact mechanism responsible for hepatocellular damage in chronic hepatitis C virus (HCV) infection remains elusive. The infected liver is infiltrated with CD8+ lymphocytes, most of which are thought to be cytotoxic T lymphocytes that can induce apoptosis of hepatocytes [1]. Both Fas and Fas ligand (FasL) expression on hepatocytes [2] and liver-infiltrating lymphocytes [3], respectively, has been confirmed in patients with chronic HCV infection. Levels in serum of the soluble form of Fas (sFas) have been shown to be related with the severity of liver inflammation in chronic hepatitis [4]. Interleukin (IL)-18, a cytokine produced by a wide range of cell types, including Kupffer cells [5], potentiates hepatocyte apoptosis [6]. Increased expression of IL-18 has been detected in liver biopsies of patients with chronic hepatitis C [5], with levels correlated with those of Fas and necroinflammatory activity [4–6].

Not only apoptosis, but also inflammation is essential for antiviral host defence. An excess inflammatory response is the mechanism commonly involved in the liver damage, resulting in tissue damage, fibrosis and cirrhosis [7]. Additionally, it has been hypothesized that the course of HCV infection is the result of the balance of Th1 and Th2 lymphocytes in the liver, with a predominance of Th2 responses [8]. The effects of IL-10, a cytokine synthesized by macrophages and Th2 lymphocytes, are both anti-inflammatory and anti-apoptotic effect [9]. Increased expression in the liver [10] and elevated serum levels of IL-10 [11] have been demonstrated in patients with chronic hepatitis C.

Treatment of chronic hepatitis C with interferon-α and ribavirin controls HCV replication and improves transaminase levels and liver histology in 40% of patients [12]. The immune effects of these drugs and their influence on

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus.

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response have only been partially established [13]. We hypothesize that chronic hepatitis therapy modulates the cytokine pattern, inducing an increase of anti-inflammatory IL-10 and a decrease of IL-18, with a diminution of the apoptosis rate in responding patients. Thus, we studied changes in the serum concentrations of these molecules after treatment and the differential pattern in responders and non-responders.

PATIENTS AND METHODS

Patients and controls

Twenty-seven patients with chronic hepatitis C, attended at the Gastroenterology Unit of the University Hospital Puerta del Mar, Cádiz, Spain, were included in this prospective study, which started in 2000.

An interpretable liver biopsy in the last 6 months with a minimum fibrosis score ≥1, according the histological index proposed by Knodell and modified by Scheuer and Desmet [14] was required before therapy. Patients with active alcohol or drug dependence, hepatitis B surface antigen, human immunodeficiency virus or other infectious, autoimmune, tumoural, biliary, or vascular-associated liver disease were excluded. Demographic, virologic and histologic characteristics of the patients are shown in Table 1.

Fifteen healthy controls were selected from hospital workers. Informed consent was obtained from patients and controls. The protocol conformed to the ethical guidelines of

Table 1 Demographic, biochemical, virological and histological characteristics of the population

Number	27
Gender (male:female ratio)	20 (74):7 (26)
Age (years)	38 (35–41)
Serum AST concentration (UI/l)	102 (84–120)
Serum ALT concentration (UI/l)	57 (49–65)
Patients with a daily alcohol consumption >50 g (%)	21.2
HCV genotypes (*n*, %)	
1	16 (59.2)
2	4 (14.8)
3	2 (7.4)
4	5 (18.5)
HCV viral load (copies × 10^5/mL)	7.1 (4.0–10.1)
Necro-inflammatory activity	5.7 (4.6–6.7)
Fibrosis score	1.2 (1.0–1.3)

Data are provided as number (*n*) and percentage (%) or as mean (95% CI).
AST, aspartate aminotransferase; ALT, alanine aminotransferase; HCV, hepatitis C virus.

Table 2 Baseline concentrations of IL-10 and IL-18 and soluble Fas in patients with chronic hepatitis C and healthy controls

		Chronic hepatitis C patients ($n = 27$)						
		Baseline			End of therapy		End of follow-up	
Parameter	Healthy controls ($n = 15$)	Overall ($n = 27$)	Responders ($n = 10$)	Nonresponders ($n = 17$)	Responders ($n = 10$)	Nonresponders ($n = 17$)	Responders ($n = 10$)	Nonresponders ($n = 17$)
IL-10 (pg/mL)	1 (0–1)*	13 (0–35)*	1 (0–2)‡	21 (0–62)‡	3 (0–9)	20 (12–57)	2 (0–5)	ND
IL-18 (pg/mL)	72 (21–122)	136 (80–193)	98 (30–166)§	147 (18–96)	24 (6–46)§	15 (2–31)	51 (69–141)§	ND
sFas (pg/mL)	549 (493–605)**	939 (861–1017)**	888 (799–977)§	911 (538–1501)	716 (652–819)§	915 (648–1341)	684 (608–761)§	ND

Data are provided as mean (95% CI) Healthy controls *vs* chronic hepatitis patients (baseline, overall): $^*P < 0.01$; $^{**}P < 0.001$.
Chronic hepatitis patients, responders *vs* nonresponders: ‡$P < 0.001$.
Patients with complete response to treatment, baseline *vs* end of therapy or end of follow-up: §$P < 0.05$.
Patients without complete response to treatment, baseline *vs* end of therapy or end of follow-up: ¶$P < 0.01$.

the 1975 Declaration of Helsinki and had been approved by the institutional human research committee.

Indications for interferon alpha plus ribavirin therapy were based on individual status according to consensus recommendation at the time of patient inclusion [15]. Twenty-one (77.8 %) patients received treatment with interferon alpha-2a (standard IFN) (Roferon-A; Hoffman-La Roche, Nutley, NJ, USA) at a dose of 3 million units thrice-weekly, plus ribavirin. Six patients (22.2 %) received treatment with pegylated interferon alpha-2a (PEG-IFN) (Pegasys; Hoffman-La Roche) at a dose of 180 μg, subcutaneously each week, plus ribavirin. Daily dose of ribavirin in patients of both groups was 800 mg. Treatment was continued for 48 weeks either because patients harboured an unfavourable hepatitis C genotype (1 or 4), or because in patients with favourable genotypes (2 or 3), HCV viral load was higher than 800 000 UI/L.

According to the response to treatment, patients were classified in three groups: (i) Non-responders, when HCV viral load remains positive at 12 weeks of treatment. (ii) Sustained responders, those with a negative HCV viral load at the end of the therapy and which continued to be negative 6 months after the cessation of treatment. (iii) Transient responders or relapsers, when the patients had a negative HCV load at the end of the therapy but showed a positive HCV viral load 6 months after the cessation of treatment.

Laboratory determinations

Sera of all subjects were reactive for anti-HCV by both a second-generation enzyme immunoassay (EIA-2) and a second-generation recombinant immunoblot assay (RIBA-2) (Ortho Diagnostic System, Raritan, NJ, USA). Plasma samples were tested for HCV RNA by reverse transcription-polymerase chain reaction (Amplicor HCV, Roche Diagnostics, Basel, Switzerland). HCV genotypes were determined by line probe assay (INNO-LiPA HCV; Innogenetics, Antwerp, Belgium).

Blood samples were centrifuged (150 g for 15 min at 4 °C) and serum was stored at −80 °C in pyrogen-free polyethylene tubes until IL-10, IL-18 and sFas were assayed with ELISA kits (R& D, Minneapolis, MN, USA), according to manufacturer's instructions, with the following detection limits (lowest positive standard): IL-10, 0.5 pg/mL; IL-18, 15 pg/mL; sFas, 20 pg/mL.

Statistical analysis

The data from two independent groups were compared with the Mann–Whitney *U*-test. Significance of parameters within each group was tested by the Wilcoxon matched-pairs signed rank test. For qualitative variables, χ^2 with Yates' correction or Fisher's exact test was used. Correlations were assessed by the Spearman's method. A *P*-value lower than

Table 3 Differential baseline characteristics of chronic hepatitis C patients in function of the response to the treatment with interferon alpha and ribavirin

Parameter	Response to treatment	
	Complete response ($n = 10$)	Relapse or absence of response ($n = 17$)
Age (years)	38 (35–41)	40 (34–47)
Men:women ratio	8 (80): 2 (20)	12 (71): 5 (29)
Platelets/μL (×1000)	178 (142–215)	160 (129–191)
Prothrombin index (%)	95 (89–100)	103 (97–109)
ALT (UI/L)	120 (87–153)	97 (70–125)
AST (UI/L)	61 (46–77)	57 (42–72)
Total bilirrubin (mg/dL)	0.7 (0.5–0.9)	0.7 (0.5–0.9)
Albumin (g/dL)	4.0 (3.5–4.4)	4.1 (3.9–4.2)
Ferritin (ng/mL)	129 (27–231)	264 (128–399)*
Genotype 1 or 4 (*n*, %)	4 (19.0)	14 (82.3)†
HCV viral load (copies x 10^5/mL)	3.6 (2.0–5.0)	9.5 (2.1–16.9)‡
Necroinflammatory activity index	5.5 (3.7–7.4)	5.8 (3.9–7.7)
Fibrosis index	1.0 (1.0–1.1)	1.3 (1.1–2.0)
IL-10 (pg/mL)	0.8 (0.0–2.2)	20.7 (0.6–62.2)§
IL-18 (pg/mL)	77.9 (0.0–165.7)	46.6 (0.0–95.6)
sFas (pg/mL)	888 (799–977)	911 (538–1501)
Treatment with pegylated interferon *vs* standard interferon (*n*, %)	3 (50) *vs* 7 (33.3)	3 (50) *vs* 14 (66.7)

AST, aspartate aminotransferase; ALT, alanine aminotransferase; HCV, hepatitis C virus.
*$P = 0.014$, †$P = 0.024$, ‡$P = 0.006$, §$P < 0.001$.

0.05 was considered significant. The statistical analysis was performed using the SPSS 11.5 program (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Increased serum concentrations of sFas and IL-18 (although a statistical significance was not reached in this latter case) were detected in chronic hepatitis patients compared with healthy controls. Likewise, and possibly as a compensatory mechanism, serum IL-10 levels were significantly elevated in patients (Table 2), as has previously been observed [13,16].

A positive correlation of serum sFas and the necro-inflammatory index ($r = 0.35$, $P = 0.03$), as well as of IL-18 with aminotransferase levels (aspartate aminotransferase, AST: $r = 0.54$, $P = 0.006$; alanine aminotransferase, ALT: $r = 0.51$, $P = 0.011$) was seen. Serum concentration of the anti-inflammatory IL-10 was negatively correlated with that of AST ($r = -0.41$, $P = 0.006$).

Patients were classified in to three groups according to their response to therapy: responders (10 cases, 37.0 %), relapsers (4 cases, 14.8 %) and non-responders (13 cases, 48.1 %). Those patients with a complete response to treatment were significantly less numerous among those with genotype 1 or 4 or presented with lower baseline serum levels of ferritin, HCV load (Table 3), as has previously been demonstrated [15].

Increased concentrations of IL-10 were detected in non-responder patients and baseline IL-10 levels in those with a complete response were similar to healthy controls. This may be because a high local concentration of IL-10, resulting from direct stimulation of this cytokine by Kupffer cells or regulatory Th2 lymphocytes in response to HCV proteins [17], may inhibit Th1 cells and the host immune response against HCV, thereby contributing to viral persistence in patients with chronic hepatitis C [18].

Changes in IL-10 and IL-18 and of sFas during treatment, were studied by grouping patients into those with a complete response to therapy (responders) and others (non-responders and relapsers). In both groups of patients, assays were performed at baseline, at the end of the treatment and, in responders, after 6 months of follow-up (Table 2). A significant decrease of serum levels of sFas was observed in responders. Increased levels persist in those in whom the HCV infection was not eliminated by the therapy.

In both responding and non-responding patients, a significant decrease of IL-18 was observed, with no differences in the decrease of this cytokine between the two groups. The hepatitis C therapy-induced decrease of this cytokine could be implicated in some of the favourable effects of interferon alpha and ribavirin on histologic damage, such as the decrease in necroinflammatory activity or in the fibrosis index observed in treated patients, although a virologic response was not found [15].

Interleukin-10 concentrations did not show statistically significant differences at the various points in the evolution. The persistent inflammatory and fibrotic liver lesion could stimulate the continued secretion of IL-10, with higher serum concentrations of this cytokine in nonresponding patients, representing a compensatory mechanism [19].

In conclusion, treatment of HCV infection has a negative influence on IL-18 concentration, independently of viral effects. A normalization of sFas, as a marker of necro-inflammatory activity and of apoptosis, is associated with a complete response. However, interferon alpha plus ribavirin were not able to modify the increased concentrations of IL-10 detected in nonresponding patients.

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